A murine model of female aggression: Escalation of aggressive behavior by midazolam and ethanol

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Abstract

The number of violent crimes associated with alcohol consumption and levels of violent crime committed by women are increasing around the world. Social aggression in rodents has been used to explore various aspects of what makes mammals fight: such as environmental conditions, the status of a conspecific, and the effects of substances of abuse. The resident-intruder confrontation in mice and rats engender species-typical territorial aggressive behaviors to examine intermale and interfemale aggression. Previous research has shown that ethanol and self-administration increases aggressive behavior in a subpopulation of outbred mice. Similar increases in aggression are seen in male mice after treatment with low doses of benzodiazepines. Most research in this field has focused on male mice. A greater understanding of the nature of female aggression is necessary as levels of violent crime committed by women have increased in the past few decades, including crime committed under the influence of alcohol. This study introduces a method of resident-intruder aggression in non-reproductively active females to understand the differences in expression in male and female aggression and to explore differences in the expression of aggression in female mice mediated by alcohol, benzodiazepines, and the quality of the stimulus animals.

Introduction

In recent decades, the level of violent crime committed by women has increased and it shows no sign of declining (**Poe-Yamagata and Butts, 1996);** (**Chesney-Lind and Pasko, 2004)**. This increase in crime includes violence committed under the influence of alcohol **(Greenfeld and Snell, 2000) (Graves, 2007)**. This phenomenon has put into perspective how little is known about female aggressive behavior, and under what conditions women express inappropriate aggression. Most preclinical research has focused heavily on male subjects. The dearth of attention given to female aggression means that a comparison is difficult. The research with males and females suggests that in many species there is a significant sex difference in the expression of aggression. There has been a paucity in the number of studies of females by the preclinical research community until recently. In 2014, the director of the NIH Office of Research on Women's Health released a statement on the lack of female subjects used in preclinical research in contrast to clinical studies **(Clayton and Collins, 2014)**. Without a more developed understanding of the sex differences in the expression of aggression, informed clinical intervention for women offenders and the violent crime committed by females is impossible. An effort must be made in preclinical research to explore the expression and mediation of aggression in females.

Current research conditions prevent the study of the mechanisms underlying aggression in human models. Current techniques of measuring molecular-level changes are either highly invasive or ethically unfeasible. Ethical considerations prevent the studying of direct forms of aggression or social confrontation in the laboratory. Because of this, human studies of aggression focus on modes of indirect expression. Techniques such as the Taylor aggression paradigm (TAP) or the avoidance of point loss procedure promulgated by Cherek (**Cherek, 1983)** have

been used to idirectly measure aggression. The TAP leads the subject to believe that he is competing against another participant in a test of reflexes. The winner of each round gives a shock to the loser as punishment. The opponent of a participant is either a confederate or is non-existent. The pattern of winning and losing is pre-determined. The TAP focuses measuring the intensity and the duration of a shock given to the opponent under provoked or unprovoked conditions as a measure of aggressive behavior (**Taylor 1967).** The Taylor aggression paradigm is sensitive enough to show a behavioral difference between male individuals given varying doses of alcohol (**Giancola and Zeichner, 1997).** Females tested using the TAP show a significant effect on willingness to administer aggressive shocks to an opponent with prior exposure to alcohol. Alcohol consumption significantly increased the duration of the shocks administered by women, but not the intensity. Men increased both the intensity and the duration of shocks they administered with alcohol consumption prior to participating in a TAP. This was interpreted by the experimenters as an expression of indirect aggression (**Giancola and Zeichner, 1995; Giancola et al, 2009).** This research proposes that women are more selective about expressing aggression. Studies with avoidance of point loss operate lead participants to believe that they are competing with a fictitious opponent. Aggressive responses are measured by participants selecting the option to make an opponent to lose points while the option to earn points in a nonaggressive manner is present **(Cherek et al, 1990).** This paradigm has shown that both males and females reveal significant individual differences in aggressive responding after consuming low doses of alcohol **(Cherek et al, 1992; Dougherty et al, 1996)** and that these were different across sexes (**Dougherty et al, 1999).** These studies provide a small window into the nature of the sex difference in aggression, but they have significant shortcomings. They do not measure social aggression. In these studies, the target of the subject's aggression is

nonexistent or unseen. This dissociates the participant from the aggressive behavior, even if they are told that they are is administering shocks to another person. These methods therefore imperfectly translate to a real-world setting because the consequences of the aggression are not available to the participant. The procedure developed by Cherek may be confounded based on the idea that participants are acting aggressively to avoid the negative outcome of a loss of points. There are more far-reaching issues to be taken into account when conducting aggression studies in humans. For example, there is no possibility for control over variables such as living condition, stressors, and prior use of drugs. The inability to ethically model direct forms of aggression in humans is problematic because as violent crime is a direct confrontation. Human research also has the added complication of a cultural biases that affect expression of aggression across sexes. In most cultures the male has been designated as the more aggressive of the two sexes. This affects the expression of aggression in both males and females. The use of laboratory animals allows for higher levels of control over a subject's environment. Animals also are uninhibited by cultural bias. Preclinical models of aggression in non-human species can help researchers better understand female aggression through the use of techniques and methodologies unfeasible in humans.

Rodents are advantageous as model species because of pre-existing models of social aggression and a large body of aggressive research. Rodent research has studied the expression of female aggression primarily in the form of maternal aggression. Maternal aggression has been an area of continued study due to the high frequency of aggressive behavior in a pregnant or post-partum rodent. The purpose of maternal aggression is to defend the young. A pregnant mouse will become increasingly more aggressive the closer she is to giving birth. The expression of aggressive behavior continues to peak for up until two weeks post-partum while she is

lactating (**St. John and Corning, 1973; Svare and Gandelman, 1973)**. After her pups are weaned, maternal aggressive behavior will disappear until she becomes pregnant again. Factors that affect maternal aggression include the presence of pups, stress level of the dam during pregnancy, and hormonal alterations both natural and experimental (**Svare and Gandelman, 1975); (Broida and Svare, 1982); (Yoshimura and Ogawa, 1991) (Gammie and Stevenson, 2006)**. Maternal aggression has been researched extensively and we can estimate the day after birth that a female mouse or rat is likely to be the most aggressive (**Lonstein and Gammie, 2002; Flannelly and Flannelly, 1987)**. This cyclic nature of the expression of maternal aggression parallel the changes seen in maternal care behavior over time. Maternal care behavior has been seen in mice independent of sex and maternal experience. Female and male mice show higher levels of maternal care behavior when pups are younger (**Noirot, 1964)**. Maternal care decreases as pups age. This relationship suggests that the maternal behavior is heavily dependent on the external cues provided by the pups such as ultrasonic cries **(Noirot, 1972)**. These types of behaviors may both be modulated by external cues coming primarily from the young. When a rodent is not actively pregnant or lactating, female aggression can be interpreted as either social or territorial aggression. Nonmaternal aggression in female mice and rats is expressed with a different hormonal profile than maternal aggression, indicating that these are two types of aggression. Maternal aggression provides evidence for a sex difference in the expression of aggression and a sex difference in the mediation of aggression via gonadal hormones.

Male mice have been shown to exhibit aggressive behavior in instances where competition is unnecessary; this came to be known as ‘spontaneous aggression’ (**Fredericson 1950).** Female mice express aggression at levels significantly lower than that of males (**Fredericson, 1952)**. This low level of spontaneous aggression in females is one factor that has

contributed to the lack of nonmaternal female aggression research. The earliest studies on female aggression used gonadal hormone manipulation, leading to the development of the hypothesis that testosterone was a key mediator of aggression in mice. Male mice were found to be more aggressive when given exogenous testosterone or in the presence of a stimulus animal who elicited pheromonal cues that contained testosterone, i.e. urine. (**Fredericson et al, 1955; Mugford and Nowell, 1972)**. Exogenous female gonadal hormone, such as estradiol, attenuated male aggressive behavior when administered to male mice (**Bronson and Desjardins, 1968).**

Studies conducted with animals that were exposed to testosterone during a critical period provided additional evidence for the argument that testosterone was necessary for aggression in both sexes. Chronic exposure to testosterone in adolescence was shown to increase aggressive behavior across sexes in mice that were given acute injections of testosterone immediately prior to an encounter with another mouse (**Edwards, 1970).** Studies that varied the amount of testosterone administered to female mice showed that females needed higher levels of testosterone as adults to exhibit similar levels of spontaneous aggression to male mice that had been castrated and administered exogenous testosterone (**Barkley and Goldman, 1977).** Studies also showed that acute exposure to testosterone in adult female mice without neonatal testosterone exposure increased spontaneous aggression (**vom Saal et al, 1976)**. More testosterone was required over a longer period to elicit the behavior (**vom Saal et al, 1976)**. The development of the idea that female mice are more aggressive when given exogenous testosterone was important, but it promoted the idea that both female and male aggression might be controlled by similar mechanisms. This had not been proven to be true by the end of the 1970's. It also suggested that females were less aggressive simply because they had less testosterone. This logic failed consider the organizational effects of gonadal hormones. Exposing

a genetic female mammal to testosterone shortly after birth will result in changes in physiological development. The natural organizational effects of the female gonadal hormones on the female body will be altered, and this primes the body for an activational effect of testosterone in the future. The level of similarity between these animals and a non-manipulated female is unknown. The results of these studies indicate that female mice can be altered to express aggression in a male-like fashion. This means that female and male aggression are mediated by the same mechanisms. However, the hormonal manipulations used in this research limit their ability to broaden our understanding of female aggression outside of the laboratory.

Later studies redefined testosterone role's in the mediation of aggression with a shift in attention to female gonadal hormone and aggressive behavior. When administered to an ovariectomized female mouse, estradiol did not elicit aggressive behavior in females **(Grimm et al, 1985).** Only when ovariectomized females were given both testosterone and estradiol, was the frequency of their aggressive behavior like that of intact females. Interestingly, this estradiol and testosterone mediated aggression did not show a decline in aggressive behavior over time which had been seen in ovariectomized females given only testosterone (**Albert et al, 1991).** The opposing effects of female gonadal hormone on female and male aggression solidifies the idea that aggression in the two sexes is mediated similarly, but not identically. There is evidence that female aggression is somewhat hormone-independent. Studies have shown that ovariectomized female rats showed no difference in aggressive behavior following the surgery (**DeBold and Miczek, 1984),** but this has not been confirmed in other rodent species. Other studies insist male and female aggression is hormone-dependent **(Albert and Walsh, 1995; Beeman, 1947; Barkley, and Goldman, 1977).** The observed differences may be attributed to laboratory housing conditions, or a myriad of other factors. Based on the information presented above, there

is evidence for a sex difference in expression of aggression and a sex difference in the effects of gonadal hormone on aggression in rodents. A characterization of this sex difference across varying aggression-altering conditions is necessary for a comparison between sexes.

The consumption of alcohol is one of many factors that contribute to violent crime committed by both males and females. It is the drug most implicated in physical aggression in humans (**Pridemore, 2004; Maldonado-Molina et al, 2010).** Both human and mouse studies have identified a sex difference in the consumption and the metabolism of alcohol. Women have been shown to have higher blood ethanol concentrations when given equivalent doses to men (**Jones and Jones, 1976; Arthur et al, 1984).** Women have more body fat and less body water per kilogram than men. Less body water means that alcohol has less volume to disperse in, and this results in higher levels of blood alcohol content in women than in men with similar body weights and equal amounts of alcohol consumed (**Marshall et al, 1983).** In fact, women achieve higher blood alcohol levels for a longer period than men across normalized doses of ethanol administered both intravenously and orally (**Baraona et al, 2001)** However, women eliminate alcohol from their bodies at a faster rate than men do due to differences in efficacy of liver enzymes. These sex differences can also be seen in rodents. Female rodents have been shown to eliminate ethanol faster than male rodents (**Erikkson, 1973; Robinson et al, 2002).** Female rodents have also been shown to drink significantly more alcohol than males in limited and continuous access paradigms (**Middaugh et al, 1999; Hwa et al, 2011).** The preservation of these sex differences indicate that rodents can serve as an appropriate model for human alcohol consumption.

Research of acute administration of ethyl alcohol or ethanol has revealed that the substance targets specific receptors in the brain and alters the activity of larger brain regions to

produce significant behavioral effects. The dose-effect curve of alcohol is biphasic, with effects varying across dose and time (**Pohorecky, 1977)**. Lower doses on the ascending limb of the curve have been known to increase aggressive behavior. Higher doses have a sedative effect on humans and rodents (**Cherek, 1985; Blanchard, 1988; Miczek et al, 1998)**. These behavioral and physiological effects are believed to be mediated by alcohol's interaction with specific receptors. Although no ethanol-specific binding site has been definitively identified, alcohol has been found to stimulate activity of the GABA-A receptor as a positive allosteric modulator (**Mehta and Ticku, 1988; Hanchar et al, 2004).** In addition, the behavioral effects of ethanol can be potentiated and attenuated by GABA-A agonists and antagonists respectively (**Lister and Linnoila, 1991, Grobin et al 1998).** Benzodiazepines, a class of anxiolytic drugs that has been used clinically for decades (**Stovener and Endresen, 1965),** are characterized by their allosteric effects on GABA-A receptors. The behavioral effects of benzodiazepines are like that of acute doses of ethanol. Today, a handful of benzodiazepines are used as anesthetics and sedatives but this class of drug was heavily prescribed in the 1960s and '70s to treat panic disorders, convulsions, and symptoms of anxiety (**Olkkola and Ahonen, 2008).** Benzodiazepines also have potential for abuse and dependence. Benzodiazepines that positively modulate GABA-A receptors have been shown to have pro-aggressive effects at small doses like alcohol (**Gardos et al 1968**; **Bond and Lader, 1988; Weisman et al 1998).** This came to be known as paradoxical aggression as benzodiazepines were most widely known for their calming effects (**Bramness et al, 2006**; **Saias and Gallarda, 2008).** This increase in hostility has been seen in a subpopulation of humans who are typically characterized as more aggressive or more anxious (**Bailey and Taylor, 1991; Ben-Porath and Taylor, 2002)**. Research has shown that benzodiazepines alter aggression similarly in male rodents. Pro-aggressive effects are seen at small or moderate doses

and they are counteracted by competitive GABA-A antagonists. (**Miczek, 1974; Miczek and O'Donnell, 1980; Rodgers and Waters, 1985; Mos et al, 1987b; Miczek et al, 1994).** The effect of benzodiazepines on aggression is context-specific (**Skolnick et al, 1985; Fish et al 2005).** Benzodiazepines have also been shown to mediate maternal aggression in mice and rats, with differential effects based on the sex of an intruder (**Mos et al, 1987a)** (**Palanza et al, 1996).** Benzodiazepines did not spontaneously illicit aggression as only subjects with a history of aggressive behavior showed increases in aggression (**Miczek, 1974; Ferrari et al, 1996).**

There is evidence to suggest that the effects of GABA-A receptors are subunit-specific. A GABA-A receptor is comprised of five subunits and can consist of a combination of: alpha (1-6), beta (1-3), gamma (1-3), delta, epsilon, pi, theta, and rho (1-3) subunits (**Sieghart, 1995; Simon et al, 2004; Sarto-Jackson and Sieghart, 2008).** Different subunit compositions have been shown to have differential sensitivities to neuromodulators like benzodiazepines and ethanol. For example, a gamma subunit must be present in a GABA-receptor for it to be sensitive to a benzodiazepine (**McKernan et al, 2000; Crestani et al 2000).** In addition, some studies report that GABA-A receptors with a delta subunit are more sensitive to ethanol than GABA-A receptors with a gamma subunit (**Wallner et al, 2003).** The research conducted with benzodiazepines and aggression in male mice indicate that benzodiazepines can mimic the effects of acute alcohol consumption at subclinical doses. The effect of benzodiazepines on maternal aggression and male aggression in mice suggests that a similar increase could be seen in nonmaternal female aggression.

