Rewarding Aggression in Ovariectomized Female Mice

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Brittany M. Blasetti

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Advisor: Marcus M. Weera

Abstract

Aggression is a powerful reinforcer across species. Like males, intact female Swiss Webster mice readily attack same-sex intruders following cohabitation with a partner. Our experiment examined whether ovariectomized females would work for the opportunity to fight. We measured stereotypical 'scalloped' patterns of fixed interval (i.e., FI) operant responding, which operationally define 'motivational states' in anticipation of fighting. No sex differences were observed, as both males and females acquired rates and scalloped patterns of operant responding to the opportunity to fight a same-sex conspecific. After each session, aggressive behavior in ovariectomized females consisted of short latencies to attack and escalated fighting patterns, consistent with historical measures of schedule-induced aggression across many species investigating males. Future research will examine the neurobiological mechanisms underlying scheduled-induced aggression and how these systems are dysregulated by ethanol and other misused substances.

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This thesis is dedicated to the memory of my younger brother, Joey M. Blasetti, who was my number one supporter. I hope I have made you proud.

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Introduction

Some people find the opportunity to aggress and defeat a conspecific to be rewarding, a phenomenon that is theorized to contribute to aberrant behaviors such as bullying and pathological violence (Chester et al., 2016; Wang et al., 2019; Osakada et al., 2024). Using an operant conditioning procedure, our lab has previously demonstrated that male mice will work towards aggressive encounters (i.e., 'aggression seeking') if a winning outcome is predictable, demonstrating that mice are valuable models for understanding the neurobehavioral underpinnings of appetitive aggression (Fish et al., 2005; Golden et al., 2019). Regrettably, aggression research has paid limited attention to female subjects (Aubry et al., 2022; Newman et al., 2019; Rieger et al., 2022; Oliveira & Bakker, 2022), even though stark sex differences are present in various neuropsychiatric disorders, which disproportionately affect women (Kessler, 2003; 1995).

Women tend to engage more in indirect forms of aggression (i.e., relational aggression) but are now experiencing higher rates of diagnosed conduct disorder (Baird et al., 2010; Denson et al., 2018; Freitag et al., 2018), a mental disorder marked by excessive aggression, suggesting that women indeed aggress outside of stereotypical gender norms. Thus, from a translational viewpoint, the motivation to engage in an aggressive confrontation within females is equally important to investigate. Recently, we began to model the critical features of aggressive behavior in reproductively naïve female mice directed toward rival females (Covington et al., 2019; Newman et al., 2019). Intact females were reinforced by the opportunity to fight a conspecific when cohabitating with a male (Newman et al., 2019). Building on these findings, the current study aimed to investigate whether ovariectomized females would similarly work for the opportunity to fight a same-sex conspecific and to distinguish if sex differences exist in the motivational and behavioral patterns of aggression under operant conditions.

Literature Review

Aggressive behavior has been extensively documented and captured under controlled laboratory conditions across species, from rodents to non-human primates (Kuske and Trainor, 2021). Aggression is an innate, conserved, and vital facet of social behavior (Covington et al., 2022; Falkner & Lin, 2014; Lin et al., 2023). Species-typical aggressive behaviors refer to the defense of territory, mating, and the overall summation of resources, but also aid in establishing social hierarchies (Covington et al., 2019; Oliveira & Bakker, 2022). In contrast, species-atypical aggression (i.e., "proactive" or "excessive") reflects a profound deviation from otherwise traditional behaviors, as it exists out of context and differs from conventional patterns (Miczek, de Boer & Haller, 2013). These behaviors include short attack latencies and dysregulated threat perception and responses, ultimately culminating in violent outbursts at the expense of the individual (Augsburger et al., 2017; Bresin & Hunt, 2023; Fortes et al., 2017).

In understanding atypical behavior through the lens of a cost-benefit analysis, exaggerated forms of aggression imply that the individual may be acting out of naivety, is spontaneously spurred to action, is potentially influenced by prior winning-losing experiences, may have a dysregulated arousal state, or may be intrinsically motivated to seek a reward (Breslin & Hunt, 2023; Hsu et al., 2005; Lischinsky & Lin, 2020; Roualt et al., 2019). This dynamic shift from otherwise stereotypical behavior toward motivated or planned responses for a perceived reward is termed "appetitive aggression" (Elbert, Schauer & Moran, 2018). More specifically, this term refers to engaging in selective, goal-oriented, and inherently hedonistic, atypical aggressive behaviors wherein the violent act may be positively reinforcing or rewarding to the individual (Golden et al., 2019).

