

**Testing for Operant Self-Administration of Aggression in Female Mice**

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PSY379H: Honors Research II

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October 7, 2021

### **Abstract**

Aggression and acts of violence incur overwhelming psychological and physical costs on societal function. In previous studies, males across species have commonly been implicated as the main perpetrators of violent behaviors, including appetitive aggression. Appetitive aggression is reward-motivated and self-administered by the perpetrator. For instance, a subset of dominant male mice have been observed to self-administer aggression by operantly lever-pressing for access to subordinate males to attack. Yet, there is a relative dearth of the examination of aggressive behavior in female mice beyond maternal, territorial, and dominance contexts. The goal of this study was to gauge whether retired, female dam mice, or “resident” females, self-administer aggression during fixed-ratio and progressive ratio testing in a resident-intruder paradigm. The results indicated that while a subset of the retired female dams showed a preference for the active lever, no residents exhibited aggressive behavior towards the intruders. Thus, retired female dams do not display appetitive aggression when presented with the opportunity to lever-press for aggressive interaction.

*Keywords:* Appetitive aggression, female mice, operant self-administration

A primary motivation for this study stems from the numerous and serious consequences of violent, pathological behavior on both its human perpetrators and victims. Homeless children, who are highly exposed to aggression due to violent parent-child relationships, are at higher risk of suffering from behavioral problems, social rejection, and aggressive attacks when interacting with peers. Among 12 to 18 year-old adolescents, witnessing or being a victim of violent aggression significantly predicts the likelihood of committing violent aggression in the future (Anooshian, 2005). Exposure to violence is also linked to increased drug use, poorer cognitive abilities, poorer school performance, and increased risk of depression and low self-esteem (Wichorek & Tisak, 2011). The effects of both physical and psychological aggression have also been observed in the healthcare and welfare system. Healthcare and welfare personnel experience extreme stress after physically and emotionally aggressive encounters by patients (Schanlon et al., 2012). Violence in schools is also a major public health concern, and many schools lack effective procedures for mitigating incidents of violence (McMahon et al., 2019).

A small percentage of antisocial individuals are perpetrators of escalated aggressive behaviors and experience appetitive arousal, or feelings of reward after committing a violent act (De Boer et al., 2016). These individuals also experience negative consequences on their health due to their aggressive behavior. Anti-social aggressive male and female adolescents have a tendency to seek fewer alternative solutions to social problems, define social problems in a hostile manner, and resort to violence (Slaby & Guerra, 1988). In addition, feelings of appetitive arousal while engaging in violent acts, and higher appetitive aggression scores, were found to be positive predictors of lifetime post-traumatic stress disorder symptom severity in German male World-War II veterans (Weierstall et al., 2012). Addictive aggression and appetitive aggression

is also maladaptive to its perpetrators due to a prevalence of impulsivity, processing problems, and low socioeconomic status in highly aggressive men (Azevedo et al., 2020).

This review will begin with a discussion about appetitive aggression in observed humans, as the costs of violent aggression in society is a primary motivation for this study. Because there are similarities between the biology of aggression between rodents and humans, and analysis of these similarities may help us to better understand appetitive aggression in humans, this literature review will then explore how studies using male mice have examined appetitive aggression in rodents (Takashi & Miczek, 2014). This review will conclude with the current state of the literature surrounding forms of aggression in females, including maternal aggression in female mice.

### **Appetitive Aggression in Humans**

Reward-motivated violence is known as appetitive aggression. Appetitive aggression, which has been observed in only a small percentage of human males, is goal-driven, premeditated, and reward-mediated. Appetitive aggression has also been associated with positive emotional affect (LaCourse et al., 2002; Weierstall & Elbert, 2011). In human males, killing other humans is emotionally arousing, and releases testosterone, serotonin, and endorphins, each of which can contribute to euphoric feelings (Elbert et al., 2010). Conversely, appetitive aggression is distinct from reactive aggression. Reactive aggression is defined as an uncontrollable response to a threat and is associated with negative emotional affect such as fear, hostility, and anger. Reactive aggression is also associated with a lack of forethought prior to the aggressive act, dissimilar to appetitive aggression (Fontaine et al., 2007).

Unlike young men, women are not often-studied subjects in surveying appetitive aggression, including violent crime, even though studies have hinted at the possibility of a

sex-driven dichotomy for aggression in humans. For instance, a study on Swiss perpetrators of violent crime found that female offenders were more likely to have suffered adverse childhood experiences, traumatic stress, and poorer mental health compared to violent male offenders (Rossegger et al, 2009). Sex differences also exist in regards to the risk of juvenile delinquency as a result of exposure to family violence. Adolescent girls who suffered child abuse were more frequently arrested for violent crimes than adolescent boys with similar family histories (Lansford et al., 2007). Additionally, the vast majority of violent offenses committed by adolescent girls were for domestic violence (Herrera & McCloskey, 2001). Violence appears to be affected by sex-differences between men and women.

Pathological aggression and sex-driven dichotomies for aggression have also been reported in rodents. Male mice in particular have been the subject of research regarding appetitive aggression.

### **Studying Pathological Aggression in Male Mice**

Engaging in combat for the defense of food, territory, and offspring characterizes aggressive behavior as evolutionarily advantageous. However, voluntarily seeking out violent encounters when there exists no selective pressure to do so, or finding acts of violence rewarding despite adverse consequences, are pathological forms of aggression. In rodents, not all forms of aggression are purely defensive or advantageous in nature. While mice will engage in violent combat in order to defend against intruders, acquire mates, and protect offspring, some aggressive male mice will also perform lever-presses to allow an intruder into their territory for combat (Fish et al., 2002; Golden et al., 2017). These behaviors can be maladaptive and result in increasingly violent attacks as observed in male mice (Golden, Heins et al., 2017).

In highly aggressive male mice, aggression can be as rewarding as imbibing drugs and alcohol. One way researchers have assessed a penchant for pathological aggression in male mice has been through the conditioned place-preference (CPP) procedure. In the CPP procedure, mice exhibit a preference for one location over another due to that location having previously been paired with rewarding events or stimuli. Using CPP, Golden and Aleyasin et al. (2017) showed that some dominant, male mice were capable of forming an appetitive preference for contexts in which they had previously defeated a subordinate mouse.

Another study has found additional evidence for appetitive aggression in male mice using the resident-intruder paradigm. The resident-intruder paradigm is a standardized test that allows for the evaluation of territorial offensive behavior, which is initiated by a resident rodent, towards an intruder rodent that has entered the resident's territory, such as the resident's home cage (Koolhas et al., 2013). Golden et al. (2017), using the resident-intruder paradigm, found that a subset of highly aggressive male mice engaged in operant self-administration of aggression. Highly aggressive male-mice, when presented with an active lever and an inactive lever, reliably pressed the active lever to gain access to a subordinate intruder male to engage in combat with. This operant self-administrative behavior was also found to resemble addiction: the aggressive males continued to press an active lever despite receiving aversive consequences (foot-shocks). An important component of self-administration of aggression, however, is that aggression-seeking mice will only lever press for access to a subordinate provided that they had been victorious in previous encounters with other male mice (Falkner et al., 2016).

Although the use of male subjects in studies of aggression has been popular, to our knowledge, there are no reports in the literature of pathological acts of aggression that have been observed or tested in female mice.

### **Displays of Female Aggression**

Although women tend to engage in less injurious and less frequent aggressive behavior compared to men, rates of violence and conduct disorder diagnoses in women have risen in Western society in recent years (De Boer, et al., 2016). In addition, the socioeconomic consequences of violence apply not only to its males but also to its female perpetrators; adolescents of both sexes who display more aggressive behavior are associated with experiencing poorer health and lower socioeconomic status in adulthood (de Jong, et al., 2014).

The expression of sex-selective, aggressive behavioral phenotypes can also appear across various species. When engaging in combat for access to limited supplies of food, *Drosophila melanogaster* females commonly use thrusting, headbutting, and fencing attacks against other females - a behavior rarely utilized by males (Nilsen et al., 2004). When defending territory against intruders of the same sex, female resident mice more frequently displayed rearing, chasing, and biting behavior compared to resident male mice (Newman et al., 2019). Female mice also tend to attack male intruders on the head and genitals, while male mice tend to attack male intruders on their back and the head (Hashikawa et al., 2018).