Acute doses of ethanol have been shown to inhibit the activity of the NMDA glutamate receptor (**Lima-Landman and Albuquerque, 1989; Lovinger et al 1989).** Like benzodiazepines and ethanol, ketamine and similar NMDA receptor antagonists have seen

clinical use as sedatives, muscle relaxants, and anesthetics (**Takahashi et al, 1984**). However, these substances have a high potential for abuse. Ketamine's recreational use is mainly attributed to the euphoric and dissociative effects of this class of drug at sub-anesthetic levels (**Smith et al, 2002).** Administration of a NMDA receptor antagonist in mice reduces aggression at high doses in mice (**Belozertseva and Bespalov, 1999)** and can alleviate symptoms caused by opiate or alcohol withdrawal (**Krystal et al, 1998).** When administered in conjunction with ethanol self-administration, the NMDA receptor antagonist memantine escalates aggressive behavior (**Newman et al 2012).** The conflicting effects of these antagonists seem to be associated with potentiating the behavioral dysregulation caused by ethanol self-administration. These effects may also be subunit-specific. NMDA receptors consist of four subunits in the form of two joined heterodimers that can consist of NR1, NR2(A-D), and NR3(A-B) subunits (**Traynelis et al 2010).** NMDA receptors have also been shown to have differential sensitivity to ethanol based on subunit composition. Receptors that contain the NR2B subunit are more sensitive to alcohol than those containing NR2C or NR2D subunits (**Masood et al, 1994; Smothers et al 2001).** The diverse effects of ethanol on glutamatergic and GABAergic neurotransmission have complicated efforts to understand ethanol's action on neuroreceptors. The use of benzodiazepines like midazolam and specific NMDA receptor antagonists such as memantine isolate specific characteristics of ethanol's modulatory abilities to help better examine behavioral effects of the substance.

The resident-intruder paradigm is a powerful model of territorial aggression in mice and rats and can serve as a protocol for characterizing aggression. Typically, a male resident rodent that cohabitates with a female performs aggressive acts onto an unfamiliar intruder male when in it is introduced to the resident's home cage (**Crawley et al, 1975)**. Almost all male residents will

inflict attack bites onto an intruder male in this paradigm (**Miczek et al, 1998)**. This paradigm can serve to model female aggression as well. It has been shown that the living conditions of residents in the resident intruder paradigm are optimal for expression of aggression in both male and female rodents. A female rat that is housed with other females will be less aggressive on average than one housed with a castrated male that has been implanted with testosterone. A female rat housed with a castrated male will be less aggressive on average than one housed with an intact male (**Albert et al, 1988).** The resident intruder model has been used to study qualitative differences in aggression when altered by substances of interest. Male rodents under the influence of low doses of alcohol were more aggressive and bit intruders in more target locations than when given a dose of water. These unique target locations were more sensitive or vulnerable than typical locations of attack bites (**Newman et al, 2015)**. The established understanding of how rodents fight is overwhelmingly male-centric (**Scott and Fredericson, 1951)** and it cannot be assumed that female mice fight in the same way. Alcohol increases aggressive behavior in male mice in the resident-intruder interaction model (**Krsiak and Borgesova, 1973**). However, only a small proportion of those animals show an elevated level of aggression after consuming low doses of alcohol prior to the confrontation with the intruder mouse (**Miczek et al 1998)**. This observation provides a basis for a potential genetic difference in mammals that results in a more aggressive phenotype (**Fagan, 1993)**. This can potentially translate as not every male human that drinks becomes violent, only a subset of them do (**Fagan, 1993)**.

Some manipulations like isolation consistently increase expression of aggression in both sexes (**Howard et al, 1981)**, but female mice appear to be more sensitive to environmental cues, particularly the presence of pheromonal testosterone in their living space (**Albert et al 1988).** In

addition, manipulation of the stimulus animal can be used to alter levels of aggression in resident mice. Male residents have been shown to express differential levels of aggression towards intruders of various conditions. This includes a varying proportion of intruders attacked, latency to attack, and length of the attacks observed (**Brain et al, 1981).** This suggests that there is a prominent effect of stimulus quality in the expression of aggression in male mice. There is also evidence for a stimulus quality effect in aggressive behavior in female mice. Research has shown that groups of Swiss female mice express aggressive behavior against a single intruder conspecific. The level of aggression in this model differs significantly based on the strain of the intruder and whether the intruder is lactating or not (**Haug and Mandel, 1978).** The results of the above study indicate that both environmental factors and the quality of the stimulus animal significantly affect aggression in both sexes. The present study aims to expand the understanding of the nature of female aggression by using the resident-intruder interaction paradigm with varying environmental conditions and stimulus qualities. In addition, we wish to explore the effects alcohol and like substances have on aggression. It is hypothesized that female residents will react to alterations in environment and stimulus quality in similar ways: intruders of different strains and novel intruders will elicit more aggression in residents and aggressive interactions in a neutral arena will show attenuated levels of aggression when compared to interaction conducted in the resident home cage. Furthermore, we expect that female residents will also express similar alterations in their aggressive behavior when exposed to the modulatory substances alcohol and midazolam. We expect a subpopulation of female residents to express alcohol-heightened aggression, and expect subclinical doses of midazolam to increase levels of aggression in residents in a dose-dependent fashion.

Methods

Subjects

Adult female and male Swiss Webster CFW mice (Charles River, Wilmington, MA) that weighed approximately 25-27 grams upon arrival, and adult female C57BL6/J (B6) mice bred in-house that derived from Jackson Laboratory stock (Bar Harbor, ME) were used in this study. Females were labeled either residents or intruders as described in Methods Subsection Resident-Intruder Confrontation. B6 females were only used as intruders and used once they reached weights between 23-25 grams. All subjects were group-housed by sex in groups of 12 in 46x24x16-cm clear polycarbonate cages for one week to allow for habituation to the laboratory environment. Access to food and water was *ad libitum* through a stainless-steel wire lid. Group-housing cages were lined with corn cob shavings. Following the termination of the habituation period, resident CFW females underwent ovariectomy. Ovariectomy surgery was conducted according to IACUC and laboratory protocol [See Appendix 1]. Post-operation resident females were singly-housed in clear polycarbonate cages (28 x 17 x 14 cm) lined with pine and cedar shavings and paired with an intact male after a recovery period. When wounds had completely closed the OVX females were paired with intact males. If a male showed aggression toward an OVX, female he was removed. Bedding in all resident cages was cleaned at least 24 hours prior to any aggression behavior testing and cages were cleaned at least once a week. Intruders were kept in larger group-housing polycarbonate cages as described above for the duration of the study. The vivarium was maintained on a 12-hour light/dark cycle (lights off at 5:30 AM, lights on at 5:30 PM). All behavioral testing occurred during the dark cycle.

Alcohol self-administration

Resident CFW female mice chosen to be characterized as alcohol-heightened aggressors or alcohol-nonheightened aggressors were conditioned to self-administer by using aluminum operant conditioning panels that fit into the animal's home cage as described in Miczek and de Almeida 2001. Prior to each self-administration session, all resident mice cages were water-restricted. Reinforcement for nose-poking consisted of the delivery of 0.5ml of tap water. Resident females were weighed and male cohabitants were removed from each home cage before the start of each session. Mice were given a nose-poke task for a water reward on a FR1 schedule (1 nose-poke = 1 aliquot of water based on body weight). When the average duration of these sessions was below 5 minutes, the FR schedule was increased to 2 and then 5 (FR5). Once an average duration of water self-administration session of less than 5 minutes was achieved, ethanol was introduced as the fluid reward. 95% ethyl alcohol was diluted with tap water to create a 6% (w/v) ethanol solution given at 1 g/kg. This dosage of ethanol was based on the concentration given to males to engender high levels of aggressive behavior in outbred mice (**Miczek et al 1998**).

Resident-Intruder Confrontation

Aggressive behavior of resident female mice was assessed in confrontation with a group-housed intruder female. Interactions between a resident and an intruder mouse took place in the resident female's home cage or in a neutral arena (84 x 51x 42 cm) with fresh pine shavings. The pair-housed male cohabiting with each resident female was removed from the home cage for the duration of the interaction. Following the introduction of the intruder mouse, bites made onto the

intruder were counted for 5 minutes after the first successful attack bite made by the resident. If no bites were counted, the session was ended at 5 minutes. If an intruder mouse exhibited aggressive behavior toward a resident mouse (tail rattling, bites), the session was terminated and that intruder was no longer used. The latency to attack and the number of bites were hand-recorded for each session.

Social instigation was used to facilitate aggressive behavior from resident females that showed low or no attack behavior towards an intruder mouse (**Potegal and Tenbrink, 1984**). An intruder mouse was placed into a perforated protective cage and this cage is inserted into the resident mouse's home cage for 5 minutes. After this 5-minute period, the intruder mouse was removed from the cage and placed into the resident's home cage unprotected and a 5-minute interaction session was completed as described above. Resident-intruder confrontations were conducted a total of three times per week on non-consecutive days. Residents were considered to exhibit stable levels of aggression when variability in number of attacks bites was less than 20% for three consecutive sessions.

Drugs

Midazolam hydrochloride was purchased from Sigma-Aldrich (**location goes here).** The drug was diluted in 0.9% saline into a 1.0 mg/ml stock solution and diluted further into 0.1, 0.3, 0.56, and 0.1 mg/ml. The drug was injected IP at a volume of .1 ml/kg. 95% ethyl alcohol was purchased from Pharmco-AAPER (Brookfield, CT)**.** It was diluted into 6% w/v ethanol solution in 250ml batches weekly and placed into syringes that fit into custom pumps that connected to each operant self-administration panel.

Video Analysis

Resident behavior during confrontations with an intruder were video recorded using two cameras: a JVC Everio GZ-MG670 digital camera for 30 frames per second (FPS) filming and a GoPro HERO3 digital camera for 120 FPS filming. All 30 FPS recordings were analyzed using *Observer* software (Noldus v. 12**)** and conducted by trained individuals (intra-observer reliability of 95%). The outset of each operationally-defined behavior was coded by depressing a key and the offset of a specific behavior was coded by release of a specific key made onto a custom keyboard. Non-aggressive behaviors included walking, self-grooming, rearing, and non-aggressive contact while aggressive behavior consisted of pursuit, sideways threats, tail rattles, and attack bites (**See Table 1**). All 120 FPS recordings were analyzed by hand to determine bite location in aggressive interactions. 120 FPS recoding was only possible in the white light, so these were conducted separately from other behavioral measures. *Observer* software was used to generate lag sequential analysis centered around the attack bite behavior at a lag state of 1 and -1. This analysis includes the generation of a probabilistic measure based on the total number of observed sequences of behavior that precede and follow an attack bite.

Statistical Analysis

All statistical analyses were conducted in SigmaPlot 13.0 (SysStat Software Inc.). Data comparing was analyzed as two-way repeated measures analyses of variance (ANOVA) to compare differences in both aggressive and non-aggressive behavior between intruder conditions (Familiar CFW v. Familiar B6, Familiar B6 v. Novel B6) and midazolam dose (vehicle, 0.1, 0.3,

0.56, 1.0 mg/kg). Data that did not meet criteria for normality or equal variance were transformed by square root or cube root function to meet those conditions.

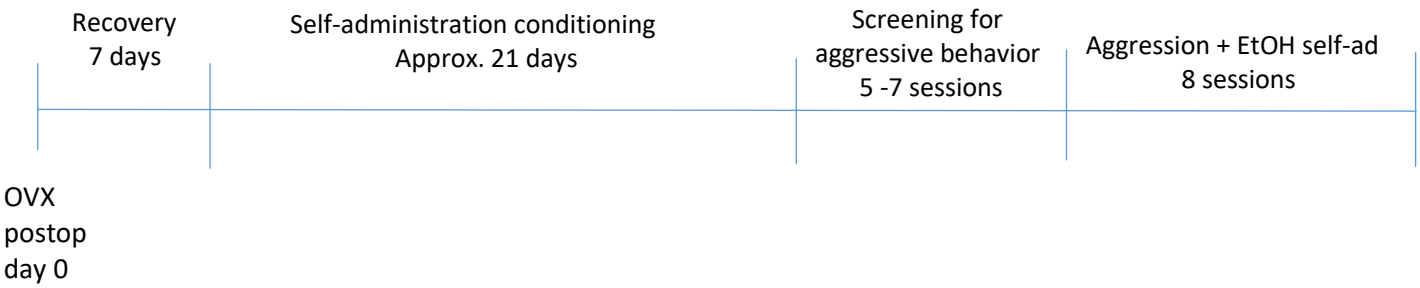
Design

Experiment 1: Aggressive behavior in confrontations of female residents vs. female intruders. The resident-intruder interaction model consistently shows that residents are dominant in interactions with group-housed intruders in males. Video analysis of many resident-intruder confrontations were conducted in order to provide information on the characteristics of inter-female aggression. This includes the proportion of residents that fought based on intruder condition, temporal distribution of aggressive attacks, a possible preference for bite location on the intruder, and the categorization of other aggressive and non-aggressive behaviors by the resident with an intruder present.

Experiment 2: The effects of ethanol on interfemale aggression. Resident females were conditioned to self-administer low doses of ethanol to examine if alcohol-heightened aggression could be detected in a subpopulation of females. An intruder was introduced into the home cage of a resident female 10 minutes after the completion of a self-administration session per a FR5 schedule of reinforcement. Eight test sessions of self-administration followed by aggressive confrontations were conducted alternating between the use of water and ethanol as fluid reinforcement. Resident females that showed a significant increase in aggression after ethanol self-administration (mean of attack bite frequency after ethanol self-administration greater than or equal to 2 standard deviations higher than the mean of attack bite frequency after water self-administration) were classified as Alcohol Heightened Aggressors (AHA). The proportion of

aggressive female residents that could be called AHA mice was compared to the proportion of AHA mice found in males (Miczek et al 1998).

Alcohol heightened aggression:

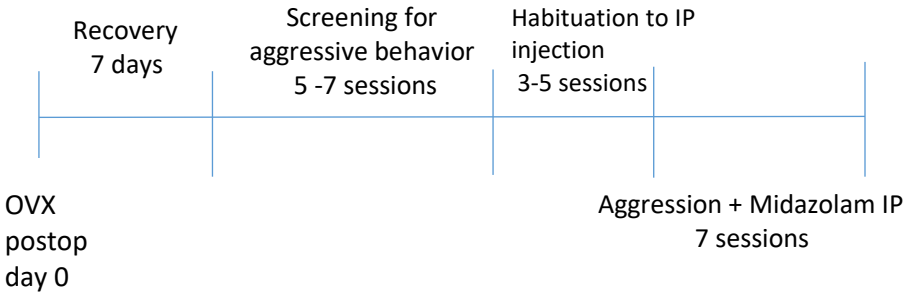


Experiment 3: Effects of strain difference of intruder and midazolam on interfemale aggression. Resident-intruder confrontations as described above were conducted using two different strains of group-housed intruders: CFW or B6 mice (Familiar CFW v. Familiar B6 condition). When resident animals showed stable levels with the same intruder for three consecutive sessions of aggression, residents were habituated to grip and injections of 0.9% saline. At least three sessions of habituation were conducted to confirm that there was no effect of handling or the injection on aggressive behavior. Each testing session consisted of an injection of either vehicle (saline) or a dose of midazolam (0.1-1.0 mg/kg) in a non-systematic format, a 13-minute period to allow for uptake of the drug, and a 5-minute interaction with an intruder. All aggressive interactions were video-recorded for behavioral analysis.

Experiment 4: Effects of intruder novelty, novel environment, and midazolam on interfemale aggression. Resident-intruder confrontations as described above were conducted using B6 intruders. Residents in the Familiar B6 condition achieved stability with one B6 intruder and

proceeded to habituation as described in earlier experiments. Residents in the Novel B6 condition were introduced to a novel B6 intruder for each intruder interaction, assessed for stability, and then moved to habituation. Residents in the Neutral Cage condition were assessed for aggressive behavior and tested in an unfamiliar neutral area (see above). Residents were tested with either vehicle or midazolam as described in Experiment 3. All aggressive interactions were video-recorded for behavioral analysis.

Midazolam and aggression:



Results

Experiment 1: The proportions of female residents that expressed aggression toward a female intruder varied based on the strain of the intruder and the level of familiarity of the intruder (Figure 1). Proportions are based on the presence of attack bites during the total number of non-manipulated aggressive sessions with an intruder in the following conditions: Familiar CFW, Familiar B6, and Novel B6. The mean proportions of aggression expressed toward B6 intruders in the Familiar and Novel condition were higher than the mean proportion of aggression expressed toward CFW intruders.