Historically, the motivation to engage in excessive aggressive behavior has been quantified by how fast, frequent, and long bouts of salient agonistic acts and postures are displayed (Miczek et al., 2001). While newer exploration into appetitive social behaviors has been investigated, more information is needed regarding the reinforcing or rewarding effects of aggression in females. Various neural regions, circuits, neurotransmitters, and genes have been hypothesized to contribute to aggression. Most notably, these include: the ventromedial hypothalamus (VMH) and medial preoptic area (mPOA), the limbic (amygdala (Amy), prefrontal cortex (PFC), and lateral septum (LS)) and midbrain (ventral tegmental area (VTA), substantia nigra (SN), and the dorsal raphe nucleus (DRN)) circuits, corticotrophin-releasing factor (CRF), serotonin (5-HT), glutamate (GluA), dopamine (DA), vasopressin (AVP), oxytocin (OXT), and the mono-amino-oxidase A (MAO-A) and catechol-o-methyltransferase (COMT) genes (Abeykoon et al., 2024; Aleyasin et al., 2018; Covington et al., 2019; Fritz et al., 2020; 2021; Hashikawa et al., 2018; Golden et al., 2019). Although an extensive amount of literature details the prevailing theories by which aggressive behavior may originate, the neural underpinnings of appetitive aggression remain undiscovered. Mentioning every locus by which typical and atypical aggressive behavior may originate would be outside the scope of this review.

However, when discussing the motivational aspects of aggression, the Mesocorticolimbic system must be incorporated as it historically relates to reward and seeking behaviors. Within this system, DA neurons from the VTA project to the nucleus accumbens (NAc), PFC, Amy, and the lateral habenula (LHb) to reinforce natural rewards like sex, food, or shelter (Aleyasin et al., 2018; Ballard et al., 2011; Blackburn et al., 1992; Golden et al., 2019; Mondoloni, Mameli & Congiu, 2022; Yamaguchi & Lin, 2018). Furthermore, this system contributes not only to the anticipation of reward but also aids in encoding both valence and salience of an experienced stimulus via DA and gamma-aminobutyric acid (GABA) projections within the central amygdala (CeA) and VTA (Kong et al., 2021). Moreover, damage to the ventromedial prefrontal cortex (vmPFC), a region nestled within this system, disrupts incentivized decision-making processing as well as emotional regulation within the amygdala (Hiser & Koenigs, 2018) and DA projections within the ventromedial striatum (VMS) have been shown to both facilitate and inhibit cognitive flexibility during effort-based decision-making tasks, suggesting that incentivized motivation may contribute to the costly behavior of excessive aggression (Aarts et al., 2011; Blair, 2022).

Beyond merely stating the neurobiological substrates of motivation and reward, it is equally essential to delineate the psychological components of associative learning and its effects on aggressive reward. Firstly, aggressive behavior can be positively or negatively reinforcing (Miczek & Meyer-Lindenberg, 2014). Reinforcement is both a psychological concept and a behavioral tool that becomes conditioned when controlled by a consequence. In this domain, Pavlovian (i.e., classical) and operant conditioning principles have influenced our understanding of how reinforcement drives motivational states. Pavlovian conditioning uses a neutral stimulus paired with an unconditioned stimulus to trigger a response, creating a conditioned stimulus that can then be used to predict a conditioned response; this type of conditioning relies on instinct (Pavlov, 1927). Alternatively, operant conditioning differs in that responding is conditioned by value (i.e., through positive and negative reinforcement and extinction), thus requiring some level of voluntary control (Lorenz, 1935; 1967; Tinbergen, 1951; Skinner, 1935).

Excessive use of violence, as measured in today’s world through increased instances of intimate partner violence, gun safety, and large-scale conflicts such as war, is a looming global health threat (Machado, Sousa & Cunha, 2024; Shapiro & Hua, 2020; White et al., 2024). As such, current studies on aggression are shifting from solely understanding instinct (e.g., species-typical aggression) and instead toward the voluntary willingness to engage in an attack, even if the confrontation is detrimental to the individual's fitness (e.g., species-atypical aggression) (Takahashi & Miczek, 2013). For these reasons, modeling operant conditioning in aggressive behavior within the laboratory is the most logical route.