Male and female mice also respond to various experiences with changes in the frequency of their aggressive behavior. In response to social isolation, males show increased aggressive behavior, while females typically exhibit decreased aggression. In response to winning experience in confrontations, male mice typically exhibit increased aggression, while females usually display decreased levels of aggression. However, the consensus in existing literature regarding female rodents' response to these experiences remains mixed overall. This is because of a relative dearth in literature reporting on female rodent aggression outside of reproductive contexts (Hashikawa et al., 2018).

The more frequently-studied forms of aggression in female rodents pertain to aggressive behaviors displayed in the context of resource-defense, competition for mates, hierarchy maintenance, and maternal protection of offspring (Newman et al., 2016). The most studied form of aggression in female rodents is known as maternal aggression.

### **Maternal Aggression in Rodents**

During the first 10 days following parturition, dams will attack intruders approaching the nest in defense of pups. On the contrary, virgin female rodents are unaggressive and rarely attack intruding conspecifics (Numan & Insel, 2003). Displays of maternal aggression by female rodents against male intruders are defined by a combination of both offensive and defensive behaviors (Lostein & Gammie, 2002). Offensive behaviors include rapid attacks, lateral threats, and even direct attacks towards the intruder's genitals. Lateral threats and jumping attacks, however, have also been considered defensive behaviors exhibited by dams. (Bosch, 2011).

Two primary extrinsic factors, including pups and intruders, heavily modulate behavioral expression of behavioral aggression. In rodent mothers, ventral trunk anesthesia (allowing for the somatosensory inhibition of suckling stimuli) diminishes displays of maternal aggression (Stern & Jolunie, 1993). The greater age and consequent development of a dam's pups is also thought to be an inverse modulator. Dams exhibited the most intense maternal aggression in the early weeks of parturition, and intensity steeply decreased with each week thereafter (Flannelly & Flannelly, 1987). In rats, the age and weight of the intruder most strongly modulate levels of maternal aggression: lactating rats fought younger, lower-weight male intruders more often than older, heavier male intruders (Flannelly & Flannelly, 1985). Extrinsic and intrinsic mediators of maternal aggression in rodents, then, have been interpreted to confer reproductive advantages, as the survival of offspring are indicative of a mother's reproductive fitness.



## Conclusions

Appetitive aggression in humans is distinct from reactive aggression in the goal-driven, deliberate intentions of the perpetrator. Appetitive aggression has also been observed in mice. While a basis has been established for the rewarding effects of aggression against subordinates as perpetrated by dominant male mice, to our knowledge, there is no literature to our knowledge that has tested for appetitive aggression in female mice.

There are also sex-selective ethological displays of aggression in animals. For instance, adolescent girls who suffered child abuse are more frequently arrested for violent crimes than adolescent boys with similar histories. In other species, female mice and fruit flies both display sex-specific of aggression and defense while engaging in combat. Studies strongly suggest that violent aggression is an extraordinarily relevant subject of study due to its deleterious effects on public health. Studies also suggest that violent aggression may be dichotomous in regards to the sexes.

The main purpose of this study is to explore whether and how female mice might exhibit appetitive aggression. The current study expands on existing research about aggression in mice by (a) examining the propensity of female mice to express appetitive aggression; (b) collecting data using an operant self-administration model, previously explored using male mice, as a basis for inferences on pathological aggression in female mice.

## Methods

### Study Design Overview

The primary question of this study was whether female mice display self-administration of aggression and therefore appetitive aggression. This was determined through investigating whether retired female dams mice can display appetitive aggression through operant self-administration in gaining access to and attacking young, virgin female mice. After being singly-housed for 10-14 days, retired dams were screened for aggression in a resident-intruder paradigm. The second phase of the study was a magazine training phase, in which retired dams were conditioned to associate a cue light with the introduction of a young female intruder into a shared chamber. Following magazine training, the retired dams underwent operant testing for appetitive aggression. This was the fixed-ratio 1 (FR1) testing phase of the study. In this phase, the retired female dams were monitored for (a) lever-pressing for access to young “intruder” females and (b) aggressive behavior upon gaining access to young “intruder” females across seven training sessions. Each of these sessions were recorded for further behavioral analysis. The next phase of the study involved a test without intruder delivery, in which resident mice were tested for their propensity to press the active lever despite no delivery of the intended reinforcer (gaining access to the intruder). The test without intruder delivery was then followed by a brief retraining phase on the residents’ acquisition of the lever-press reward-contingency to account for behavioral extinction. Data collection concluded with progressive ratio schedule testing, in which the residents’ motivation for acquiring (and receiving) the intended reinforcer was tested.

The primary independent variable was the number of operant testing sessions for FR1, test without intruder delivery, and PR tests. The primary dependent variables included measures for appetitive aggression during each FR1. The first dependent variable was the number of active

lever-presses residents made during each test in order to gain access to an intruder. The second and third dependent variables included the number of attacks residents made towards intruders and the residents' preferences for the active lever. It was hypothesized that retired dams would display appetitive aggression through operant self-administration of access to young intruder females.

### **Animals**

The 42 subjects of the study included 12 retired dams, or retired breeder mice (aged 27 to 41 weeks old), and 30 young, virgin females (aged 10 to 15 weeks old). The retired dams were C57BL/6 mice. The young, virgin females were C57BL/6J congenic *Drd1a*-tdTomato transgenic mice (hemizygous or non-carriers of the transgene). Retired dams were used due to older, 10 month-old female mice having been found to be more aggressive than younger females and observance of intense maternal aggression (Hashikawa et al., 2018; Numen & Insel, 2003). C57BL/6 mice were used because they are considered to be a moderately aggressive strain of mice (Lidster et al., 2019). Young, virgin female mice were used to simulate the effects of dominance hierarchies, which facilitates aggressive behavior (Williamson et al., 2019). Tomato mice were used because of their availability in the lab. Experimental protocols were approved by The Institutional Animal Care and Use Committee (IACUC) at the University of Texas at Austin,

### **Materials**

The study used operant chambers, which were used during each phase of the study except for the aggression screening phase, in order to assess appetitive self-administration of aggression in retired dams. Specifically, the purpose of the operant chamber was to determine whether retired dams pressed an active lever in order to gain access to an intruder and engage in aggressive behavior.

### ***The Operant Chamber***

The operant chamber used for this study was The Modular Test Chamber – Standard with Modified Top for Mouse (Med Associates Inc.). The operant chamber was 6” in length, 5.25” in width, and 5” in height. Its door, back, and top was made of clear polycarbonate. The base of the chamber was made of a white, propylene base. The Classic Mouse Chamber (Med Associates Inc.) was used for the floor of the chamber and consisted of stainless steel grid rods. The operant chamber was also equipped with response levers, a camera, a house light, tray and a cue light.

A cue light was activated to provide additional reinforcement when an active lever was pressed, and a camera was used to film trials for additional review. Presses on the inactive lever did not reward the retired dams with access to the intruder.

The software MED-PC IV was used to program and automate operant chamber processes, such as the timed extension and retraction of levers for magazine and operant training sessions. The program was also used to record the number of active and inactive presses done by mice in operant training sessions. Sound Attenuating Cubicles (Med Associates Inc.) were used to contain the operant chambers. The cubicles were 23.5” in width, 15” in height, and had a depth of 14”.

## **Procedures and Measures**

### ***Aggression Screening Phase***

The first phase of the study was the aggression screening phase. This phase of the study modeled the procedures used in Covington et al. (2018) for screening male mice for stable levels of aggression. The purpose of this phase was to establish a baseline level of aggression in the residents as exhibited towards intruders introduced into their home cage. Retired dam mice were singly-housed in home cages for 14 days prior. This was done in an attempt to promote

aggressive behavior due to isolation (Hashikawa et al., 2018). After this 14-day period, a young, virgin female intruder mouse was introduced daily into each retired dam's home cage for 5-minute confrontational sessions following the resident-intruder paradigm. Young, virgin female intruder mice were rotated per each session to ensure retired dams did not become habituated to a particular intruder (Covington et al., 2018). Each retired dam underwent one session per day for a total of 5 days.

If injuries were observed, the session in which the injury was observed was to be immediately halted and the intruder mouse removed. The young intruders were also rotated throughout aggression screening sessions to minimize the amount of stress and wounds intruders sustained upon exposure to the resident females.

**Measures.** If any attacks were observed (biting or scratching as initiated by the resident towards the intruder) during the confrontational sessions were recorded by a human observer.