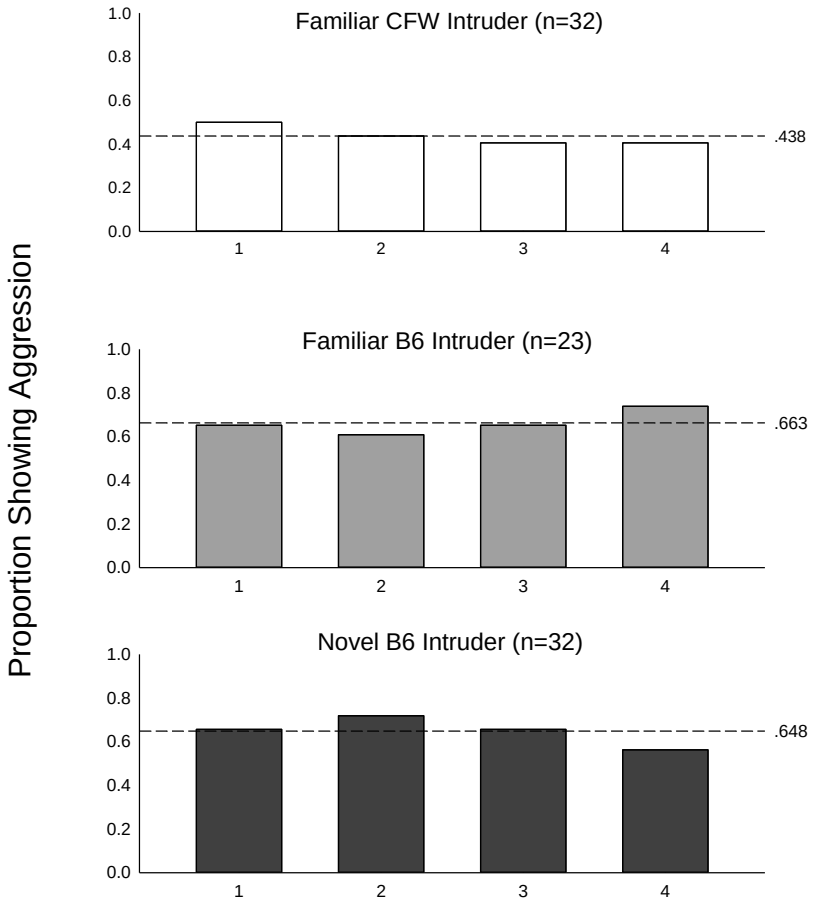


Fig. 1 Proportions of female residents that showed aggressive behavior in assessment sessions of aggression across intruder conditions. Data are expressed are average proportion per session with the mean of the condition present as a horizontal reference line.

Lag sequence analysis of 270 total observations conducted at state lag 1 or -1 were compared across doses of midazolam and intruder conditions. Sideway threat behavior was found to be the observed behavior most likely to precede an attack bite and walking behavior was found to be the observed behavior most likely to follow an attack bite. The probability of sideways threat behavior preceding an attack bite varied across intruder condition and midazolam dose (Figures 2 and 3).

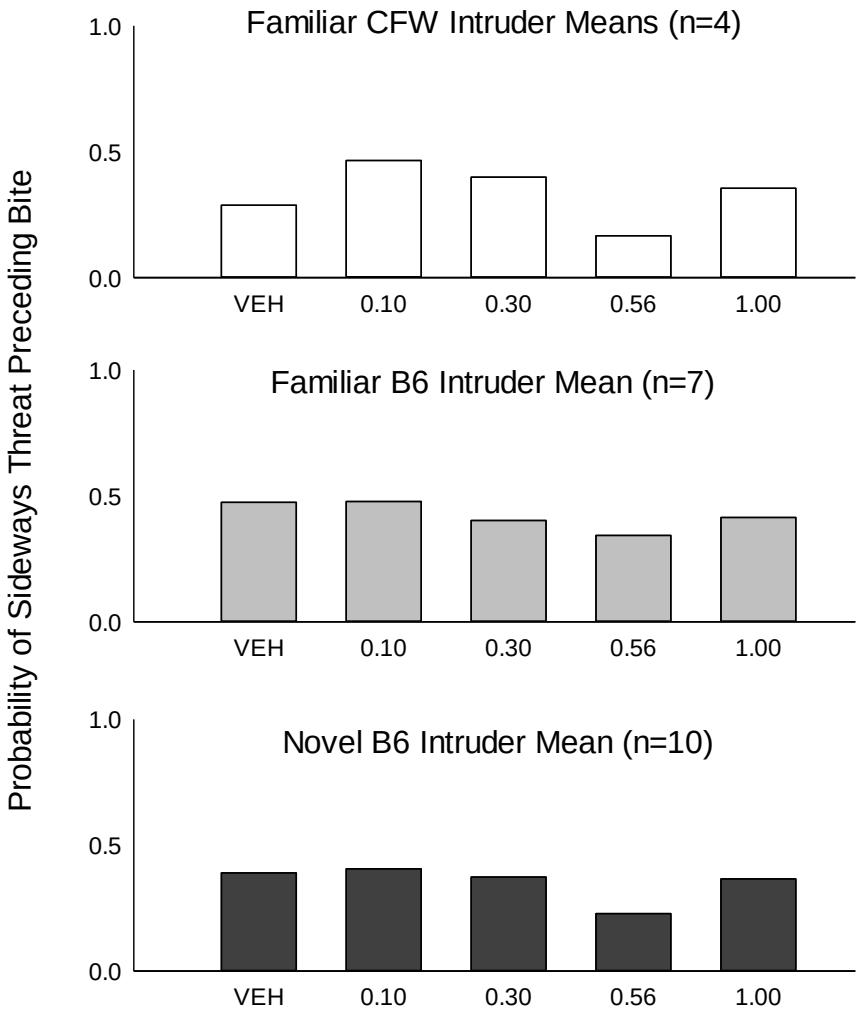


Fig. 2 Probability of sideways threat behavior preceding an attack bite varies across doses of midazolam and between intruder conditions. Data are presented as group averages of probability at the given dose.

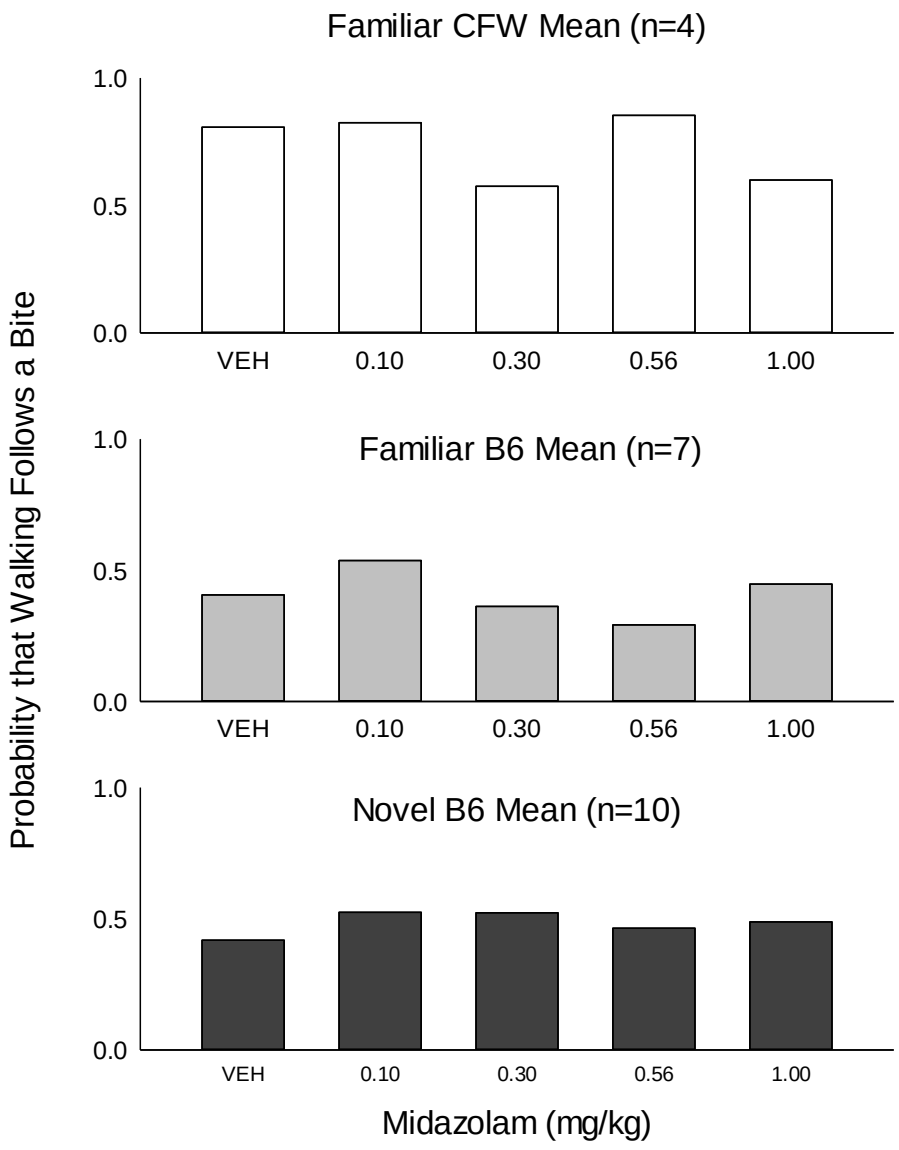


Fig. 3 Probability of walking behavior following an attack bite varies across doses of midazolam and between intruder conditions. Data are presented as group averages of probability at the given dose.

Experiment 2: Of the animals that underwent alcohol-heightened aggression characterization, only eight animals showed consistent level of aggression across the eight testing sessions. Of this group, two animals met the criteria for classification as alcohol-heightened aggression. The means and standard errors of the seven measured behaviors of the two subpopulations were measured via video analysis (Table 2, Figure 4). Slow motion videos

analyzed for each animal in the characterization experiment showed individual differences in attack bite location between post-water and post-ethanol self-administration intruder interactions (Figure 5).

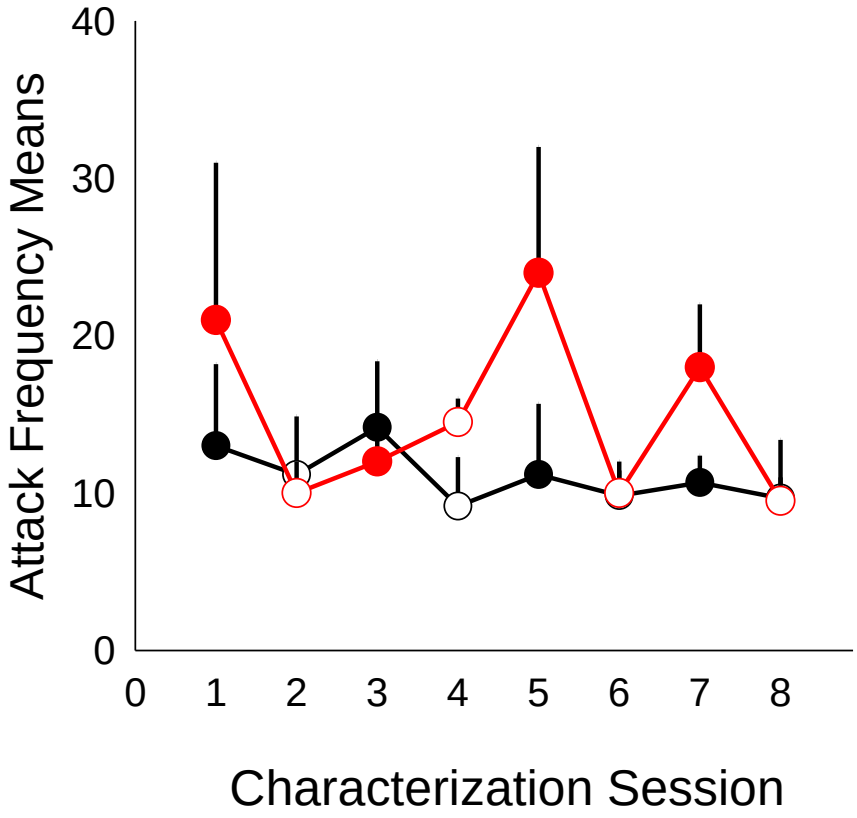


Fig 4. The average mean and standard error of attack bite frequency plotted against session number for both AHA (red) and ANA (black) resident females. Closed circles indicate a session preceded by ethanol self-administration (odd numbers) and open circles indicate a session precede by water self-administration (even numbers).

Table 2. Effects of Ethanol (1.0 g/kg) on Resident Behavior During Encounters with Familiar CFW Intruders

	ANA (n=6)		AHA (n=2)	
	H2O	EtOH	H2O	EtOH
Aggressive Behaviors (Frequency)				
Sideways Threat	14.88 ± 2.38	14.72 ± 3.51	9.5 ± 2.5	18.83 ± 11.17
Bites	4.5 ± 2.06	9 ± 1.58	8.67 ± 0.67	8.83 ± 4.5
Tail Rattle	9.0 ± 6.36	5.89 ± 0.67	13.17 ± 0.83	3.83 ± 1.17
Non-Aggressive Behaviors (Durations in seconds)				
Self-Groom	6.90 ± 1.12	12.94 ± 2.09	9.49 ± 4.15	28.36 ± 17.37
Rear	56.58 ± 7.33	53.67 ± 7.61	54.50 ± 7.03	42.79 ± 0.24
Walking	21.13 ± 8.43	16.04 ± 5.29	6.66 ± 0.39	6.58 ± 0.84
Non-Aggressive Contact	54.24 ± 7.03	62.42 ± 10.91	83.48 ± 11.29	65.48 ± 10.55

Data for each behavior are Mean ± SEM.

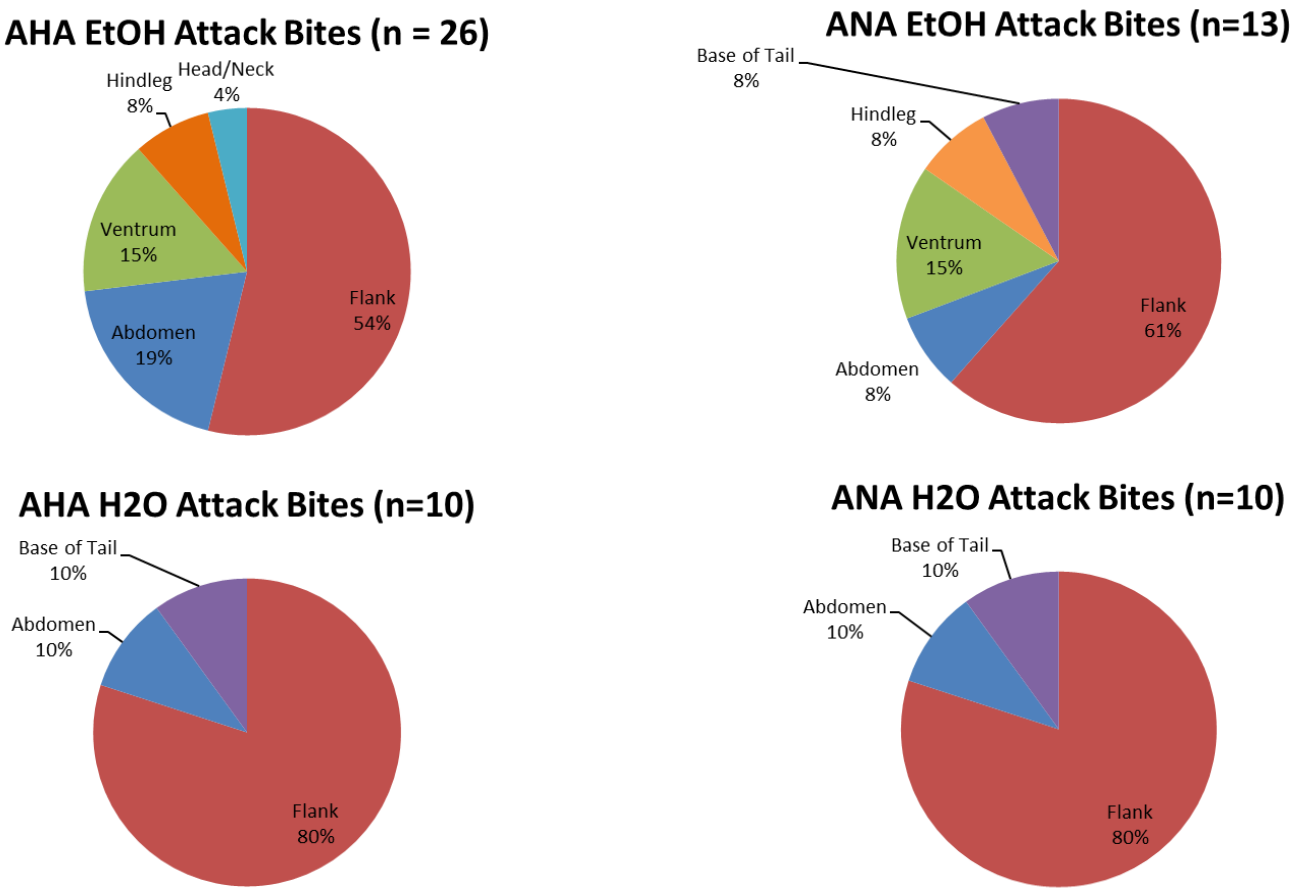


Fig. 5 Representative animals from the AHA and ANA characterization groups shown with the number of attacks bites observed in the slow motion video recordings and the correlation location on the body of the intruder.