Operant conditioning dictates how a given stimulus and its associated responses and reinforcers drive state changes that directly impact behavior (Herrnstein, 1977). In early behavioral studies that utilized visual representations of conspecifics, researchers were able to prompt instrumental agonistic displays in both Siamese fighting fish (*Beta splendens*) and fighting cocks (Thompson 1964, 1966; Thompson & Sturm, 1965a; Thompson & Sturm, 1965b). When paired with a visual representation of an opponent, the conditioned stimuli were enough to provoke outwardly combative displays in these animals. Although these early studies proved that an individual's aggressive behavior could be reliably reinforced and escalated, no physical contact was recorded.

Murine models of both aggression-seeking and consummatory performance behavior came later. Beginning with the classification of aggressive behaviors in male mice who readily attacked opponents even when subjected to an electrical grid to gain access to a conspecific (Lagerspetz, 1964), several studies followed which explored the motivation to pursue and engage in physical aggression under paired contexts (Golden et al., 2019; Takahashi & Miczek, 2013), before culminating in the widely used resident-intruder paradigm (Miczek and O'Donnell, 1978), of which has been known as the industry "gold standard" for studying aggressive performance.

For an act to be considered rewarding, the following conditions must be met: (1) the act and its subcomponents must be reinforcing, (2) it must provide an incentive to engage, and (3) it must elicit an affective response in the individual (Covington et al., 2019). This schema has been modeled under several contexts: for natural rewards, such as food, in rats trained to bar press for access to a nutritional liquid (Hutt, 1954); in the rewarding properties underlying drug abuse, such as with conditioned place preference (CPP), in which an animal frequently returns to the conditioned-paired chamber for access to the drug of choice (van der Kooy, 1987; Tzschentke, 1998; 2007); in self-administration studies associating reinforcement with drug use (Arena et al., 2019; Armstrong et al., 2023; Boyson et al., 2014; Burke & Miczek, 2015; Covington & Miczek, 2005; Miczek et al., 2004; Tidey & Miczek, 1997); and perhaps most importantly, in aggression, such as with Syrian hamsters who preferentially chose to engage in attack (Meisel & Joppa, 1994), in pigeons who were instrumentally reinforced by both food and attacking an opponent (Cherek, Thompson and Heistad, 1973) and more recently, in male and female rodents who preferentially returned to contexts where they could defeat an opponent (Aleyasin et al., 2018; Börchers et al., 2023; Golden et al., 2016; Flanigan et al., 2017). However, despite the abundant literature concerning the relationship between reinforcement and reward, operant conditioning has only recently begun to be associated with appetitive aggression. This has been most prominently demonstrated with operant self-administration using a fixed interval ("FI"; access to the reward after a determined interval) or fixed ratio ("FR"; access to the reward after a determined ratio has been met) schedule of reinforcement.

Specific conditions must be met for aggressive behavior to be considered rewarding. Using FI/FR schedules of reinforcement allows researchers to capture step-by-step, time-gated quantitative measurements of both seeking and consummatory performances (Covington et al., 2019), which can later be quantitatively dissociated. Deviations from the defensive functioning of a confrontation, such as when neither food nor shelter is required, permit more offensive encounters, further reinforcing an association between conflict and reward. Thus, excessive aggressive behaviors outside these confines suggest that working for and participating in the opportunity to fight an opponent must rely on conditioned contexts that are operantly reinforced by the chance for an attack (Covington et al., 2019). This type of reinforcement has been demonstrated in a variety of species ranging from rodents to monkeys, fish, and fruit flies (Azrin et al., 1965; Connor, 1975; Hoopfer, 2016; Jackson et al., 2023; Thompson, 1964; Takahashi & Miczek, 2014; Trannoy et al., 2015).

Initially, to attempt to decipher the neurobiology underlying motivation, male mice were operantly conditioned to respond to the opportunity to attack a conspecific according to an FR schedule of reinforcement (Fish et al., 2002). The findings from this study suggested that the encounter was highly arousing for the animal and that an operant schedule of reinforcement could produce escalated bouts of aggression. Subsequently, additional studies in males on the pharmacological effects of ethanol and neurosteroids in operant conditioning for aggression suggested not only dose-dependent effects on behavior but also that both GABA and corticosterone modulated performance (Fish et al., 2002; 2005). These studies were the first to suggest a potential reinforcing effect of aggression. They hinted that appetitive and consummatory states could be quantitively segregated, which prompted a replication using a variable-ratio scale in combination with SCH-23390, a D2 antagonist injected into the NAc (Couppis & Kennedy, 2008), followed by additional studies on time-dependent schedules of reinforcement in male mice, which further affirmed the reinforcing effects of aggression (May & Kennedy, 2009).