### ***Magazine Training***

The next phase of the study was the magazine training phase. The purpose of this phase of the study was to train the resident mice to associate pressing the active lever with the presentation of a reward (the introduction of the intruder into the operant chamber). Each resident mouse underwent three, 5-minute-long sessions of magazine training. Each session was separated by an hour.

The resident was placed into the houselit operant chamber at the beginning of each session. After 10 seconds had elapsed, the cue light flashed for 2 seconds and was followed by prompt, manual insertion of a young intruder mouse into the operant chamber. If the resident attacked the intruder before the end of 5 minutes, the session ended immediately and the intruder returned to its home cage. At the completion of each session, both the resident and intruder

mouse were returned to their home cages. The young intruder females were rotated for use throughout these sessions to minimize stress and injury upon exposure to the older resident females.

**Measures.** The number of attacks (if any) observed perpetrated by residents towards an intruder were recorded for each session and resident by a human observer.

### ***FR1 (Fixed-Ratio 1) Schedule Testing Phase***

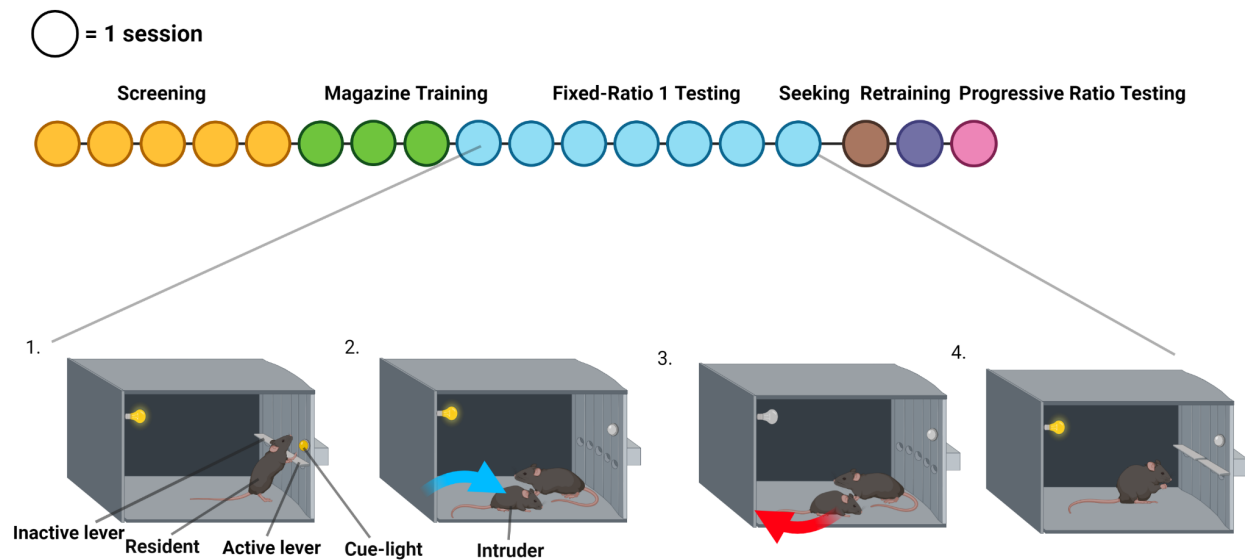
The next phase of the study assessed whether the residents exhibit appetitive aggression, which was to be indicated as an increasing number of trials in which residents lever pressed for and attacked the young intruder females. This phase of the study closely modeled the procedures utilized in Golden et al. (2017), which examined operant self-administration in male mice. See Figure 1 for a depiction of this protocol and an overall timeline of the study. Residents each underwent daily, 40-minute operant-testing sessions (following a FR1 schedule) in the operant chamber over 7 days for a total of seven sessions. Each 40-minute session consisted of ten, 4-minute trials.

An active lever and inactive lever was available to retired dams for a span of 2 minutes at the beginning of each trial, and a house light was on for the first 3 minutes of the trial. When a dam pressed down on the lever, a cue light was illuminated, the levers retracted, and the intruder was manually introduced into the operant chamber, signaling the beginning of the reward phase of the trial. The intruder female was allowed to remain in the operant chamber with the dam until 3 minutes had elapsed since the beginning of the trial (provided the retired dam did not attack the intruder before then). If the resident attacked the intruder, the intruder female was immediately removed from the operant chamber and returned to its home cage, signaling the end of the trial. If the resident did not attack the intruder, then the intruder was not removed from the

operant chamber until the house light automatically turned off at 3 minutes into the trial. The trial ended once 4 minutes had elapsed since the beginning of the trial.

### Figure 1

#### *FR1 (Fixed-Ratio 1) Testing and Experimental Design Overview*



*Note.* Study design and the sequence of tests. The figure also details the procedure for one trial of FR1. If the resident pressed the active lever, the intruder was introduced into the chamber. Once the intruder was removed, a new trial began.

Retired dams were returned to their home cages at the end of each session. A camera was used to film each session. The number of trials per session in which a retired dam pressed the active lever, along with the number of trials in which a resident attacked (bit or scratched) the young female intruder (had there been an attack), was to be recorded over seven days.

**Measures.** The number of active and inactive presses each resident made over each of the seven days of FR1 testing was also recorded by a human observer. Each session was also recorded as video data for further behavioral analysis.

**Behavior Video Scoring.** The reward phases of the Day 7 session of FR1 for each resident was scored for the proportions of time the resident had spent engaged in various behaviors. Because some residents did not active-lever press on Day 7 of FR1, there was no video data containing reward phases to score for these residents on that day. For these residents, the video for the last day on which residents had active lever-pressed and entered reward phases was scored for behavior. For example, if a resident did not make a single active lever-press on Day 7 of FR1 and therefore did not enter a reward phase that day, but the same resident had made at least one active lever-press on its Day 6 of FR1, the reward phase for that session was scored for behavior.

Behaviors were manually scored by a human observer with JWatcher, which was used to calculate the duration of time residents spent engaged in mutually exclusive behaviors according to an ethogram. The behaviors that were scored included “exploring” behavior, “circling” behavior, anogenital-sniffing, frontal-sniffing, allogrooming (whether the resident groomed the intruder or was groomed by the intruder were scored separately), self-grooming, and unspecified intruder-directed behavior. The ethogram which was used to identify and score these behaviors is detailed in the Table 1 below.



**Table 1***Behavioral Ethogram for FR1 Reward Phase Video Scoring*

Behavior	Description
Anogenital-sniffing	Resident sniffs the intruder's anogenital area.
Circling	Resident quickly moves in a circular path around intruder, as if "corralling" the intruder.
Exploring	Resident is not oriented towards the intruder; resident is oriented towards the operant chamber's features.
Frontal-sniffing	Resident's nose is pointed towards the intruder's nose while the resident sniffs the intruder.
Groomed by intruder	Resident is being groomed by the intruder (the intruder's paws are on the resident's back, and the intruder is observed licking the resident's fur).
Groomed intruder	Resident grooms the intruder (the resident's paws are on the intruder's back, and the resident is observed licking the intruder's fur).

Self-grooming	Resident grooms itself. The resident points its head down and strokes its fur on its head. The resident also licks its body.
Unspecified intruder-directed	Resident is oriented towards the intruder but is not sniffing or grooming the intruder.

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*Note.* The descriptions for each type of behavior as exhibited by residents during the reward phase of a trial. These descriptions were used to identify and score residents' behaviors during the reward phases across each trial, or within a single session of FR1, for each resident.

### ***Test Without Intruder Delivery***

The test without intruder delivery immediately followed the FR1 testing. The purpose of this test was to assess how motivated residents were to lever-press despite receiving no reinforcement, or the introduction of the intruder into the operant chamber. Each resident mouse underwent three, 30 minute-long tests without intruder delivery in the operant chamber. The active and inactive levers were extended for the full duration of each session and were not retracted at any point, unlike prior phases of the study. When the resident mice pressed the active lever, the cue light flashed, but there was no presentation of the intended reinforcer; no intruder was introduced into the chamber. Resident mice were returned to their home cages at the end of each session.

**Measures.** The number of active presses each resident mouse made was recorded and treated as an index of the residents' motivation for gaining access to an intruder despite no reward presentation. The number of presses of the inactive lever residents made was also recorded.