Experiment 3: There was a near-significant biphasic effect of midazolam dose on attack bite frequency in the Familiar CFW Intruder condition [(F (1, 4) = 2.584, p=0.053); Table 3, Table 4, Figure 6], with increased levels of mean attack bite frequency seen at the 0.3 mg/kg dose. There was no effect seen from intruder condition in this comparison.

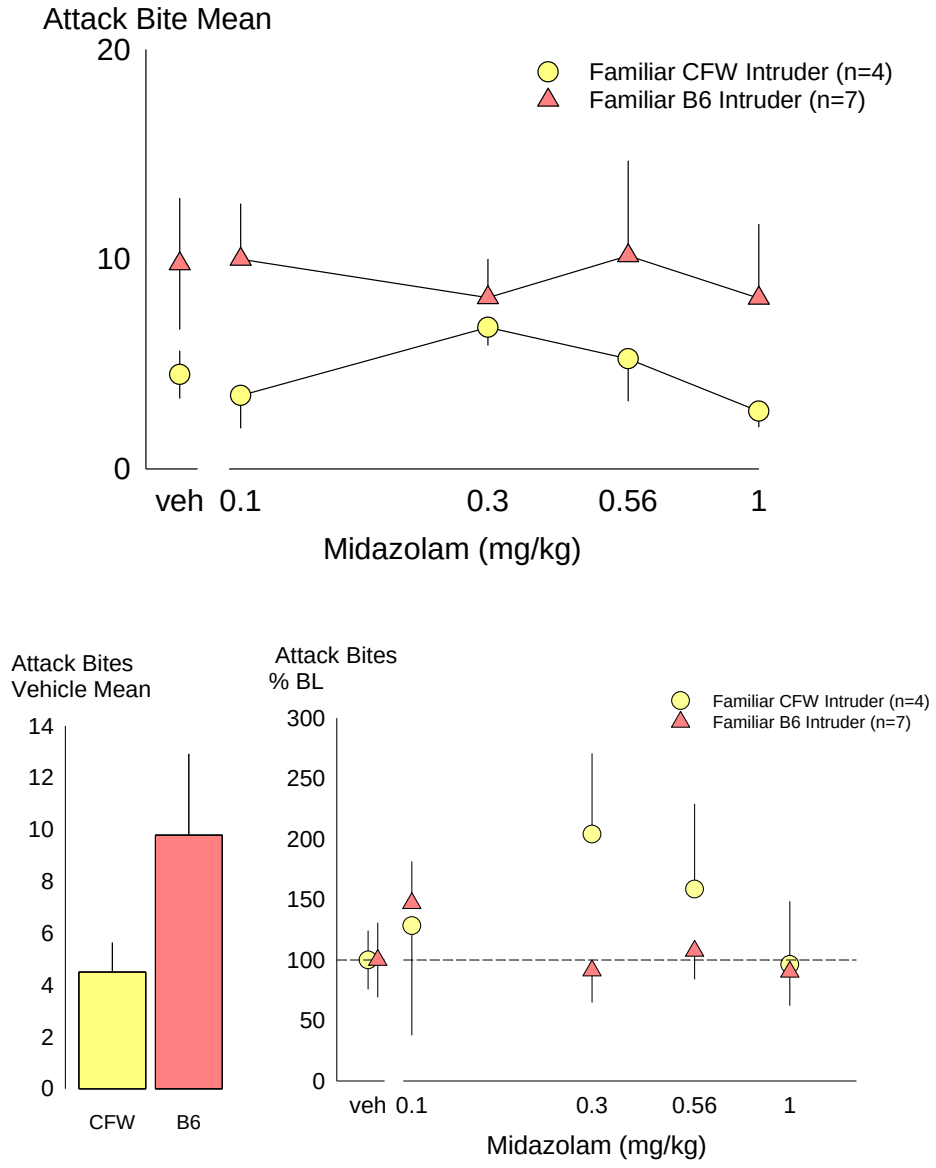


Fig 6. The attack bite frequency means across doses show less change in the Familiar B6 condition compared to the Familiar CFW condition across midazolam dose. The vehicle means and standard error of the mean (SEM) for the Familiar CFW and the Familiar B6 condition and the percent change from the vehicle baseline across doses suggest that aggression towards familiar CFW intruders is more sensitive to midazolam.

Table 3. Effects of Midazolam on Resident Behavior During Encounters with Familiar CFW Intruders

Dose (mg/kg)	V	0.1	0.3	0.56	1.0
Aggressive Behaviors (Frequency)					
Sideways Threat	11.7 ± 3.63	6.0 ± 3.7	21.8 ± 6.94	15.3 ± 4.0	8.0 ± 2.7
Bites	4.3 ± 1.16	3.5 ± 1.56	7.5 ± 1.56	5.25 ± 2.02	2.75 ± 0.75
Tail Rattle	4.92 ± 1.87	2.0 ± 0.82	12.25 ± 6.65	10.0 ± 5.79	4.0 ± 1.87
Non-Aggressive Behaviors (Duration in seconds)					
Self-Groom	18.1 ± 4.69	4.63 ± 2.07	15.86 ± 6.72	9.0 ± 4.14	9.85 ± 5.34
Rear	20.1 ± 2.6	25.34 ± 4.07	31.02 ± 6.35	24.78 ± 6.92	30.96 ± 12.39
Walking	7.0 ± 0.90	23.54 ± 15.83	5.85 ± 1.04	12.04 ± 2.88	12.02 ± 3.13
Non-Aggressive Contact	40.25 ± 6.33	51.71 ± 6.15	15.86 ± 6.72	8.97 ± 4.14	9.85 ± 5.34

Data for each behavior are Mean ± SEM.

Table 4. Effects of Midazolam on Resident Behavior During Encounters with Familiar B6 Intruders

Dose (mg/kg)	V	0.1	0.3	0.56	1.0
Aggressive Behaviors (Frequency)					
Sideways Threat	7.93 ± 1.93	6.60 ± 1.17	11.83 ± 4.14	8.67 ± 3.52	8.14 ± 2.53
Bites	9.43 ± 3.20	10.0 ± 2.65	14.17 ± 5.57	10.17 ± 4.53	8.14 ± 3.54
Tail Rattle	3.57 ± 1.07	1.8 ± 0.74	4.0 ± 1.94	2.17 ± 1.22	1.43 ± 0.75
Non-Aggressive Behaviors (Duration in seconds)					
Self-Groom	21.6 ± 5.22	21.7 ± 5.41	33.67 ± 8.47	29.65 ± 8.75	13.28 ± 4.56
Rear	82.22 ± 10.72	97.33 ± 12.34	54.65 ± 5.69	65.33 ± 17.34	62.72 ± 13.12
Walking	40.10 ± 3.37	46.40 ± 5.87	47.53 ± 5.26	41.55 ± 6.03	52.70 ± 4.95
Non-Aggressive Contact	58.73 ± 11.52	55.95 ± 4.02	67.60 ± 13.47	76.98 ± 18.82	85.31 ± 19.42

Data for each behavior are Mean ± SEM.

Tail rattle behavior differed significantly at 1.0 mg/kg of midazolam in the Familiar B6 condition and an interaction effect was seen between intruder condition and midazolam dose at the 0.3 and the 0.56 mg/kg doses [(F (1, 4) = 3.569, p=0.015); (F (1,4,) = 3.477, p = 0.17); Figure 7], with more similar levels of tail rattle frequency seen at vehicle and 0.1 mg/kg doses.

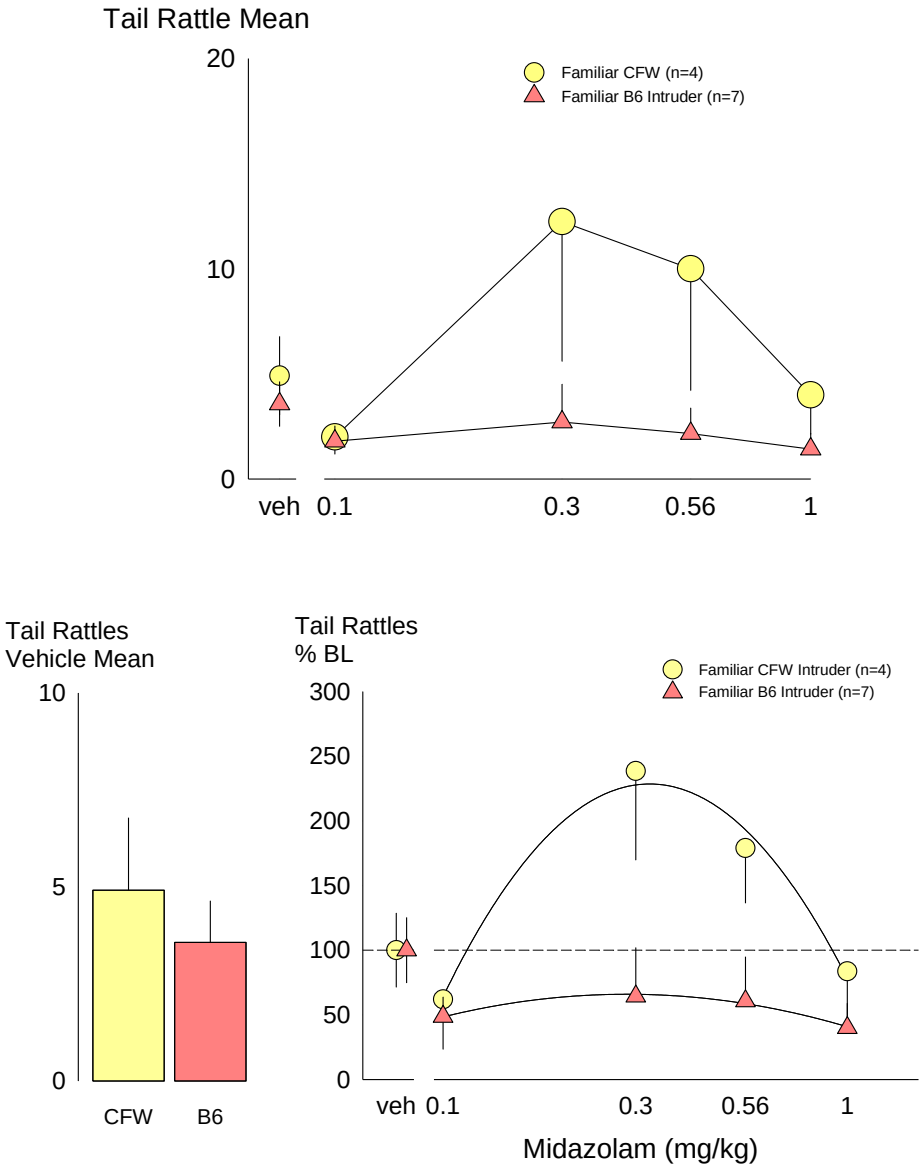


Fig 7. The tail rattle frequency means across doses between the two intruder conditions show less change in the Familiar B6 condition across midazolam dose compared to the Familiar CFW condition. The vehicle means and standard error of the mean (SEM) for the Familiar CFW and the Familiar B6 condition and the percent change from the vehicle baseline across doses suggest that aggression towards familiar CFW intruders is more sensitive to midazolam.

A significant effect of midazolam dose but not intruder condition was seen in sideways threat behavior in the comparison between the Familiar CFW and the Familiar B6 conditions [(F (1, 4) = 3.01, p=0.016); Figure 8], with a trend towards higher levels of sideways threat behavior seen in the Familiar CFW condition at baseline, 0.3, and the 0.56 mg/kg doses.

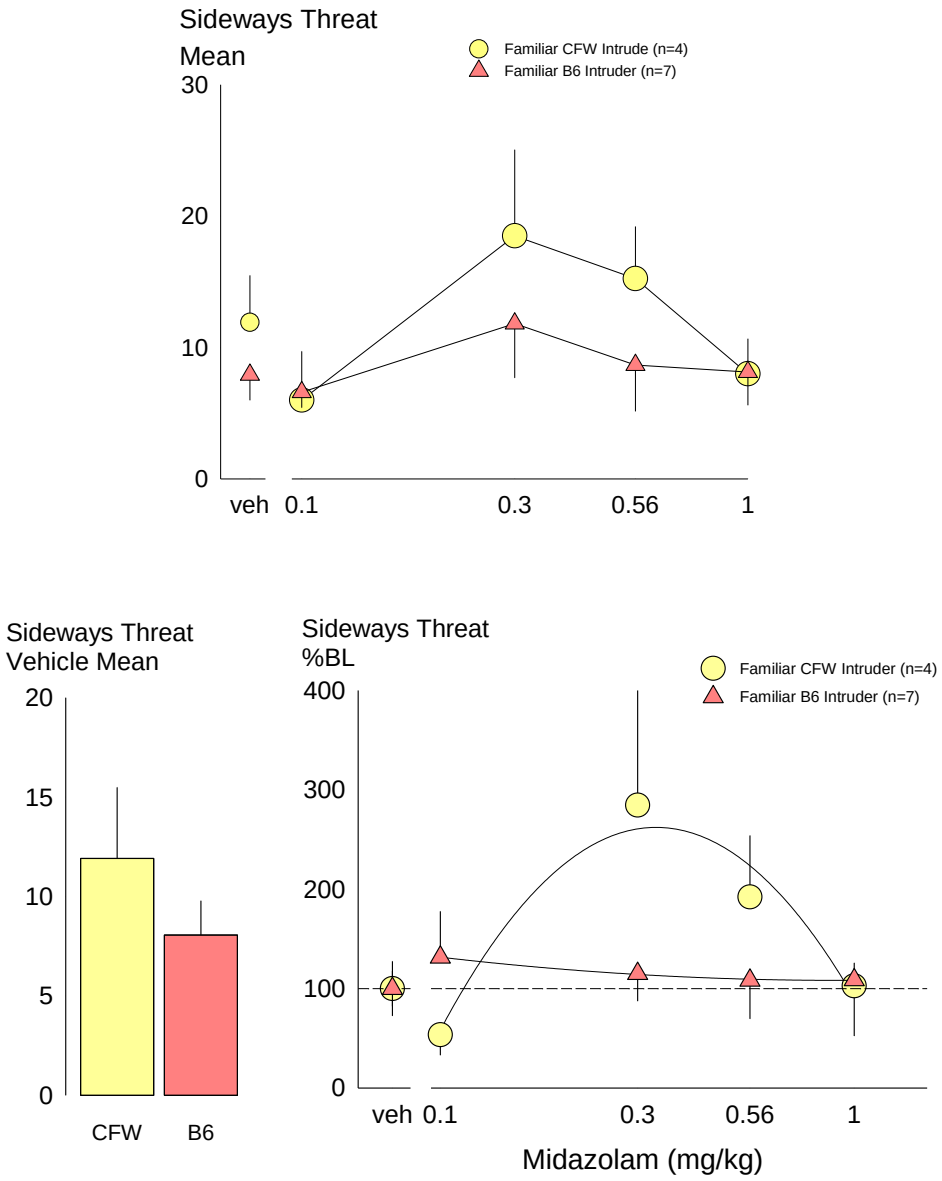


Fig 8. The sideways threat frequency means across doses between the two intruder conditions show less change in the Familiar B6 condition across midazolam dose compared to the Familiar CFW condition. The vehicle means and standard error of the mean (SEM) for the Familiar CFW and the Familiar B6 condition and the percent change from the vehicle baseline across doses suggest that aggression towards familiar CFW intruders is more sensitive to midazolam.

Some non-aggressive behaviors were significantly different across intruder conditions, but were less effected by midazolam dose. Walking duration different significantly across intruder condition [F (1, 1) = 35.036, p<0.001); Figure 9], with levels of the behavior on average four times higher across all doses measured.

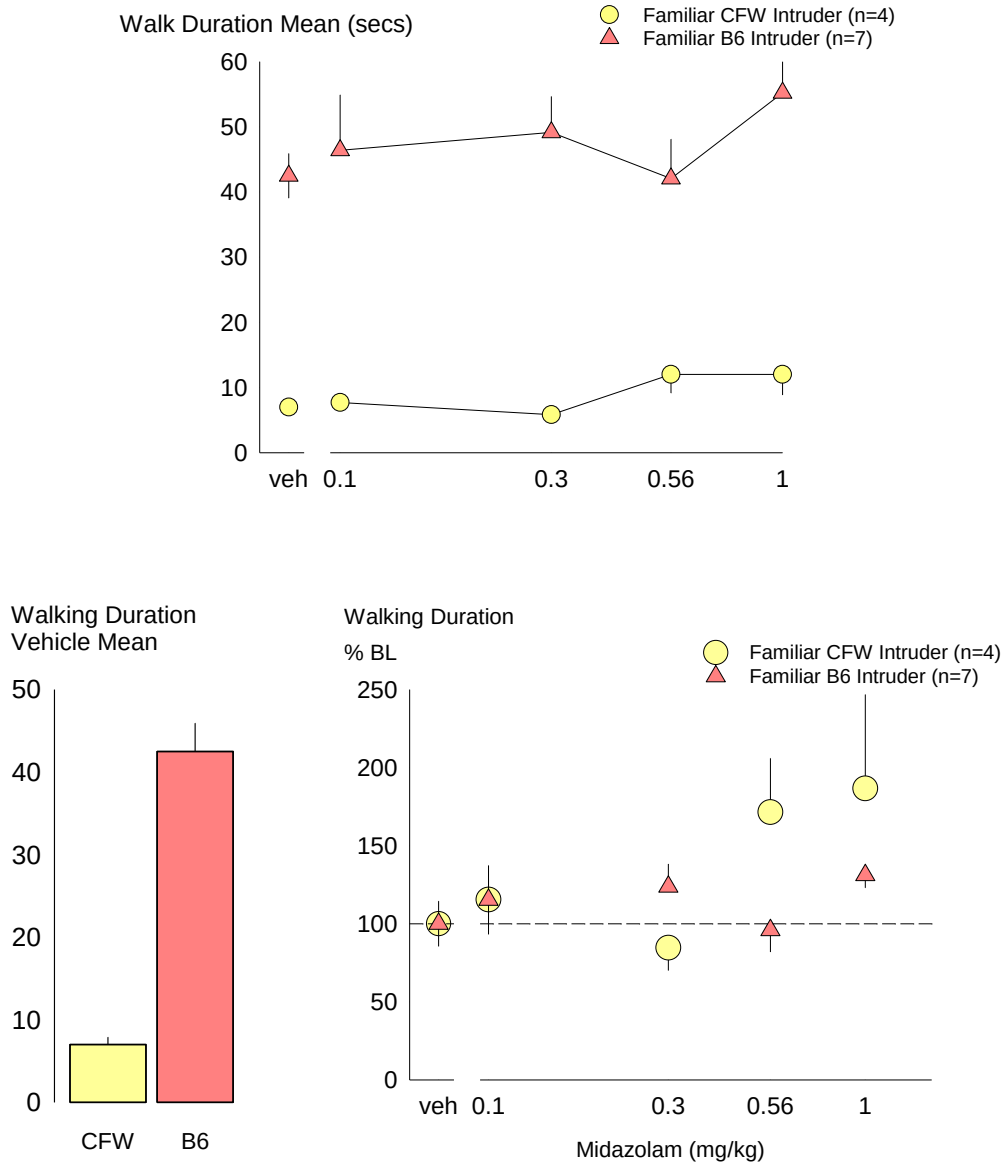


Fig 9. The walking duration means across dose between the two conditions show remarkable difference based on the strain of the intruder. The percent change from vehicle baseline indicate that non-aggressive behaviors may not be as sensitive to the effects of midazolam as aggressive behaviors.

A significant effect of intruder condition was also seen in duration of rearing behavior [F (1, 1) = 9.13, p= 0.014); Figure 10]. Resident females in the Familiar B6 condition reared for longer durations at baseline and 0.1 mg/kg doses compared to other doses measured.

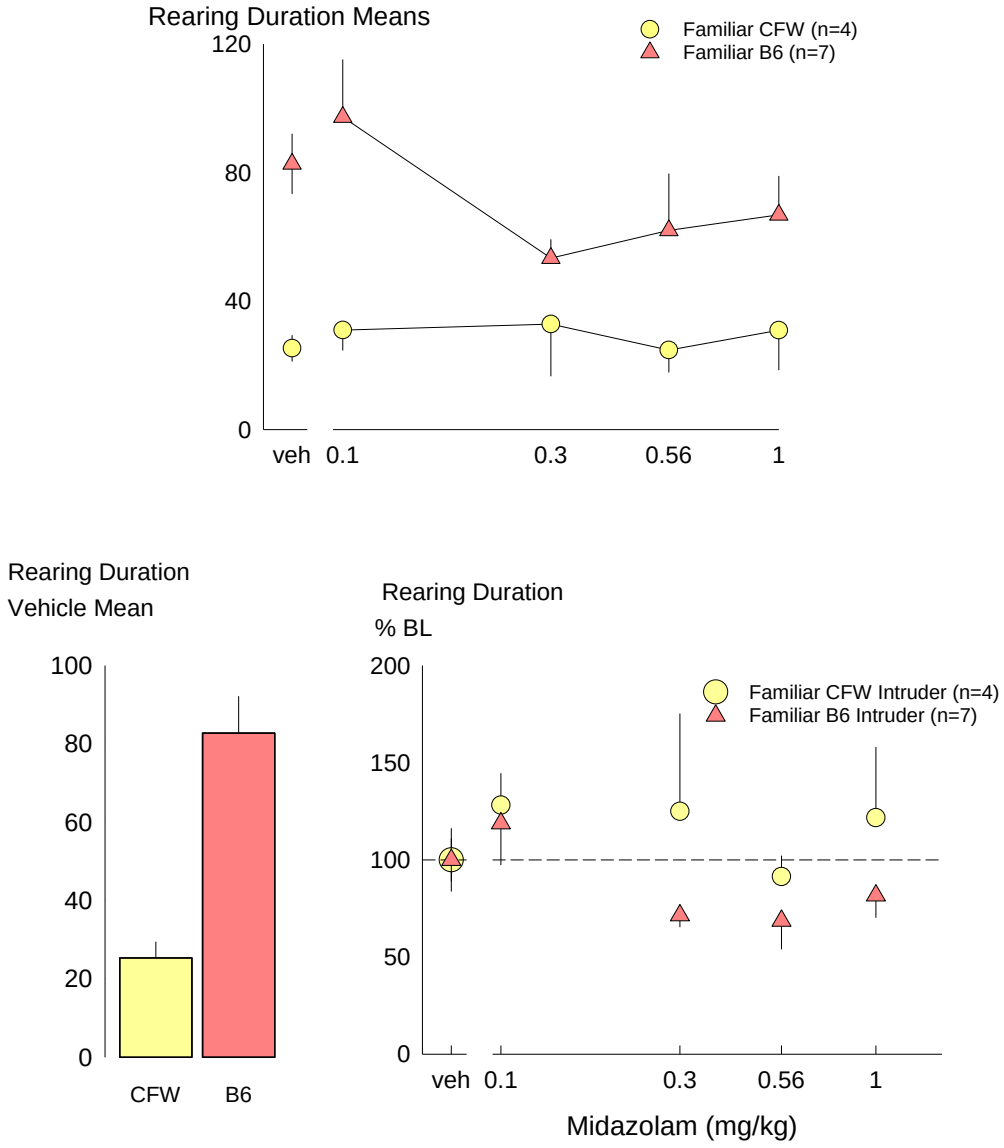


Fig 10. The rearing duration means across dose between the two conditions show a clear difference based on the strain of the intruder. The percent change from vehicle baseline indicate that non-aggressive behaviors may not be as sensitive to the effects of midazolam as aggressive behaviors.

No effect of intruder condition or midazolam dose were seen in the other two non-aggressive behaviors measured: self-groom duration and non-aggressive contact duration.

Experiment 4: There was a near significant effect of intruder condition and no effect of midazolam dose on attack bite frequency in the comparison between the Familiar B6 and the Novel B6 conditions (F (1,1) = 3.885, p=0.067); Table 5, Figure 11), with a trend of resident females in the Novel B6 condition expressing higher attack bite behavior across all midazolam doses.

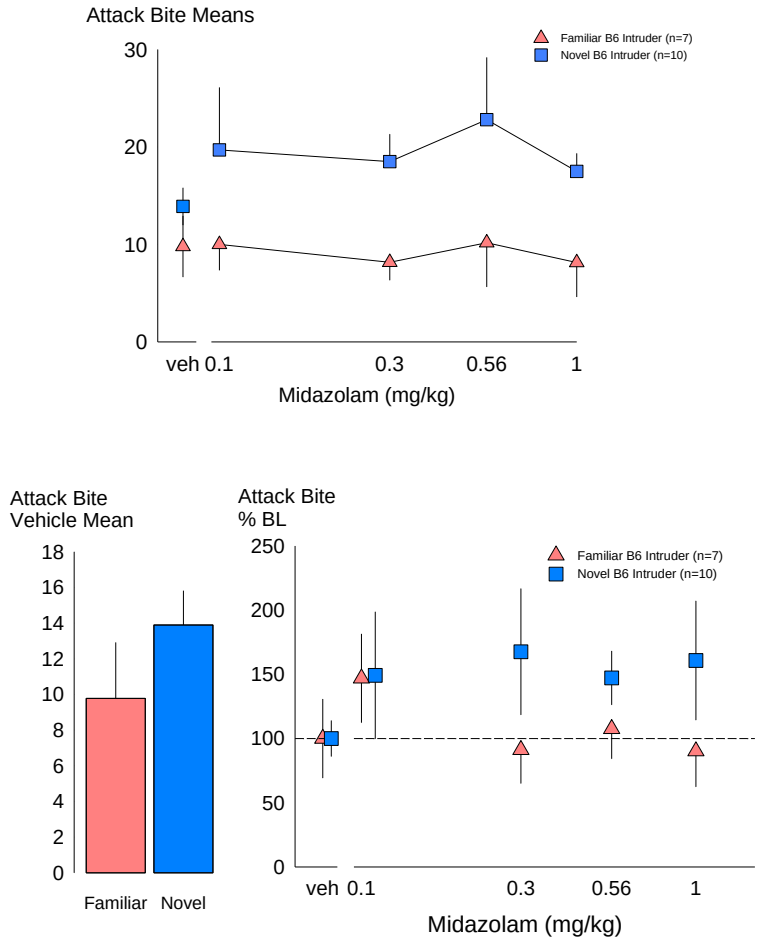


Fig 11. The attack bite frequency means across doses between the two conditions indicate that novelty-induced aggression may be heightened if the strain of the intruder differs from the strain of the resident.

Table 5. Effects of Midazolam on Resident Behavior During Encounters with Novel B6 Intruders

Dose (mg/kg)	V	0.1	0.3	0.56	1.0
Aggressive Behaviors (Frequency)					
Sideways Threat	32.48 ± 4.35	56.00 ± 13.72	49.14 ± 13.49	47.86 ± 8.83	44.83 ± 5.65
Bites	15.92 ± 2.39	25.86 ± 8.10	21.29 ± 3.23	28.0 ± 8.51	19.5 ± 1.89
Tail Rattle	12.40 ± 3.0	21.86 ± 7.01	18.71 ± 8.90	17.29 ± 4.66	14.83 ± 3.64
Non-Aggressive Behaviors (Duration in seconds)					
Self-Groom	11.68 ± 2.19	9.26 ± 1.88	9.75 ± 3.65	8.76 ± 3.64	16.91 ± 2.47
Rear	48.18 ± 7.60	39.16 ± 16.84	27.55 ± 8.35	30.28 ± 8.99	30.69 ± 11.37
Walking	43.87 ± 5.15	25.74 ± 6.84	30.67 ± 6.05	30.0 ± 7.46	33.64 ± 7.40
Non-Aggressive Contact	28.73 ± 4.94	25.01 ± 9.67	22.51 ± 9.37	29.27 ± 7.48	55.90 ± 23.89

Data for each behavior are Mean ± SEM.

Resident females in the Novel B6 condition expressed higher levels of sideways threat and tail rattle behavior across all doses compared to the Familiar B6 condition [F (1, 1) = 27.304, p<0.001); Figure 12, [F (1, 1) = 11.127, p= 0.005; Figure 13].

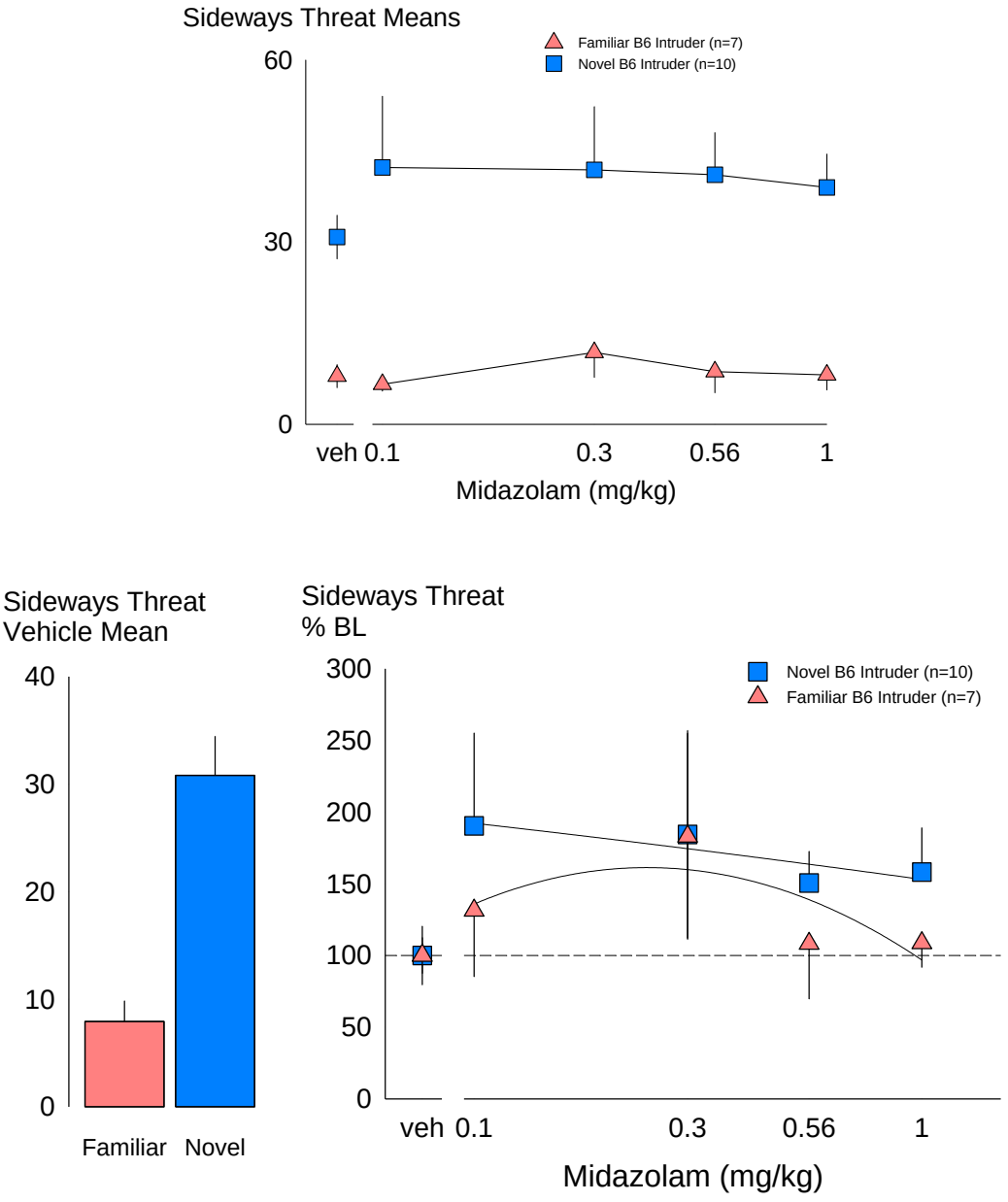


Fig 12. The sideways threat frequency means across dose between the two conditions show a large difference a result of the novelty of the intruder. The percent change from vehicle baseline means across dose indicate that novelty-induced increased aggressive behavior may interfere with a midazolam effect.