### ***Retraining Phase***

The retraining phase of the study followed the test without intruder delivery in order to counteract any behavioral extinction as a result of a lack of a reward presentation in the test without intruder delivery. The retraining phase also served to retrain the residents' associations between pushing the active lever and the presentation of a reward in preparation for progressive ratio testing. Each resident mouse underwent a single session of retraining. The retraining phase sessions exactly followed the procedures for a single day of FR1 testing: mice gained access to an intruder upon pressing the active lever, and the cue-light was illuminated.

**Measures.** The number of active and inactive presses residents made, as well as whether there was an attack, was recorded for each session. Mice were returned to their home cages at the end of each session, and intruder mice were rotated through each session.

### ***Progressive Ratio Schedule Testing Phase***

The Progressive Ratio (PR) schedule testing phase began after the retraining phase of the study. The purpose of this test was to assess the levels of motivation each resident had for obtaining the reward (gaining access to the intruder through its introduction into the operant chamber). Each resident mouse underwent a single session of PR testing. This testing phase of the study resembled FR1 testing in which resident mice were presented with the introduction of an intruder into the operant chamber. However, residents were required to make an increasingly higher number of active lever-presses after each consecutive reward-presentation in order to earn another reward. The cumulative number of presses required for each consecutive reward and the presses required between rewards each resident needed to make, in sequence, is shown in the Table 2 below. For example, according to the schedule, a resident that pressed the active lever 22

times during a session (and therefore received four rewards) must press the active lever 9 more times in order to receive another reward.

**Table 2**

*Number of Active lever-presses Needed for Reward*

Cunulative presses	2	6	12	21	33	48	68	93	125	165	215	277	354
Presses for next reward	2	4	6	9	12	15	20	25	32	40	50	62	77

*Note.* The cumulative number of presses (top row) residents must make for each consecutive reward and the number of presses residents must make between each reward presentation (bottom row).

The length of each PR testing session varied depending on how often resident mice pressed the active lever, but the maximum length of a session was 1.5 hours. Every time a resident mouse pressed the active lever before 30 minutes had elapsed (either since the beginning of the session or since earning the last reward), the session was extended for up to another 30 minutes. If 30 minutes had elapsed since the beginning of the session or since the resident had earned the last reward, and the resident had not pressed the active lever, the session was ended, and the resident was considered to reach its breakpoint.

**Measures.** The number of active and inactive lever-presses each resident made for each session was recorded. The number of rewards earned, as well as the breakpoint (the greatest active lever-pressing reward requirement met) reached for each resident, was also recorded. Mice were returned to their home cages at the end of each session.

### **Statistical Analyses**

Because no aggression was observed as exhibited by residents towards the intruders, the residents were split into two groups, “high-” and “low-pressers,” for usage in further analyses intended to reveal what may have motivated the residents to lever-press. Residents were split into the two groups using a median split using the number of active lever-presses residents made during the last four days (Days 4-7) of FR1 testing. The median number of active lever-presses made by all residents for Day 4, Day 5, Day 6, and Day 7 of FR1 were calculated for each day. If the number of active lever-presses a resident made independently exceeded the respective median number of active lever-presses for at least three out of the four days (Days 4-7), the resident was classified as a high-presser. Residents that did not meet this requirement were classified as low-pressers. The active lever preferences each resident had for each day of FR1 testing, tests without intruder delivery, retraining tests, and PR tests were also calculated as the proportion of active lever-presses out of the total amount of lever-presses made by each resident in a particular test.

A two-way repeated measures ANOVA was conducted in order to analyze the relationship between the presser-type of mice (high- or low-presser) and the number of active lever-presses residents made across each day of FR1 testing (time). An additional two-way repeated measures ANOVA assessed the effect of presser-type and time on the proportion of active lever-presses residents made each day of FR1 testing.

A series of t-tests were used to compare operant activity between high and low-presser mice. Two-tailed two-sample t-tests analyzed the number of active lever-presses made, inactive lever-presses made, and active lever preference between high- and low-presser residents during the tests without intruder delivery. Additional two-tailed two-sample t-tests compared the total

number of active lever-presses made, inactive lever-presses made, active lever preference, rewards earned, and breakpoints reached between high-presser and low-presser residents during PR testing.

Two-tailed two-sample t-tests analyzed and compared the proportion of time spent engaging in non-aggressive behaviors (anogenital sniffing, frontal-sniffing, exploring, circling, allogrooming of the resident by the intruder, allogrooming of the intruder by the resident, self-grooming, and unspecified intruder-directed behavior) between high- and low-pressers during the reward phase of FR1 testing.

## Results

### Testing for Aggression

No aggressive behavior as exhibited by the residents directed towards the intruders was observed in any of the tests (aggression screening, magazine training, fixed-ratio 1, tests without intruder delivery, retraining, and progressive ratio). Residents were instead observed engaging in other social behaviors, such as allogrooming and sniffing of the intruders. Further analyses were thus conducted in order to examine other factors that may have motivated the residents to engage in lever-pressing behavior.

### FR1 Testing Results

#### *Number of Active Lever-Presses*

A two-way repeated measures ANOVA was conducted to assess the effect of time (day of FR1, or Day) and presser-type (high- versus low-presser residents) on the average number of active lever-presses made by residents across each day of FR1. An extremely significant main effect was found for presser-type and Day on the average number of active lever-presses made by residents  $F(1, 10) = 45.98, p < .0001$ . That is, residents' classification of either presser-type had an effect on the average number of active lever-presses residents made. High-presser residents, on average, made more active lever-presses than low-presser residents on each day of FR1.

Moreover, the interaction between presser-type and Day was found to be extremely significant,  $F(6, 60) = 9.01, p < .0001$  (see Figure 2). A one-way repeated measures ANOVA yielded an extremely significant main effect for Day on the average number of active lever-presses made by high-presser residents,  $F(4, 24) = 4.26, p = .0095$ . Conversely, an additional one-way repeated measures ANOVA revealed that there was no main effect for Day

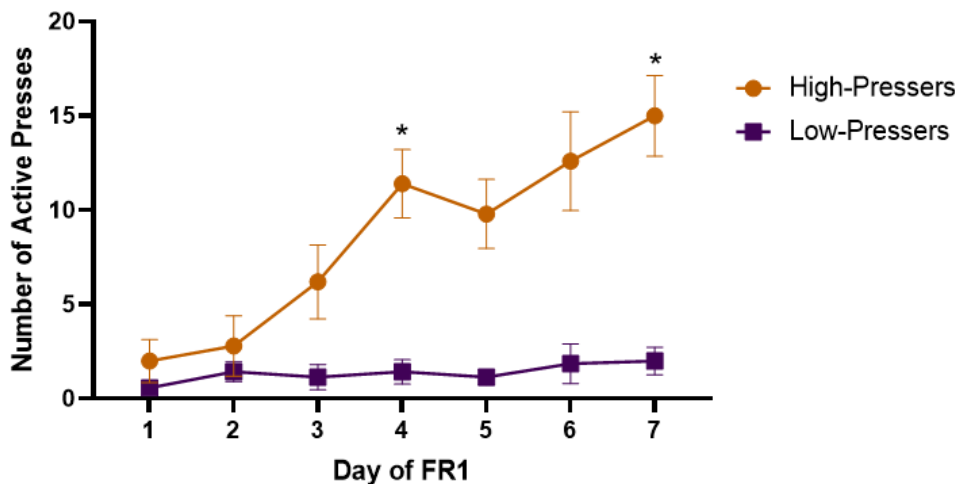
on the average number of active lever-presses made by low-pressers,  $F(6, 36) = 0.78, p = .58$ .

The average number of active lever-presses residents made tended to increase over each day of FR1 testing for high-presser, but this was not the case for low-presser residents.

Bonferroni multiple comparisons tests found that, on Day 4 and Day 7, the average amount of active lever-presses made by high-presser residents ( $M = 11.40, 15$ , respectively), compared to the average number of active lever-presses made by low-presser residents ( $M = 1.42, 2$ , respectively) was significantly greater, Day 4:  $t(5.19), p = .02$ , Day 7:  $t(5.74), p = .01$ .

**Figure 2**

*Active Lever-Presses Between High- and Low-Pressers During FR1*



*Note.* The number of active presses high- and low-presser residents made on each day of FR1.

There was a significant main effect for presser-type ( $p < .0001$ ) and a significant interaction with Day ( $p < .0001$ ) on the number of active lever-presses residents made on each day. \* $p < .05$ , high-presser versus low-presser, Bonferroni multiple comparisons. Data are  $M \pm SE$ .

### *Active Lever Preference*

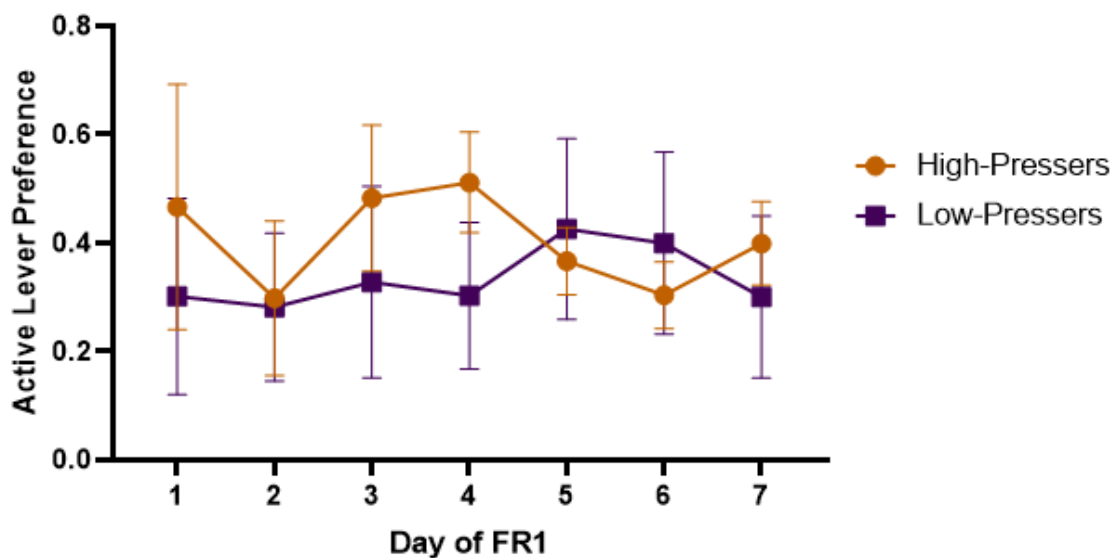
A two-way repeated measures ANOVA found no main effect for both presser-type,  $F(1, 10) = 0.77, p = .39$ , and Day of FR1,  $F(3.76, 37.66) = 0.14, p = .95$ , on active lever preference.



The high- and low-presser residents' average active lever preference did not differ from each other significantly on any day of FR1. There was also no interaction between presser-type and Day of FR1 on residents' average active lever preference,  $F(6, 60) = 0.28$ ,  $p = .94$  (see Figure 3). A one-sample t-test showed that high-presser residents ( $M = 0.404$ ) did not have a preference for either lever significantly differing from what would have been expected by chance (Hypothetical Mean = 0.5),  $t(1.72)$ ,  $p = .16$ . However, another one-sample t-test found that low-presser residents, on average, had a preference for the inactive lever ( $M = 0.67$ ),  $t(3.08)$ ,  $p = .02$ .

**Figure 3**

*Active Lever Preference Between High- and Low-Pressers During FR1*



*Note.* The average active lever preference of high- and low-presser residents on each day of fixed-ratio 1 (FR1) testing. There was no main effect for presser-type nor Day of FR1 on residents' active lever preference. There was no interaction between presser-type and Day of FR1. Low-pressers showed a preference for the inactive lever. Data are  $M \pm SE$ .

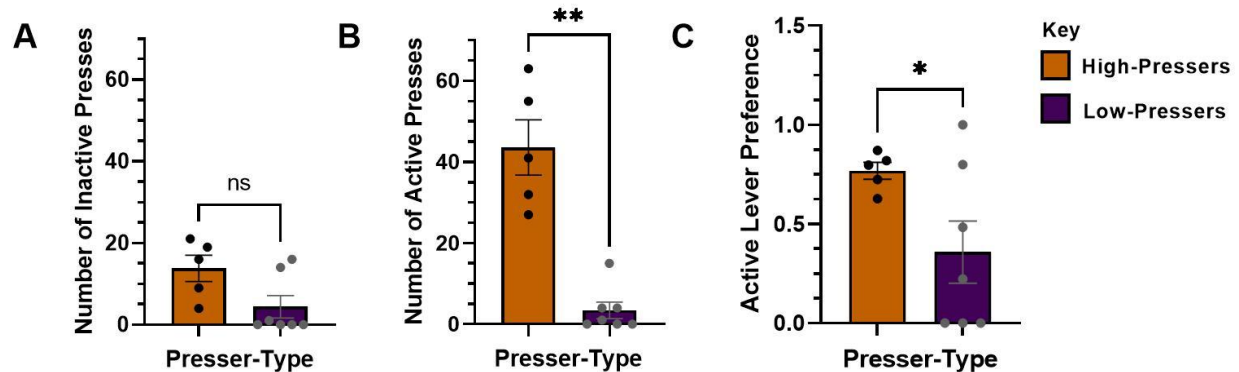
### Tests without Intruder Delivery Results

As demonstrated by a two-tailed, two-sample t-test, the average number of active presses made between high- ( $M = 43.60$ ) and low-presser ( $M = 3.429$ ) residents during the tests without intruder delivery significantly differed,  $t(5.66)$ ,  $p = .002$  (Figure 4B). The average number of inactive presses made between high- ( $M = 13.80$ ) and low-presser ( $M = 4.42$ ) residents did not differ significantly in the tests without intruder delivery,  $t(2.22)$ ,  $p = .05$  (Figure 4A).

The average active lever-preference between high-presser ( $M = 0.76$ ) and low-presser ( $M = 0.35$ ) residents during the tests without intruder delivery differed significantly,  $t(2.53)$ ,  $p = .03$ . High-presser residents, on average, had a significantly greater preference for pressing the active lever over the inactive lever compared to low-presser residents during the tests without intruder delivery (Figure 4C).

#### Figure 4

*Residents' Lever-Presses and Active Lever Preference During Tests without Intruder Delivery*



*Note.* Residents' lever-pressing behaviors in the test without intruder delivery. (A). There was no significant difference in the number of inactive presses made between high- and low-pressers. (B). High-pressers, on average, made significantly more active lever-presses than low-pressers. (C). High-pressers had a significantly greater preference for the active lever compared to low-presser. \* $p < .05$ . \*\* $p < .001$ . Data are  $M \pm SE$ .

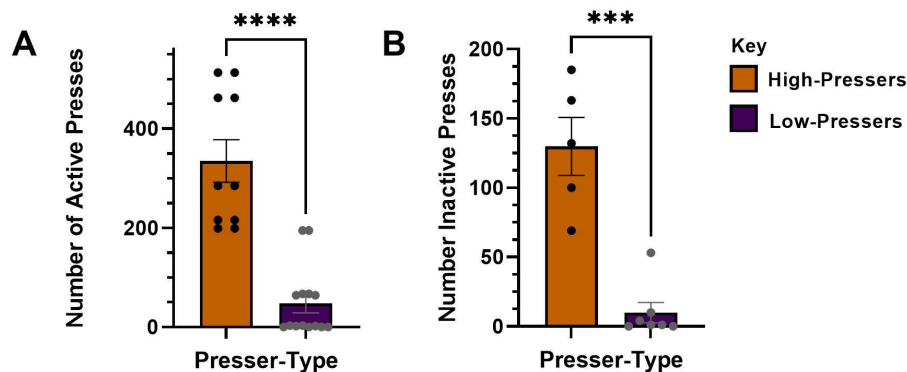
## PR Testing Results

### *Active and Inactive Lever-Presses*

Using a two-tailed two-samples t-test, it was found high-presser ( $M = 335$ ) resident mice, on average, pressed the active lever significantly more frequently than the low-presser ( $M = 47.29$ ) resident mice during PR testing,  $t(6.82)$ ,  $p < .001$  (Figure 5A). Additionally, the number of inactive presses made between high-presser ( $M = 13.80$ ) and low-presser ( $M = 4.42$ ) residents was also found to significantly differ,  $t(2.22)$ ,  $p = .05$ ; high-presser residents, on average, also pressed the inactive lever significantly more during PR testing compared to the low-presser residents (Figure 5B).