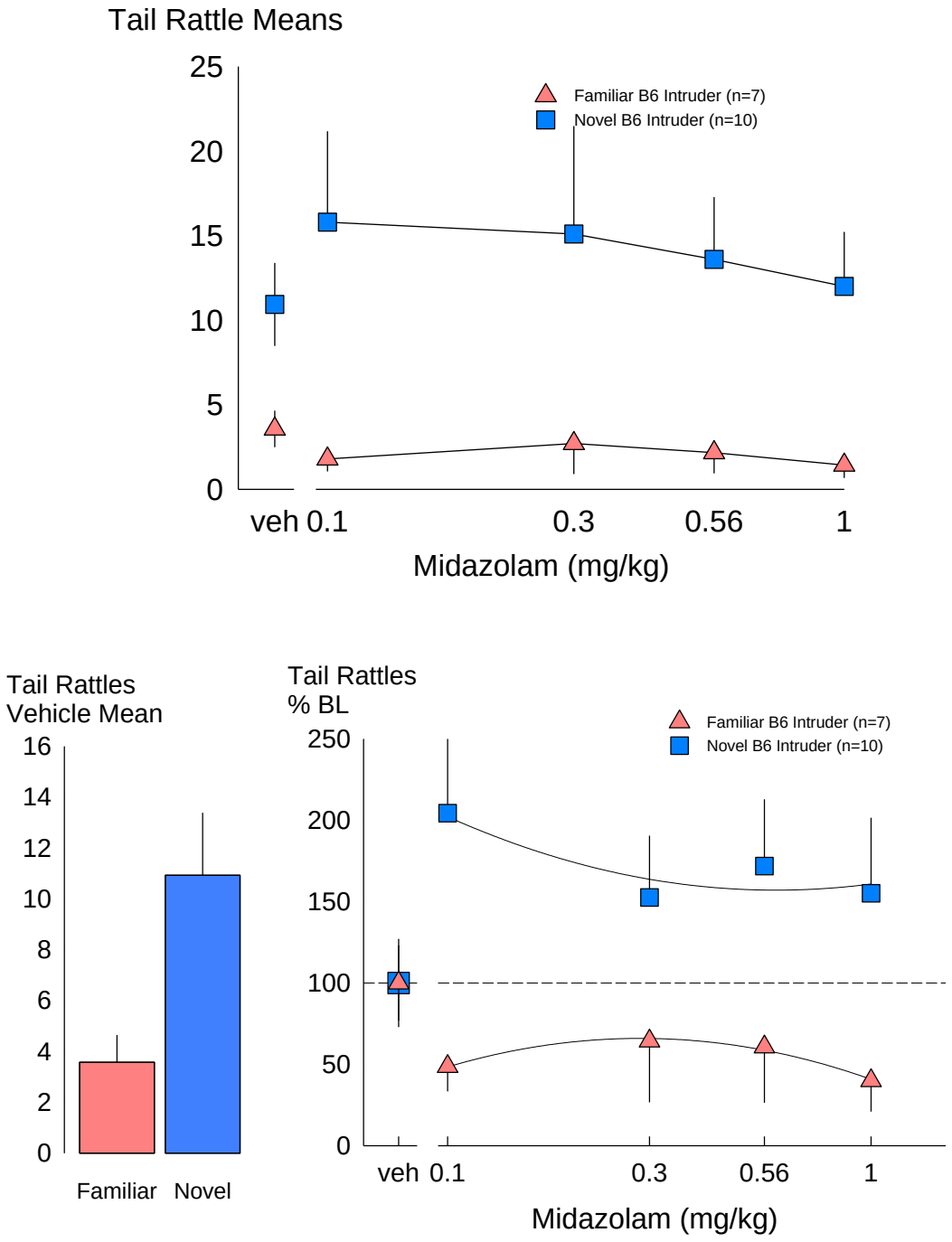


Fig 13. The tail rattle frequency means across dose between the two conditions show a large difference in when compared at each midazolam dose, but a smaller difference at vehicle doses. The percent change from vehicle baseline means across dose indicate that novelty-induced increased aggressive behavior and aggression altered by an intruder strain difference may interfere with a midazolam effect.

Walking behavior showed a significant difference in intruder condition, midazolam dose, and a condition x dose interaction [*F* (1, 1) = 10.124, p=0.006), *F (*1, 4) = 2.775, p=.035), *F* (1, 4) = 2.917, p=.028); Figure 14]. Resident females in the Familiar B6 condition walked more compared to residents in the Novel B6 condition across all midazolam doses, and there was a significant difference between walking behavior at the 0.56 and the 1.0 mg/kg doses within the Novel B6 intruder condition.

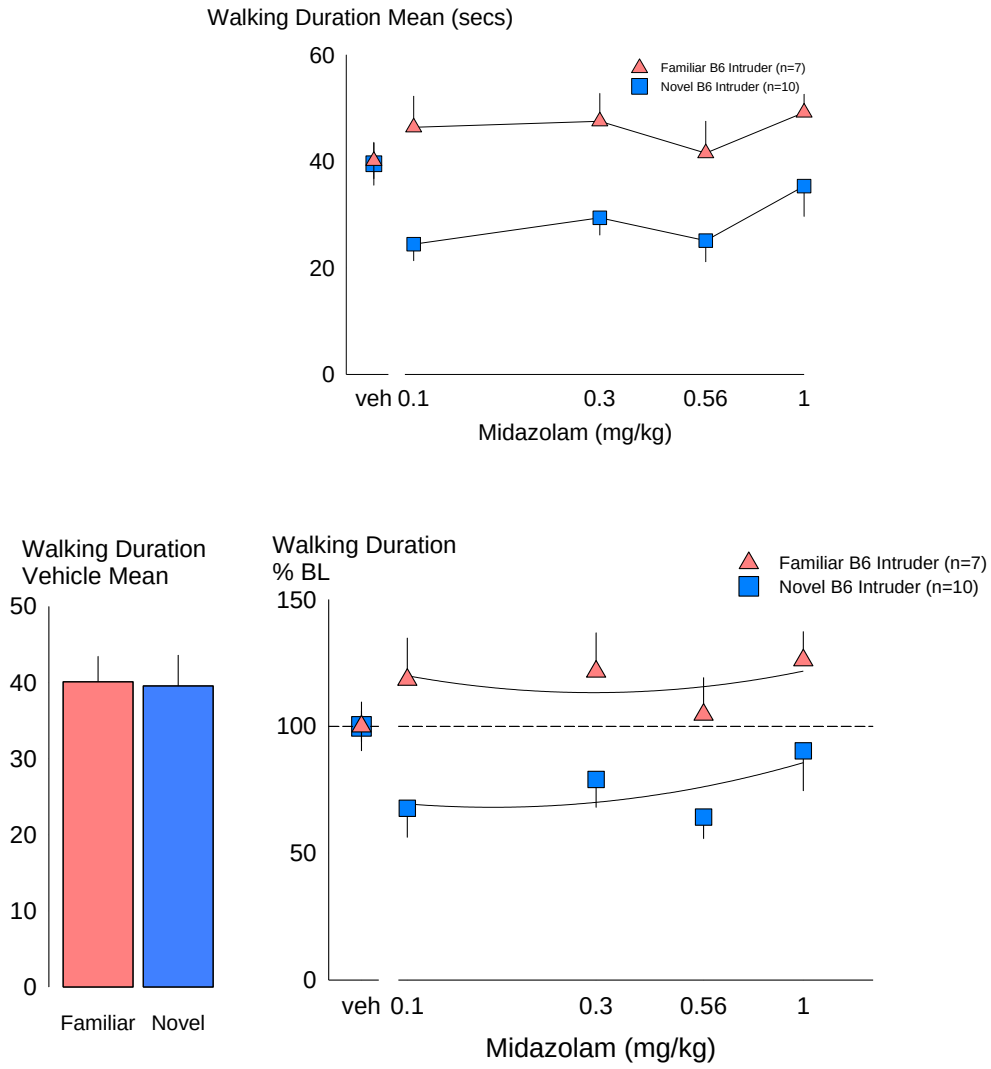


Fig 14. The walking duration means across dose between the two conditions show a dose-dependent and intruder condition difference, indicating that the effect of midazolam and the effect of intruder condition can potentially be individually identified.

Both rearing duration and non-aggressive contact duration were significantly different based on intruder effect and midazolam dose, with residents in the Familiar B6 condition performing these behaviors for a longer duration than the residents in the Novel B6 condition [(Rearing: (F (1, 1) = 5.434, p=0.034, F (1, 4) = 4.69, p=.002); Figure 15); (Non-aggressive contact: (F (1, 1) = 5.402, p = 0.35, F (1, 4) = 3.76, p = 0.009); Figure 16).

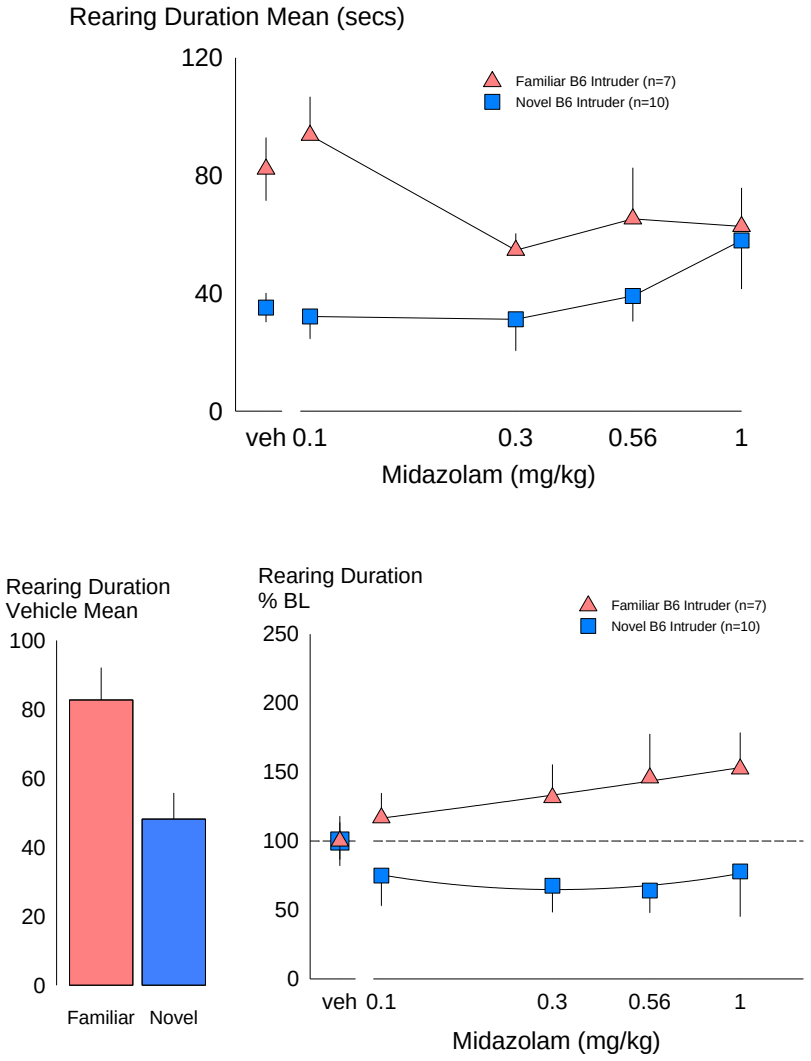


Fig 15. The rearing duration means across dose between the two conditions show a dose-dependent and intruder condition difference, indicating that the effect of midazolam may be different as dose increases in both intruder conditions.

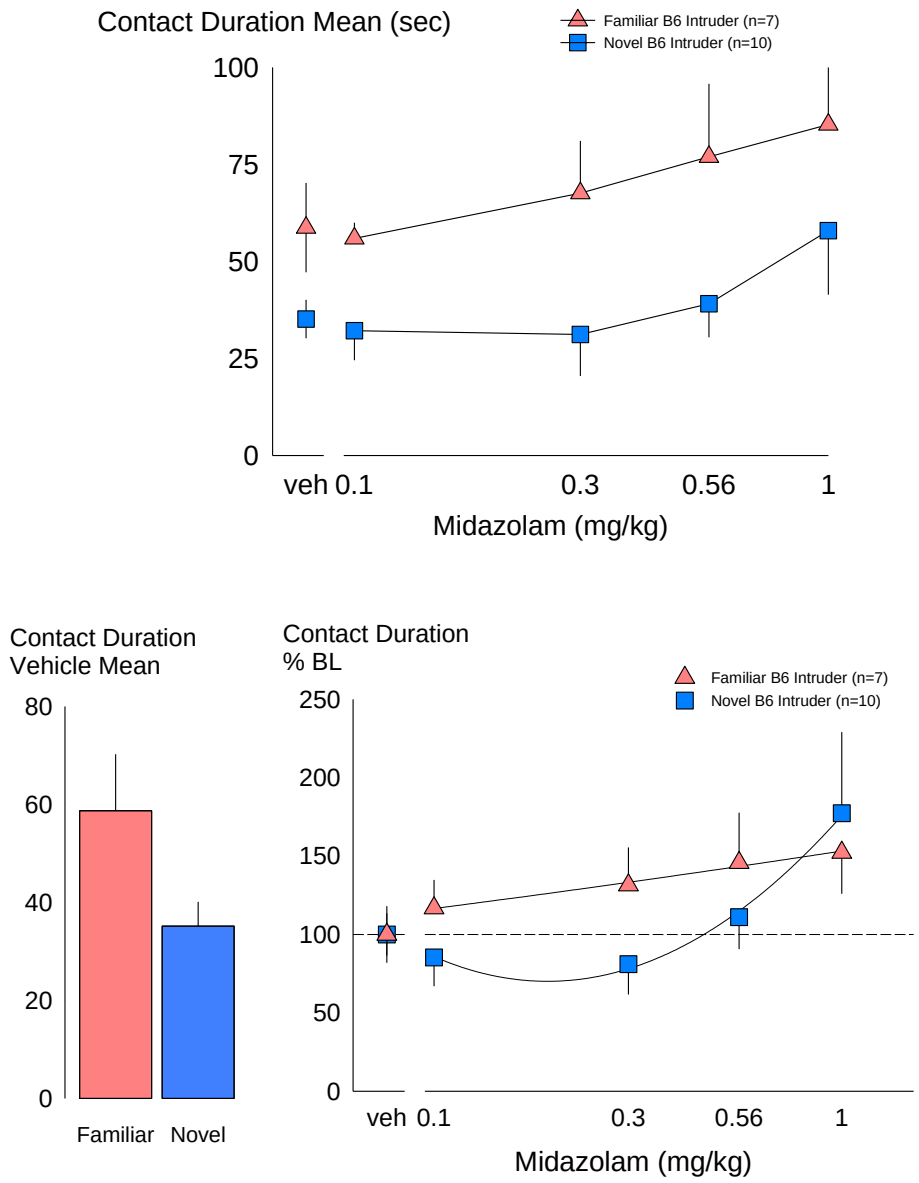


Fig 16. The non-aggressive contact duration means across dose between the two conditions show a dose-dependent and intruder condition difference, indicating that the effect of midazolam may be different as dose increases in both intruder conditions.