## Figure 5

### *Progressive Ratio Testing*

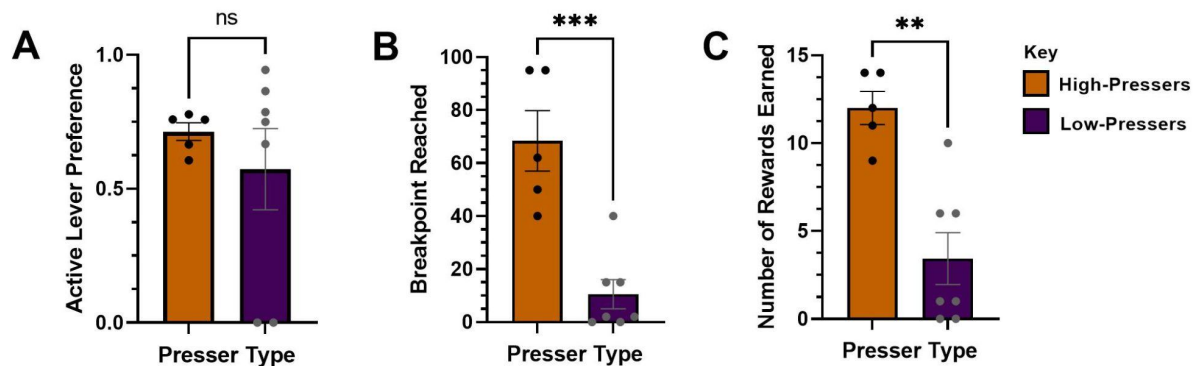


*Note.* The average number of active and inactive lever-presses made by high-presser residents and low-presser residents. (A). High-pressers made significantly more active lever-presses than low-pressers. (B). High-pressers also made significantly more inactive lever-presses than low-pressers. \*\*\* $p < .001$ . \*\*\*\* $p < .0001$ . Data are  $M \pm SE$ .

***Active Lever Preference, Breakpoints Reached, and Number of Rewards Earned***

The results of a two-tailed two-sample t-test showed that high-presser residents' ( $M = 0.71$ ) and low-presser residents' ( $M = 0.57$ ) average active lever preference during PR testing did not significantly differ from each other,  $t(0.93)$ ,  $p = .39$  (Figure 6A). However, a one-sample t-test found that high-presser residents' average preference for the active-lever ( $M = .71$ ) was significantly greater than what would be expected by chance (Hypothetical Mean = 0.5),  $t(6.44)$ ,  $p = .003$ . An additional one-sample t-test found that low-presser residents, on average did not have a preference for the active lever,  $t(0.48)$ ,  $p = .64$ .

The results of a two-tailed two-sample t-test showed that high-presser residents, on average, reached a significantly higher breakpoint ( $M = 68.40$ ) than low-pressers ( $M = 10.57$ ) did during PR testing,  $t(5.01)$ ,  $p = .0005$ . High-presser residents achieved a significantly higher ratio response-requirement (Figure 6B). The results of an additional two-tailed two-sample t-test showed that high-presser residents earned a significantly greater average number of rewards compared to low-pressers during PR testing,  $t(4.41)$ ,  $p = .0013$ . High-presser residents lever-pressed the active lever and gained access to the intruder (the reward) significantly more than low-presser residents. See Figure 6C.

**Figure 6***Active Lever Preference, Max Breakpoints, and Rewards Earned during PR Testing*

*Note.* (A). High-presser and low-presser residents' average preference for the active lever did not significantly differ during PR testing. However, high-pressers' active lever preference was significantly greater than what was expected by chance, but not for low-pressers. (B).

High-pressers reached a significantly greater breakpoint than low-pressers during PR testing.

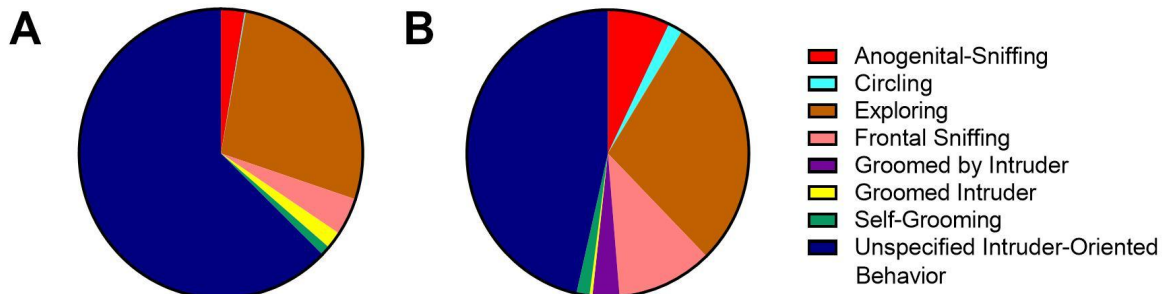
(C). High-pressers earned significantly more rewards than low-pressers during PR testing.  $**p < 0.01$ .  $***p < .001$ . Data are  $M \pm SE$ .

### **Social Behavioral Analysis Results**

Video-recording scores of residents' behaviors during FR1 testing were analyzed. Figure 7 shows, for each presser-type, the proportion of time residents spent engaging in each type of behavior. Multiple two-tailed, two-sample t-tests were run to compare the proportion of time high- and low-presser residents spent engaging in various behaviors during the reward phase (in which residents were accompanied by the intruder in the operant chamber). Low-pressers, on average, were found to have spent a lower proportion of time engaged in anogenital-sniffing of the intruders than high-pressers. No significant differences were found comparing the proportion of time spent on other behaviors between high- and low-presser residents. Notably, only high-presser residents were observed being groomed by the intruders. The results of the t-tests are summarized below in Table 3.

**Figure 7**

*Distribution of Residents' Behaviors During The Reward Phase of FRI*



*Note.* The proportion of time low- and high-presser residents spent performing different behaviors when accompanied by the intruder during (the reward phase) of FRI testing. (A). The low-presser residents' average distribution of behaviors. (B). The high-presser residents' average distribution of behaviors.

**Table 3***Results of Multiple t-tests Comparing High- and Low-Presser Distributions of Behavior*

Behavior	High-Pressers		Low-Pressers		<i>t</i> (10)	<i>p</i>
	<i>M</i>	<i>SEM</i>	<i>M</i>	<i>SEM</i>		
Anogenital-sniffing	0.07	0.01	0.02	0.01	2.29	.04
Circling	0.01	0.01	0.01	0.001	1.01	.36
Exploring	0.28	0.02	0.26	0.06	0.30	.76
Frontal-sniffing	0.10	0.04	0.04	0.01	1.60	.17
Groomed by intruder	0.03	0.01	0.00	0.00	1.68	.16
Groomed intruder	0.07	0.07	0.01	0.01	0.74	.59
Self-Grooming	0.01	0.009	0.01	0.01	0.31	.76
Unspecified intruder-directed	0.46	0.06	0.61	0.08	1.25	.23

*Note.* The results of t-tests comparing the proportion of time high- ( $n = 5$ ) and low-presser residents ( $n = 7$ ), on average, spent engaged in various behaviors while accompanied by the intruder during FR1 testing.

### Discussion

The retired female dams, or residents, did not exhibit any aggressive behavior towards the young virgin intruders during any of the tests in this study. Although none of the residents behaved aggressively after gaining access to the intruders in their home cages and the operant chamber, residents were still observed engaging in lever-pressing behavior. In order to understand what may have motivated residents to lever-press, residents were split into either the high-presser or low-presser groups using a median split of their active lever-pressing counts during FR1 testing. Further analyses were then run measuring various aspects of residents' lever-pressing behavior (lever-press counts, active lever preference, etc.). Moreover, while residents did not engage in aggressive behavior at any point in the study, residents were observed engaging in other behaviors. These behaviors were scored and analyzed with the intent of revealing whether these behaviors could have motivated residents to lever-press.

An extremely significant main effect was found for presser-type on the number of active lever-presses residents made during FR1 testing. There was also a main effect for time on the number of active-lever presses made by high-pressers but not for low-pressers. There was also main effect for both presser-type and time on residents' active lever preference across each day of FR1 testing. Additionally, low-pressers had a preference for the inactive lever during FR1 testing, but high-pressers had no preference for either lever. In the tests without intruder delivery, it was found that high-pressers made significantly more active lever-presses and had a greater active lever preference than low-pressers. PR tests showed that high-pressers had a significantly greater number of active and inactive lever-presses, rewards earned, and breakpoints reached than low-pressers. Comparisons of the residents' distributions of behaviors during the reward



phase of FR1 found that high-pressers engaged in significantly more anogenital-sniffing of the intruders than low-pressers.

### **Absence of Aggressive Behavior**

A primary goal of the study was to test for appetitive aggression in residents using a variety of assessments (aggression screening tests, FR1 testing, tests without intruder delivery, and PR tests). However, none of the retired dams (residents) were observed to have engaged in aggressive behavior towards any of the young intruder females at any phase in the study. This finding was somewhat unexpected due to the dearth of existing literature assessing the aggressive behavior of female mice towards other female mice beyond maternal, territorial, hierarchical, and mate competition contexts. Another reason why this finding was somewhat unexpected was due to the fact that the residents used in this study were older, retired dams (females that were once breeders) and were singly-housed; literature has suggested that social isolation and older age in female mice increases aggressive behavior (indicated as increased frequency of attacks and attack latency), although these effects of these factors remain controversial (Hashikawa et al., 2018). The mixed nature of these previous study findings could potentially explain why no aggressive behavior was observed in the residents.

While confounds such as the residents' familiarity with the intruders were minimized by ensuring that (a) residents did not interact with intruders that were its offspring and (b) intruders were rotated throughout the study, other confounds were not accounted for in this study. For example, the estrous cycles of the residents and the intruders were not determined. Therefore, hormonal changes associated with aggression could have also contributed to the absence of aggressive behavior by residents towards intruders. Ultimately, the absence of aggressive behavior in this study suggests that, under the conditions of this study, retired dam mice will not

engage in aggressive behavior directed towards young intruder females in a resident-intruder paradigm.

### **Motivation to Active Lever-Press**

It is notable that while high-pressers were found to have pressed the active lever significantly more than low pressers during FR1, high-presser residents did not show a preference for the active lever during FR1 sessions. That is, high-presser residents pressed the active lever and the inactive lever essentially the same number of times. Therefore, these results may suggest that high-presser mice did not lever-press for some aspect of the experimentally-intended reward (the introduction of the intruder into the operant chamber), but instead for some other contingency shared between the pressing of the inactive and the active lever that high-pressers may have found rewarding (such as some aspect of lever-pressing itself, excluding the flashing of the cue light, which activated upon the pressing of only the active lever).

Interestingly, the findings from the PR test potentially suggest an opposite scenario. While the active lever preferences of the high and the low-presser residents during PR did not significantly differ from one another, high-pressers pressed the active lever significantly more than would be expected by random chance (50% of total presses) during PR. Moreover, high-presser residents not only reached a significantly greater breakpoint than low-pressers, but also acquired a significantly higher number of rewards during PR testing. In other words, high-presser residents worked significantly harder than low-pressers to earn a significantly greater number of rewards than low-pressers.

These PR findings support the potential and opposing argument that high-pressers were (a) in fact motivated to press the active lever over the inactive lever (as opposed to indiscriminate lever-pressing behavior, as results from FR1 testing and tests without intruder delivery may

suggest), and (b) motivated to press the active lever by some aspect of the intended experimental reward (such as the introduction of the intruder into the operant chamber) unique to pressing of the active lever, at least in the PR tests. Following this logic, there also exists the possibility that residents may have been motivated to press the active lever over the inactive lever because the flashing of the cue light was found to be rewarding by the residents (the cue light flashed only upon pressing the active lever). It is also possible that high-presser residents were motivated to lever-press the active lever for something else entirely. What residents may have been motivated to lever-press for ultimately remains unclear and is the purpose of the behavioral video analyses.

### **Explanations for the Discrepancy between FR1 and PR Results**

One possible explanation for why an active lever preference was found in high-pressers during the PR tests, but not in high-pressers during the FR1 tests, could be related to the self-paced session versus the imposed trial design of PR and FR1 testing, respectively. The self-paced session design for the PR test consisted of a 30-90 minute session during which the active lever never retracted despite being pressed. On the other hand, each FR1 session consisted of ten trials during which the active lever was available for a maximum of 2 minutes; in each trial, once a resident pressed the active lever, the active lever immediately retracted until the trial restarted. Therefore, residents had less opportunity to press the active-lever during FR1 testing compared to PR testing. Furthermore, high and low-presser residents may have been at various stages in acquiring an understanding of the lever-press and reward contingency. These potential factors could have deflated the average reported active lever preferences of the residents during FR1 testing and ultimately contributed to a discrepancy between the residents' average lever preference between FR1 and PR.

Another explanation for why PR tests may have yielded a significant active lever preference but FR1 results did not could potentially be due to the fact that the progressive ratio reinforcement schedule inherently demanded for mice to press the active lever more (as the ratio of lever-presses -to-reward requirement increased with each consecutive reward earned). This could have resulted in the inflation of the number of active lever-presses residents made, and, ultimately, the active lever preferences of the residents during PR relative to FR1 active lever preferences.

### **Tests without Intruder Delivery and Addiction-like Behavior**

In the tests without intruder delivery, high-presser residents showed a preference for the active lever despite no delivery of the intruder. However, the cue light had flashed upon pressing the active lever. Therefore, one possible argument is that, assuming a) the intended reinforcer (the introduction of the intruder into the operant chamber) was highly rewarding and b) the residents were motivated to press for the flashing of the cue light, the residents had acquired addiction-like behavior for pressing the active lever due to acquiring an association of the cue light with the intended reinforcer. This is because highly-rewarding stimuli can cause animals to lever-press for stimuli associated with delivery of the highly-rewarding stimuli, even if the reward is not delivered. An opposing possible interpretation of the high-pressers' active lever preference could be that the cue light itself was reinforcing to begin with; in this case, it is possible that residents had no acquired addiction-like behavior for pressing the active lever but instead lever-pressed to self-administer the flashing of the cue light.

### **Scored Video Data**

Comparisons of the proportions of time high and low-pressers spent engaging in various behaviors during the reward phase of FR1 found that high-pressers performed significantly more

anogenital-sniffing of the intruders than low-pressers. It has been found that, at least in rats, sniffing of a conspecific may be used as a means of to convey hierarchical dominance over the conspecific (Wesson, 2013). It is therefore possible that the residents, which were older than the intruders, were conveying dominance through anogenital and frontal-sniffing of the intruders. However, why high-presser residents sniffed the intruders significantly more than low-presser residents remain unclear. Comparisons of high- and low-pressers' behaviors when accompanied by the intruders in FR1 also found that only high-presser residents were groomed by the intruders. It is known that allogrooming, or social grooming between members of the same species, is affiliative and can be used to console distressed conspecifics (Wu et al., 2021). This suggests that positive social interactions could have reinforced the high-pressers' active lever-pressing, especially because the residents had been deprived of social interaction by being singly-housed. It is also possible that this seemingly exclusive behavior was observed by chance.

Differences in the proportions of various behaviors high- and low-pressers exhibited during the FR1 reward phase could also potentially be explained by the nature of the video data that had been used to compare high- and low-pressers. The majority of the video data for the high-presser residents were recorded on the last day of FR1 testing (Day 7). Because low-presser residents pressed the active lever much less frequently than the high-pressers, several low-presser residents did not have any video data of the reward phase on Day 7 of FR1 testing; as a result, video data from prior days of FR1 testing (as far back as Day 2) were scored and analyzed for comparison. The fact that not all high- and low-pressers' behavioral video data came from the same day of FR1 testing could have introduced confounds that may contribute to statistical findings comparing the residents' behaviors during the reward phase of FR1.

Ultimately, although the findings from analyses of behavioral video data of high- and low-presser residents during FR1 suggest that positive social interactions may have motivated the high-presser residents to lever-press the active lever significantly more than the low-pressers, future studies are needed to test this possibility.

### **Limitations and Future Study Directions**

Inadequate counterbalancing of the active and the inactive lever during FR1, tests without intruder delivery, and PR tests in which pressing the active lever, but not the inactive lever, resulted in the flashing of the cue light introduced a confounding variable in assessing whether high-presser residents were motivated by some aspect of the introduction of the intruder into the operant chamber. The fact that residents were singly-housed could have also been a confounding variable; it is possible that residents lever-pressed for social interaction.