There was no significant effect of midazolam dose or intruder condition on self-grooming behavior.

Discussion

This set of experiments demonstrated that a majority of CFW female mice express aggression as residents in a resident-intruder interaction paradigm. This aggression toward a submissive female intruder was sensitive to manipulations including varying the stimulus animal, ethanol, and the benzodiazepine midazolam.

A key feature of this study is that all female residents were ovariectomized as adults that did not have reproductive experience. We assume that all organizational effects of female gonadal hormones took place and all female residents had experienced hormonal cycles prior to any measured aggressive behavior. This also means that none of the aggression seen in this study can be attributed to fluctuations in adult hormonal level. Aggression in female mice is not dependent on endogenous ovarian hormone levels, although it is heavily mediated by them as it is in rats (**DeBold and Miczek, 1984)**. In addition, these experiments illustrate that not all female aggression is related to maternal defense of the young. Females do not need to experience the hormonal changes that accompany pregnancy to express aggressive behavior. However, resident females in this study did receive an exogenous hormone input and the hormonal profile of these residents is not similar to a female mouse in the wild. All resident females were housed with intact males. Pair-housing with an intact opposite sex conspecific has been shown to heighten aggressive behavior in female rats (**Albert et al, 1988)** and male mice (**Scott and Fredericson, 1951)**. This is presumed to be caused by either a reaction to pheromones put off by the intact cohabitant or the gonadal hormone in the urine of the intact cohabitant. It is possible that the expression of aggressive behavior in mice may be activated in some way by sexual behavior. The theory lends itself more to an understanding of why male mice begin to fight, as the changes in gonadal hormone concentration after sexual behavior in males are more pronounced than in

females. The nature of the housing conditions in these experiments do not provide evidence for the argument that aggression in female mice is hormone-independent. Female mice outside of the laboratory typically are in a state of hormonal flux due to natural cycling or at high consistently high levels of female gonadal hormone if lactating, pregnant, or possibly taking hormonal contraceptives. A study that observed the aggressive behavior of residents with cycling hormone levels or biologically relevant elevated hormone levels would be more translatable compared to this.

At no point in this experiment did the addition of a pharmacological agent elicit spontaneous aggression. This speaks to the idea that aggression is not inducible via drug action, it can only be modulated by it. However, changing the nature of the stimulus quality via manipulating the intruder condition altered the proportion of animals that were aggressive per condition and the magnitude of the aggression they expressed. These observations bring up a number of questions about the purpose of social aggression in mice. It is likely that female nonmaternal aggression in mice is performed for the same reasons that male mice express aggression: defending territory and asserting dominance. Male mice exhibit more aggressive behavior than female mice in the resident-intruder paradigm. This serves as a reflection of the importance of maintaining both territory and dominance for the male mouse. The ability to drive away individuals of the same sex is fundamental, regardless of their strain or level of familiarity. Therefore, it should be expected that male mice express high levels of aggression towards an intruder of any kind. Female mice, however, must be able to differentiate between targets of territorial aggression and targets of dominance aggression. The typical harem breeding strategy seen in mice lends itself to the idea that prolonged aggressive behavior towards familiar conspecifics in females is unnecessary. Aggressive behavior in the rat shows us that dominance

can be established with only a few displays of aggressive behavior and that a dominant rat will not continue to express aggression towards a same-sex conspecific if it displays submissive behavior (**Dijkstra et al, 1984)**. This is likely due to the natural living conditions of rats. Rats form social groups with dominant and submissive males and females (**Blanchard et al, 1984).** Mouse living conditions are similar to these with respect to female; a female mouse is likely to be living with other female mice whereas males disperse potential rival males. It makes sense then to assume that any aggression between two female mice of the same strain would be shorter in nature and involve less injurious behavior on the part of the aggressor. The low levels of individual attack bites compared to overall aggressive behaviors of female residents towards familiar CFW intruders could be explained through this logic.

The increased proportion of resident females that displayed aggressive behavior towards both familiar and novel C57BL6/J intruders compared to CFW intruders may indicate that familiarity is a key factor in understanding why female mice fight. This again brings up the concept of a fundamental difference between territorial and dominance that female mice are more sensitive to. The presentation of a submissive intruder of a mouse strain different from the resident results in the resident performing aggressive acts to indicate that the intruder is not welcome. This aggressive behavior must be of a greater magnitude than dominance aggression because its purpose is dispersal. If the intruder does not leave, the resident has failed to effectively protect her territory and must continue to be aggressive.

Novelty-induced aggression is a phenomenon seen in male mice. Male mice will eventually show stable levels of aggression towards a specific intruder after multiple aggressive interactions. Aggression levels will increase dramatically upon the introduction of a novel intruder and return to stable levels through repeated aggressive sessions. In addition, introduction

of a novel intruder for subsequent interactions will maintain the heightened levels of aggressive behavior in male mice. This was shown to be present in female mice in this study. However, this was demonstrated using B6 intruders. A future direction of this research would be to explore novelty-induced aggression using CFW intruders.

The highest levels of aggression seen in this study were in the Novel B6 intruder condition. This is understood to be territorial aggression, greater than that seen in dominance aggression, potentiated by the novelty of the intruders presented. When comparing the Familiar B6 and the Novel B6 groups, there was a significant effect of intruder condition in all but one of the behaviors measured, and in that behavior, there was a trend towards a difference. More aggressive behaviors were expressed by residents in the Novel B6 group compared to Familiar B6 and lower levels of non-aggressive behaviors were expressed. The Familiar B6 and Novel B6 groups saw no significant effects of midazolam dose on attack bite and sideways threat frequency, behaviors that are most associated with aggression. There was a significant effect on tail rattling behavior, but this behavior is correlated with physical arousal in addition to aggressive behavior. This may mean that the novelty-induced aggression seen in the Novel B6 group interfered with a midazolam effect. Residents in the Familiar B6 group spent more time walking and rearing than residents in the Novel B6 group. This could indicate that intruders that are more familiar to an intruder are less stimulating. Familiar B6 residents also spent more time participating in non-aggressive contact with an intruder. This suggests that the residents require less contact to determine if an intruder is an appropriate target for aggressive behavior.

When comparing the Familiar B6 and the Familiar CFW groups, walking duration and rearing differed significantly between them, with residents in the Familiar B6 condition walking more and rearing more. This could suggest a significant portion of the strain difference seen in

this study is related to the difference in behavior between the strains of intruders. CFW mice on average are larger and move less than B6 mice. When encountering an intruder that moves more, residents in the B6 conditions may have needed to increase their own walking behavior to maintain contact. The fact that walking duration of residents in the Novel B6 condition falls between those of Familiar B6 and Familiar CFW walking durations may suggest that the novelty of the intruder influences non-aggressive behaviors as well. It could be understood that residents encountering novel intruders of a more active strain need to move more but are also on average performing more aggressive behavior, attenuating durations of non-aggressive behavior. The lack of significant difference of intruder condition in attack bite and sideways threat behavior could indicate that there is no difference in the types of aggression being expressed towards familiar CFW and B6 intruders. However, there is a significant interaction between midazolam dose and intruder condition with respect to tail rattle behavior at 0.3 and 0.56 mg/kg. Tail rattle behavior is lower in the Familiar B6 group compared to the Familiar CFW group. These doses have been previously shown to increase aggressive behavior above baseline in male CFW residents encountering CFW intruders (**Fish et al 2005).** In context of the Familiar B6 group, it may mean that tail rattling is functioning as a non-injurious cue presented by the resident for the intruder to receive. If the goal of the aggression is to make an intruder disperse, less tail rattling could mean that the resident will rely on more aversive forms of aggressive behavior like attack bites and sideways threats to relay the message. Familiar B6 intruders also received the most non-aggressive contact than any other group; this was expressed as a difference in percent of change from vehicle baseline of contact duration. This could be interpreted as a resident requiring more time in contact with an intruder of a different strain to establish a social connection compared to the time needed for a conspecific. The Familiar B6 and Familiar CFW

groups saw a midazolam dose effect at all aggressive behaviors, providing further evidence for the idea that novelty-induced aggression interfered with the midazolam effect in the Novel B6 group. There was no effect of midazolam in these groups at any of the non-aggressive behaviors, indicating that a subclinical dose of midazolam in female mice can increase aggressive behavior similar to male mice.

Further refinement of the experiments conducted and more analysis will be necessary to come to a more definitive conclusion on the nature of female aggression in mice. A potential source of variation in this study is the age at which mice were first tested for aggressive behavior. Animals that were tested with midazolam in the Familiar CFW condition were first trained to self-administer ethanol via operant conditioning panel. The animals tested in the Familiar B6 and Novel B6 conditions were not. This training procedure takes on average 3-4 weeks to complete. The Familiar CFW condition was the only one preceded by ethanol self-administration, further adding to the potential sources of variability in the data collected.

Characterization of female residents as alcohol-heightened aggressors or alcohol non-heightened aggressors proved difficult due to the effect of intruder familiarity on repeated tests of aggression post water and ethanol self-administration. A potential future direction of this study would be to characterize residents as AHA or ANA using novel intruders for each characterization session. There is also a potential issue with the dose of ethanol used for characterization. The 1.0 g/kg does of ethanol was used because it has been shown that a subpopulation of male mice self-administering this dose will best express heightened levels of aggression in a resident intruder interaction 10 minutes following the administration (**Miczek et al 1998).** It is unknown if the 1.0 g/kg dose is optimal for female self-administration and characterization. As mentioned earlier, a sex difference in blood alcohol concentration from the

same dose of alcohol has been observed in both humans and mice. A dose-effect curve using alcohol-heightened aggressive female mice in a resident-intruder interaction paradigm would be able to answer this question.

An additional shortcoming of this study is the lack of comparability to similar experiments conducted in male mice. Resident-intruder interaction procedures conducted with male mice are most similar to the Familiar CFW condition in this study. Novel intruders are not used because it leads to a novelty-induced aggression discussed earlier with a potential ceiling effect in the levels of aggressive behaviors expressed by male residents. The two B6 conditions (Familiar and Novel) have one and two additional manipulations respectively when compared to traditional male resident-intruder interaction procedures. An important note to mention was that a ceiling effect was not seen in female aggression when using novel B6 intruders. The use of the C57BL/6J mouse intruder in this study subtracts from the translatability of the levels of aggressive behavior measured. Aggression towards an intruder of the same strain more closely resembles a human aggressive encounter than aggression towards a different strain of the same species.

Statistical analysis of the data collected in this study proved difficult due to the number of animals in each of the conditions. More subjects characterized as alcohol-heightened aggressors and non-heightened aggressors using both CFW and B6 intruders will be necessary to report statistical differences in the nature of this aggression. This would also allow for more powerful analysis of bite location. Further, additional analysis of lag sequence is required develop a prototypical or average sequence of behaviors leading up to and immediately following an attack bite delivered to a female intruder by a female resident. The nature of this study was that of optimizing a procedure for a population of animals whose aggressive behavior was relatively

unknown. A total of 58 mice were used in this study and about 40% of them were not consistently aggressive enough to test systematically. This led to lower than desirable numbers of animals in each condition. This became more problematic as it led to high levels of variability and in the data collected and led to issues with data meeting the standards for normality and equal variance required for statistical analysis.

Despite these shortcomings, this study showed a significant effect of strain of intruder on aggressive behavior in CFW female mice pair-housed with an intact male, providing further evidence for the idea that stimulus quality plays a fundamental role in determining why mice fight. The subtle differences in the conditions used in this study suggest that the effect of intruder strain difference and novelty-induced aggression mediate aggressive and non-aggressive behavior in different and distinguishable ways. In addition, this set of experiments explored trends of increased aggressive behavior under the influence of acute ethanol self-administration and systemically injected benzodiazepines. This confirms the idea that intermale and interfemale aggression in mice and mediated by similar mechanisms, but the levels of sensitivity of the aggressive behaviors between sexes differ.

Works Cited

Albert, D.J, Dyson, E.M., Petrovic, D.M., & Walsh, M.L. (1988). Activation of Aggression in Female Rats by Normal Males and by Castrated Males with Testosterone Implants. *Physiology & Behavior, 44,* pp. 9-13.

Albert, DJ., Jonik, RH., & Walsh, ML. (1991). Hormone-dependent aggression in the female rat: testosterone plus estradiol implants prevent the decline in aggression following ovariectomy. *Physiology & Behavior, 49,* (4), pp. 673-677.

Albert, DJ., Walsh, ML. (1995). Aggression is attenuated by ovariectomy in pregnant female rats given progesterone and estradiol replacement to maintain pregnancy. *Physiology & Behavior, 57,* (6), pp. 1035-1038.

Arthur, M.J.P., Lee, A., & Wright, R. (1984). Sex differences in the metabolism of ethanol and acetaldehyde in normal subjects. *Clinical Science, 67,* (4), pp. 397-401.

Bailey, D.S. & Taylor, S.P. (1991). Effects of alcohol and aggressive disposition on human physical aggression. *Journal of Research in Personality, 25,* (3), pp. 334-342.

Baraona, E., et al. (2001). Gender difference in pharmacokinetics of alcohol. *Alcoholism: Clinical and Experimental Research, 25,* (4). pp. 502-507.

Barkley, MS., & Goldman, BD. (1977). Testosterone-induced aggression in adult female mice. *Hormones and Behavior, 9,* pp. 76-84.

Beeman, EA. (1947). The effect of male hormone on aggressive behavior in mice. *Physiological Zoology, 20,* (4), pp. 373-405.

Belozertseva, I.V. & Bespalov, A.Y. (1999). Effects of NMDA receptor channel blockade on aggression in isolated male mice. *Aggressive Behavior, 25,* (5), pp.381-396.

Ben-Porath, D.D. & Taylor, S.P. (2002). The effects of diazepam and aggressive disposition on human aggression: an experimental investigation. *Addictive Behaviors, 27,* pp. 167-177.

Blanchard, D.C., Fukunagastinson, C., Takahashi, L.K., Flannelly, K.J., & Blanchard, R.J. (1984). Dominance and aggression in social groups of male and female rats. *Behavioral Processes, 9,* pp. 31-48.

Blanchard, R.J. (1988). Ethanol and aggression. *Aggressive Behavior, 14,* (2). Pp. 145-145.

Bond, A. & Lader, M. (1988). Differential effects of oxazepam and lorazepam on aggressive responding. *Psychopharmacology, 95,* pp. 369-373.

Brain, P.F., Benton, D., Childs, G., & Parmigiani, S. (1981). The effect of the type of opponent in tests of murine aggression.

Bramness, J.G., Skurtveit, S., & Mørland, J. (2006). Flunitrazepam: psychomotor impairment, agitation, and paradoxical reactions. *Forensic Science International, 159,* (2-3), pp. 83-91.

Broida, J. & Svare, B. (1982). Postpartum aggression in C57BL/6J and DBA/2J mice: experiential and environmental influences. *Behavioral and Neural Biology, 35,* (1), pp. 76-83.

Bronson, FH., & Desjardins, C. (1968). Aggression in adult mice: modification by neonatal injections of gonadal hormone. *Science, 161,* 705-706.

Cherek, D.R. (1983). Evaluating the effects of commonly used drugs on human aggressive behavior. *Aggressive Behavior, 9,* (2), pp. 116-117.

Cherek, D.R., Steinberg, J.L., & Manno, B.R. (1985). Effects of alcohol on human aggressive behavior. *Journal of Studies on Alcohol, 46,* (4), pp. 321-328.

Cherek, DR., Spiga, R., & Egli, M. (1992). Effects of response requirement and alcohol on human aggressive responding. *Journal of the Experimental Analysis of Behavior, 58,* (3), pp. 577-587.

Cherek, DR., Spiga, R., Steinberg, JL., & Kelly, TH. (1990). Human aggressive responses maintained by avoidance or escape from point loss. *Journal of the Experimental Analysis of Behavior, 53,* (2), pp. 293-303.

Chesney-Lind, M., & Pasko, L. (2013). *The Female Offender: Girls, Women, and Crime.* SAGE: Los Angeles.