One way this study could be improved would be by disallowing the active lever from retracting upon being pressed during FR1 testing, as was the case in PR testing. This would produce similar levels of active-lever availability in both tests, allowing residents a more equal opportunity to press the active-lever. Additionally, programming both the active and the inactive lever to flash cue lights upon being pressed, respectively, in future studies could provide greater insight into whether residents found the cue light rewarding (as opposed to the introduction of the intruder into the operant chamber). Another future study direction could control for the need for the residents' need for social interaction by separately operantly testing two groups of residents (residents that are singly-housed versus residents that are group-housed). If the differences in the sensory stimuli associated with presses on each lever were controlled for, and only the socially-isolated (singly-housed residents), compared to the group-housed residents, showed a preference for the active lever, then this would suggest that social interaction is

reinforcing to female when they have been socially-isolated. A limitation to this study was the feasibility of scoring and analyzing all video data of the behaviors residents engaged in throughout the study. Analyzing and averaging the proportions of time residents spent in various behaviors across every day of FR1 testing, for instance, may provide a better understanding of the behaviors residents engaged in. Additionally, the behaviors of the intruders, which were not scored in this study (asides from intruders' grooming of the residents), could be of interest.

The estrous cycles of the residents and the intruders were also not recorded in this study. Because the estrous cycle could have impacted resident and intruder social behavior, careful consideration of the residents' and intruders' estrous cycles could be of benefit in future studies.

### **Conclusions**

Retired dams did not exhibit aggression towards young intruder females in a resident-intruder paradigm. However, a subset of resident dams was observed to show a significantly greater preference for the active lever than other residents. It is unclear if residents were motivated to press the active lever for the flashing of the cue light, some aspect of social interaction, or something else entirely.

### References

- Anooshian, L.J. (2005). Violence and aggression in the lives of homeless children: A review. *Aggression and Violent Behavior, 10*(2), 129-152.
- Archer, J. (1991). The influence of testosterone on human aggression. *British Journal of Psychology, 82*(1), 1-28.
- Azevedo, J., Vieira-Coelho, M., Castelo-Branco, M., Coelho, R., & Figueiroedo-Braga, M. (2020). Impulsive and premeditated aggression in male offenders with antisocial personality disorder. *PLoS One*.
- Bosch, O.J. (2011). Maternal nurturing is dependent on her innate anxiety: The behavioral roles of brain oxytocin and vasopressin. *Hormones and Behavior, 59*(2), 202-212.
- De Boer, S.F., Buwalda, B., & Koolhas, J.M. (2016). Aggressive behavior and social stress. *Handbook of Stress Series, 1*, 293-303.
- De Jong, T.R., Beiderbeck, D.I., & Neumann, I.D. (2014). Measuring virgin female aggression in the female intruder test (FIT): Effects of oxytocin, estrous cycle, and anxiety. *PLoS ONE, 9*(3).
- Elbert, T., Weierstall, R., & Schauer, M. (2010). Fascination of violence: On mind and brain of man hunters. *European Archives of Psychiatry & Clinical Neuroscience 260*(2).
- Falkner A.L., Grosenick L., Davidson T.J., Deisseroth K., Lin D. Hypothalamic control of male aggression-seeking behavior. *Nat Neurosci.*, 9(4), 596-604.
- Fish, E.W., De Bold, J.F., & Niczek, K.A. (2002). Aggressive behavior as a reinforcer in mice: Activation by allopregnanolone. *Psychopharmacology, 163*, 459-466.



- Flannelly, K.J., & Flannelly, L. (1985). Opponents' size influences maternal aggression. *Psychological Reports, 57*(3), 883-886.
- Flannelly, K.J., & Flannelly, L. (1987). Time course of postpartum aggression in rats (*Rattus norvegicus*). *Journal of Comparative Psychology, 101*(1), 101-103.
- Fontaine, R.G. (2007). Disentangling the psychology and law of instrumental and reactive subtypes of aggression. *Psychology, Public Policy, and Law, 13*(2), 143–165.
- Golden, S.A., Aleyasin, H., Heins, R., Flanigan, M., Heshmati, M., Takahashi A., Russo, S.J., & Shaham, Y. (2017). Persistent conditioned place preference to aggression experience in adult male-sexually experienced CD-1 mice. *Genes, Brain and Behavior, 16*, 44-55.
- Golden, S.A., Heins, C., Venniro, M., Caprioli, D., Zhang, M., Epstein, D.H., & Shaham, Y. (2017). Compulsive addiction-like aggressive behavior in mice. *Biological Psychiatry, 82*, 239-248.
- Hashikawa, K., Hashikawa, Y., Lischinsky, J., & Lin, D. (2018). The neural mechanisms of sexually dimorphic aggressive behaviors. *Trends in Genetics, 18*(10), 755-776/
- Herrera, V.M., & McCloskey, L.A. (2001). Gender differences in the risk for delinquency among youth exposed to family violence. *Child Abuse & Neglect, 25*(8), 1037-1051.
- Koolhaas, J. M., Coppens, C. M., de Boer, S. F., Buwalda, B., Meerlo, P., &

- Timmermans, P. J. (2013). The resident-intruder paradigm: a standardized test for aggression, violence and social stress. *Journal of visualized experiments: JoVE*, 77. doi: <https://doi.org/10.3791/4367>
- LaCourse, E., Cote, S., Nagin, D.S., Vitaro, F., Brendgen, M., & Tremblay, R.E. (2002). A longitudinal-experimental approach to testing theories of antisocial behavior development. *Cambridge University Press*, 14(4), 909-924.
- Lansford, J.E., Miller-Johnson, S., Berlin, L.J., Dodge, K.A., Bates, J.E., & Pettit, G.S. (2009). Early physical abuse and later violent delinquency: A prospective longitudinal study. *Child Maltreatment*, 12(3), 233-245.
- Lidster, K., Owen, K., & Browne, W.J. (2019). Cage. aggression in group-housed laboratory male mice: an international data crowdsourcing project. *Sci Rep*.
- McMahon, S.D., Peist, E., Davis, J.O., Bare, K., Martinez, A., Reddy, L.A., Espelage, D.L., & Anderman, E.M. (2019). Physical aggression toward teachers: Antecedents, behaviors, and consequences. *Aggressive Behavior*, 46(1), 116-126.
- Natarajan, D., de Vries, H., Saaltink, D., de Boer, S.F., & Koolhas, J.M. (2008). Delineation of violence from functional aggression in mice: An ethological approach. *Behavior Genetics*, 39, 73-90.
- Newman, E.L., Covington III, H.E., Junghyup, S., Bicakci, M.B., Ressler, K.J., DeBold, J.F., & Miczek, K.A. (2019). Fighting females: Neural and behavioral consequences of social defeat stress in female mice. *Biological Psychiatry*, 86(9), 657-668.
- Nilsen, S.P., Chan, Y., Huber, R., & Kravitz, E.A. (2004). Gender-selective patterns of aggressive behavior in *Drosophila melanogaster*. *PNAS*, 101(3), 12342-12347.

- Numan, M. & Insel, T.R. (2003). *The Neurobiology of Parental Behaviour*. New York, NY: Springer.
- Rossegger, A., Wetli, N., Urbaniok, F., Elbert, T., Cortoni F., & Endrass, J. (2009). Women convicted for violent offenses: adverse childhood experiences, low level of education and poor mental health. *BMC Psychiatry*, 9(81).
- Schanlon, A., Zeh, A., Wendeler, D., Peters, C., Wohlert, C., Harling, M., & Nienhaus, A. (2012). Frequency and consequences of violence and aggression towards employees in the German healthcare and welfare system: A cross-sectional study. *BMJ Open*, 2(5).
- Stern, J.M., & Kolune, J.M. (1987). *Maternal aggression of rats is impaired by cutaneous anesthesia of the ventral trunk, but not by nipple removal. Physiology & Behaviors*, 54, 861-868.
- Takahashi, A., & Miczek, K.A. (2014). Neurogenetics of aggressive behavior – Studies in rodents. *Current Topics in Behavioral Neurosciences*, 17, 3-44.
- Weierstall, R., Huth, S., Knecht, J., Nandi, C., & Elbert, T. (2012). Appetitive aggression as a resilience factor against trauma disorders: Appetitive aggression and PTSD in German World War II veterans. *PLoS One*, 7(12).
- Weierstall, R. & Thomas, E. (2011). The appetitive aggression scale - development of an instrument for the assessment of human's attraction to violence. *European Journal of Psychotraumatology*, 2(1).
- Wichorek, M.G., Tisak, M.S., & Tisak, J. (2011). Relation between exposure to and consequences of aggression: U.S. national sample of adolescents. *Journal of School Violence*, 10(4).

Williamson, C.M., Lee, W., DeCasien, A.R. *et al.* (2019). Social hierarchy position in female mice is associated with plasma corticosterone levels and hypothalamic gene expression. *Sci Rep* 9.