Clayton, J.A. & Collins, F.C. (2014, May 14). Policy: NIH to balance sex in cell and animal studies. Retrieved from http://www.nature.com/news/policy-nih-to-balance-sex-in-cell-and-animal-studies-1.15195

Crawley, JN., Schleidt, WM., & Contrera, JF. (1975). Does social environment decrease propensity to fight in male mice. *Behavioral Biology, 15,* (1), pp. 73-83.

Crestani, F., Martini, J.R., Mohler, H., & Rudolph, U. (2000). Resolving differences in GABA-A receptor mutant mouse studies. *Nature Neuroscience, 3,* (11), pp. 1059-1059.

DeBold, JF., & Miczek, KA. (1984). Aggression persists after ovariectomy in female rats. *Hormones and Behavior, 18,* pp. 177-190.

Dijkstra, H., Olivier, B., & Mos, J. (1984). Dominance maintenance and intruder attack in laboratory colonies of rats: different models for the psychopharmacological control of aggression. *Aggressive Behavior, 10,* (2), pp. 149-149.

Dougherty, DM., Bjork, JM., Bennett, RH., & Moeller, FG. (1999). The effects of a cumulative alcohol dosing procedure on laboratory aggression in women and men. *Journal of Studies on Alcohol and Drugs, 60,* pp. 322-329.

Dougherty, DM., Cherek, DR., Bennett, RH. (1996). The effects of alcohol on the aggressive responding of women. *Journal of Studies on Alcohol, 57,* (2), pp. 178-186.

Edwards, DA. (1970). Post-neonatal androgenization and adult aggressive behavior in female mice. *Physiology and Behavior, 5,* pp. 465-467.

Erikkson, C.J.P. (1973). Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. *Biochemical Pharmacology, 22,* (18), pp. 2283-2292.

Fagan, J. (1993). Interaction among drugs, alcohol, and violence. *Health Affairs, 12,* (4), pp. 65-79.

Ferrari, P.F., Palanza, P., Rodgers, R.J., Mainardi, M., & Parmigiani, S. (1996). Comparing different forms of male and female aggression in wild and laboratory mice: an ethopharmcological study. *Physiology & Behavior, 60,* (2), pp. 549-553.

Fish EW, Faccidomo S, Miczek KA (1999). Aggression heightened by alcohol or social instigation in mice: reduction by the 5- HT1B receptor agonist CP-94,253. *Psychopharmacology* 146: 391–399

Fish, E.W., DeBold, J.F., & Miczek, K.A. (2005). Escalated aggression as a reward: corticosterone and GABA-A receptor positive modulators in mice. *Psychopharmacology, 182,* pp. 116-127.

Flannelly, KJ. & Flannelly, L. (1987). Time course of postpartum aggression in rats (*Ratus norvegicus). Journal of Comparative Psychology, 101,* (1), pp. 101-103.

Fredericson, E. (1950). The Effects of Food Deprivation Upon Competitive and Spontaneous Combat in C57 Black Mice. *The Journal of Psychology, 29* (1), pp. 89-100.

Fredericson, E. (1952). Aggression in female mice. *Journal of Comparative and Physiological Psychology, 45,* (3), pp. 254-257.

Fredericson, E., Story, AW., Gurney, NL., & Butterworth, K. (1955). The relationship between heredity, sex, and aggression in two inbred mouse strains. *Journal of Genetic Psychology, 87,* pp. 121-130.

Gammie, SC., & Stevenson, SA. (2006). Effects of daily and acute restraint stress during lactation on maternal aggression and behavior in mice. *Stress: The International Journal on the Biology of Stress, 9,* (3), pp. 171-180.

Gardos, G., DiMascio, A., Salzman, C., & Shader, R.I. (1968). Differential actions of chlordiazepoxide and oxazepam on hostility. *Archives of General Psychiatry, 18,* (6), pp.757-760.

Giancola PR. & Zeichner, A. (1995). An investigation of gender differences in alcohol-related aggression. *Journal of Studies on Alcohol, 56,* (5), pp. 573-579.

Giancola, PR & Zeichner, A. (1997). The biphasic effects of alcohol on human physical aggression. *Journal of Abnormal Psychology, 106,* (4), pp. 598-607.

Giancola, PR., Levinson, CA., Corman, MD., Godlaski, AJ., Morris, DH, Phillips, JP., & Holt, JCD. (2009). Men and Women, Alcohol and Aggression. *Experimental and Clinical Psychopharmacology, 17,* (3), pp. 154-164.

Graves, K. (2004). Not always sugar and spice: expanding theoretical and functional explanations for why females aggress. *Aggression and Violent Behavior, 12,* pp. 131-140.

Greenfeld, LA. & Snell, TL. (2000) Women offenders. *Bureau of Justice Statistics – U.S. Department of Justice.*

Grimm, V.E., Haltmeier, H., Shuib, Z.B., & Brain, P.F. (1985). Lasting Effects of Neonatal Treatments with Testosterone, Estradiol, or 5-(alpha) DHT on the Abilities of Steroid Implants to Induce Social Aggression in Ovariectomized Mice. *Aggressive Behavior, 11,* (2), pp. 164-165.

Grobin, A.C., Matthews, D.B., Devaud, L.L., & Morrow, A.L. (1998). The role of GABA-A receptors in the acute and chronic effects of alcohol.

Hanchar, H.J., Wallner, M., & Olsen, R.W. (2004). Alcohol effects on gamma-aminobutyric acid type A receptors: are extrasynaptic receptors the answer? *Life Sciences, 76,* (1), pp. 1-8.

Haug, M. & Mandel, P. (1978). Strain differences in aggressive behavior of female mice against lactating and non-lactating individuals. *Neuroscience Letters, 7,* pp. 235-238.

Howard, SM., Gandelman, R., & Rosenthal C. (1981). Isolation potentiates the aggression-activating property of testosterone in female mice. *Physiology & Behavior, 26,* pp. 971-972.

Hwa, L.S., Chu, A., Levinson, S.A., Kayyali, T.M., DeBold, J.F., & Miczek, K.A. (2011). Persistent escalation of alcohol drinking in C57BL/6J mice with intermittent access to 20% ethanol. *Alcoholism: Clinical and Experimental Research, 35,* (11), pp. 1938-1947.

Jones, B.M., & Jones, M.K. (1981). Ethanol absorption, elimination and peak blood-alcohol levels in male and female social drinkers as a function of age. *Alcoholism: Clinical and Experimental Research, 5,* (1), pp. 156-156.

Krsiak, M. & Borgesova, M. (1973). Effect of alcohol on behavior of pairs of rats. *Psychopharmacologia, 32,* pp. 201-209.

Krystal, J.H., et al. (1998). Dose-related ethanol-like effects of the NMDA antagonist, ketamine, in recently detoxified alcoholics. *Archives of General Psychiatry, 55,* (4), pp. 354-360.

Lima-Landman, M.T.R. & Albuquerque, E.X. (1989). Ethanol potentiates and blocks NMDA-activated single-channel currents in rat hippocampal pyramidal cells. *FEBS Letters, 247,* pp. 61-67.

Lister, R.G. & Linnolia, M. (1991). Alcohol, the chloride ionophore and endogenous ligands for benzodiazepine receptors. *Neuropharmacology, 30,* (12B), pp. 1435-1440.

Lonstein, JS., & Gammie, SC. (2002). Sensory, hormonal, and neural control of maternal aggression in laboratory rodents. *Neuroscience and Biobehavioral Reviews, 26,* pp. 869-888.

Lovinger, D.M., White, G., & Weight, F.F. (1989). Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science, 243,* pp. 1721-1724.

Maldonado-Molina, M.M, Jennings, W.G., & Komro, K.A. (2010). Effects of alcohol on trajectories of physical aggression among urban youth: an application of latent trajectory modeling. *Journal of Youth and Adolescence, 39,* (9), pp. 1012-1026.

Marshall, A.W., Kingstone, D., Boss, M., & Morgan M.Y. (1983). Ethanol elimination in males and females: relationship to menstrual cycle and body composition. *Hepatology, 3,* (5), pp. 701-706.

Masood, K., Wu, C., Brauneis, U., & Weight, F.F. (1993). Differential ethanol sensitivity of recombinant *N*-methyl-D-aspartate receptor subunits. *Molecular Pharmacology, 45,* pp. 324-329.

McKernan, R.M., et al. (2000). Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA-A receptor alpha (1) subtype. *Nature Neuroscience, 3,* (6), pp. 587-592.

Meta, A.K., & Ticku, M.K. (1988). Ethanol potentiation of GABAergic transmission in cultured spinal cord neurons involves γ-Aminobutyric $Acid_A$-gated chloride channels. *The Journal of Pharmacology and Experimental Therapeutics, 246,* (2), pp. 558-564.

Miczek, K.A. & O'Donnell, J.M. (1980). Alcohol and chlordiazepoxide increased suppressed aggression in mice. *Psychopharmacology, 69,* (1), pp. 39-44.

Miczek, K.A. (1974). Intraspecies aggression in rats: effects of d-amphetamine and chlordiazepoxide. *Psychopharmacologia, 39,* (4), pp. 275-301.

Miczek, K.A., Weerts, E., Haney, M., & Tidey, J. (1994). Neurobiological mechanisms controlling aggression: preclinical developments of pharmacotherapeutic interventions. *Neuroscience and Biobehavioral Reviews, 18,* (1), pp. 97-110.

Miczek, KA., Barros, HM., Sakoda, L., & Weerts, EM. (1998). Alcohol and heightened aggression in individual mice. *Alcoholism: Clinical and Experimental Research, 22,* (8), pp. 1698-1705.

Middaugh, L.D., Kelley, B.M., Bandy, A.E., & McGroarty, K.K (1999). Ethanol consumption by C57BL/6J mice: influence of gender and procedural variables. *Alcohol, 17,* (3), pp. 175-183.

Mos, J., Olivier, B., & Vanderpoel, A.M. (1987). Modulatory actions of benzodiazepine receptor ligands on agonistic behavior. *Physiology & Behavior, 41,* (3), pp. 265-278.

Mos, J., Olivier, B., and van Oorschot, R. (1987). Maternal aggression towards different sized male opponents: effect of chlordiazepoxide treatment of the mothers and d-amphetamine treatment of the intruders, *Pharmacology Biochemistry & Behavior, 26,* pp. 577-584.

Mugford, RA. & Nowell, NW. (1972). The dose-response to testosterone propionate of preputial glands, pheromones and aggression in mice. *Hormones and Behavior, 3,* (1) pp. 39-46.

Newman, E.L., Chu, A., Bahamón, B., Takahashi, A., DeBold, J.F., & Miczek, K.A. (2012). NMDA receptor antagonism: escalation of aggressive behavior in alcohol-drinking mice. *Psychopharmacology, 224,* pp. 167-177.

Noirot, E. (1964). Changes in responsiveness to young in the adult mouse: the effect of external stimuli. *Journal of Comparative and Physiological Psychology, 57,* pp. 97-99.

Noriot, E. (1972). Ultrasounds and maternal behavior in small rodents. *Developmental Psychobiology, 5,* (4), pp. 371-387.

Olkkola, K.T. & Ahonen, J. (2008). Midazolam and other benzodiazepines. In *Modern Anesthetics: Handbook of Experimental Pharmacology, Vol. 182*. (pp. 335-362). Berlin: Springer-Verlag.

Palanza, P., Rodgers, R.J., Ferrari, P.F., & Parmigiani, S. (1996). Effects of chlordiazepoxide on maternal aggression in mice depend on experience of resident and sex of intruder. *Pharmacology Biochemistry & Behavior, 54,* (1), 175-182.

Poe-Yamagata, E. & Butts, JA. (1996). Female Offenders in the Juvenile Justice System: Statistics Summary. *U.S. Department of Justice.*

Pohoercky, L.A. (1977). Biphasic action of ethanol. *Biobehavioral Reviews, 1,* pp. 231-240.

Potegal M, Tenbrink L (1984). Behavior of attack-primed and attack-satiated female golden hamsters (Mesocricetus auratus). *Journal of Comparative Psychology* 98:66–75

Pridemore, W.A. (2004). Weekend effects of binge drinking and homicide: the social connection between alcohol and violence in Russia. *Addiction, 99,* (8), pp. 1034-1041.

Robinson, D.L., Brunner, L.J., & Gonzales, R.A. (2002). Effect of gender and estrous cycle on the pharmacokinetics of ethanol in the rat brain. *Alcoholism: Clinical and Experimental Research, 26,* (2), pp. 165-172.

Rodgers, J. & Waters, A. (1985). Effects of benzodiazepines and their antagonists on social and agonistic behavior in male mice. *Aggressive Behavior, 11,* (2), pp. 184-184.

Saias, T. & Gallarda, T. (2008). Paradoxical aggressive reactions to benzodiazepine use: a review. *Encephale: Revue de Psychiatrie Clinique Biologique et Therapeutique, 34,* (4), pp. 330-336.

Sarto-Jackson, I. & Sieghart, W. (2008). Assembly of GABA-A receptors (review). *Molecular Membrane Biology, 25,* (4), pp. 302-310.

Scott, J.P. & Fredericson, E. (1951). The causes of fighting in mice and rats. *Physiological Zoology, 24,* (4), pp. 273-309.

Sieghart, W. (1995). Structure and pharmacology of gamma-aminobutyric acid-a receptor subtypes. *Pharmacological Reviews, 47,* (2), pp. 181-234.

Simon, J., Wakimoto, H., Fujita, N., Lalande, M., & Barnard, E.A. (2004). Analysis of the set of GABA-A receptor genes in the human genome. *Journal of Biological Chemistry, 279,* (40), pp. 41422-41435.

Skolnick, P., Reed, G.F., & Paul, S.M. (1985). Benzodiazepine-receptor mediated inhibition of isolation-induced aggression in mice. *Pharmacology Biochemistry & Behavior, 23,* (1), pp. 17-20.

Smith, K.M., Larive, L.L., & Romanelli, F. (2002). Club drugs: methylenedioxymethamphetamine, flunitrazepam, ketamine hydrochloride, and gamma-hydroxybutyrate. *American Journal of Health-System Pharmacy, 59,* (11), pp. 1067-1076.

Smothers, C.T., Clayton, R., Blevins, T., & Woodward, J.J. (2001). Ethanol sensitivity of recombinant human *N*-methyl-D-aspartate receptors. *Neurochemistry International, 38,* pp. 333-340.

St. John, RD. & Corning, PA. (1973). Maternal Aggression in Mice. *Behavioral Biology, 9,* pp.635-639.

Stovener, J. & Endresen, R. (1965). Intravenous anesthesia with diazepam. *Lancet, 2,* pp. 223-227.

Svare, B. & Gandelman, R. (1973). Postpartum aggression in mice: experiential and environmental factors. *Hormones and Behavior, 4.* pp 323-334.

Svare, B. & Gandelman, R. (1975). Postpartum aggression in mice: inhibitory effect of estrogen. *Physiology & Behavior, 14,* (1), pp. 31-35.

Takahashi, R.N., Morato, G.S., & Montierodelima, T.C. (1984). Effects of ketamine on experimental animal models of aggression. *Brazilian Journal of Medical and Biological Research, 17,* (2), pp. 171-178.

Taylor, S.P. (1970). Aggressive behavior and physiological arousal as a function of provocation and the tendency to inhibit aggression. *Journal of Personality, 35* (2), pp. 297-310.

Traynelis, S.F., et al. (2010). Glutamate receptor ion channels: structure, regulation, and function. *Pharmacological Reviews, 62,* pp. 405-496.

vom Saal, FS., Svare, B., & Gandelman, R. (1976). Time of neonatal androgen exposure influences length of testosterone treatment required to induce aggression in adult male and female mice. *Behavioral Biology, 17,* pp. 391-397.

Wallner, M., Hanchar, H.J., & Olsen, R.W. (2003). Ethanol enhances alpha(4)-beta(3)-delta and alpha(6)-beta(3)-delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans. *Proceedings of the National Academy of Science of the United States of America, 100,* (25), pp. 15218-15223.

Weisman, A.M., Berman, M.E., & Taylor, S.P. (1998). Effects of clorazepate, diazepam, and oxazepam on a laboratory measurement of aggression in men. *International Clinical Psychopharmacology, 13,* pp. 183-188.

Yoshimura, H. & Ogawa, N. (1991). Ethopharmacology of maternal aggression in mice: effects of diazepam and SM-3997. *European Journal of Pharmacology, 200,* (1), pp. 147-153.