FI and FR schedules of reinforcement establish reliable patterns and response rates that can assess the underlying motivational state and provide a compelling dissociation of the anticipatory seeking versus the actual performance of pursuits, threats, and attacks after a given period (Covington et al., 2019). Furthermore, key similarities have been noted between drug and reward-seeking behaviors (i.e., as with aggression) in response to stimuli. The components required for an act to be considered rewarding strongly mirror the addiction cycle; namely, both require (1) the compulsion to seek, (2) a loss of control, and (3) some affective emotion (Covington et al., 2019; Koob & Le Moal, 2008). Many have likened the subcomponents of appetitive aggression to an addiction-like phenotype, suggesting the cause to be potential dysregulation in reward circuitry (Golden et al., 2019; Koob, 2015).

As appetitive aggression involves reinforcement, it incentivizes an individual's motivation to seek an aggressive encounter despite the consequences (Covington et al., 2018; Golden et al., 2017a, 2019b), and classical models of rodent drug addiction can be used to study excessive aggressive behavior. For example, CD-1 mice were first trained to operantly respond to a chance to attack an opponent and then underwent a period of forced abstinence; these mice had higher relapse rates in aggression-seeking behavior (Golden et al., 2017). Similarly, to study the influence of DA on reinforcement, mice were trained to respond to access to an intruder, which induced high DA neural activity within the NAc. Inhibition of GABAergic dopamine (Drd)-1 expressing neurons decreased operant responding and seeking, indicating a cell-specific role of DA in appetitive aggression (Golden et al., 2019). However, the most detailed evidence for the aggression-addiction link comes from studies using CPP, a validated behavioral tool typically used in addiction studies, in aggression-paired CPP contexts (aCPP).

Historically, CPP assessed the reinforcing quality of morphine on associative learning processes under a paired context in rats (Beach, 1957). To briefly summarize, an animal is initially placed in an environment where a drug is administered, followed by removal and an opportunity to choose between a cue-laden or cue-deprived environment. Unsurprisingly, the animal often prefers the drug-cued environment and will avoid aversive stimuli. Increasing time spent in the paired context suggests it is rewarding (van der Kooy, 1987). Decades of behavioral research have demonstrated the reinforcing effects of food, sex, and drugs of abuse (for a comprehensive analysis of CPP, see Bardo, Rowlett & Harris, 1995; Bardo & Bevins, 2000) through CPP paradigms. The first evidence of the benefit of the aCPP paradigm came from Syrian hamsters who were conditioned to an environment involving either aggression or sexual behavior; these females preferred environments that were aggressively or sexually rewarding (Meisel & Joppa, 1994). Since then, several studies have explored the reinforcing effects of aCPP, predominantly in males (Golden et al., 2019). Only recently has aCPP begun to be studied in female mice and rats, with results suggesting conflicting evidence on its rewarding properties in this population.

However, CPP has its limitations. First, it measures an associative learning process that largely relies on cues; therefore, it does not indicate volitional motivation (Bardo & Bevins, 2000). In one study of male rats who were operantly conditioned to respond to both SA intravenous (IV) amphetamine and IV amphetamine injection under a paired context, the magnitude of the drug association in CPP and the rate of responding in drug-SA were not correlated, suggesting a distinction between CPP and operant self-administration in pursuing reward (Bardo, Valone & Bevins, 1999). Moreover, an individual's emotional state must be considered when measuring the motivation to seek a reward for it to apply to humans (Koob, 2015). Additionally, CPP is not influenced by feedback from gaining access to the reward compared to operant SA tests (Dixon et al., 2013).

Secondly, the methodology behind CPP also requires scrutinization. CPP requires active cue administration by a trained experimenter, whereas SA utilizes training the subject to exert personal control over the situation (Bardo & Bevins, 2000). Thus, one may question if the paired context warrants aggression or if the novelty of exploration influences the outcome, suggesting that aggression is simply a byproduct of unfamiliarity. If the latter is true, a follow-up question must address whether it is merely an extension of innate social behavior, as animals naturally prefer both arousing and novel contexts and unfamiliar social interactions (Haywood & Wachs, 1967; Nadler et al., 2004).

Lastly, predicting and measuring behavior between contexts and subjects is difficult to discern. At the individual level, it can be challenging to ascertain if a behavior directly correlates to the associated context due to a preference or an aversion (Bardo & Bevins, 2000). This issue is most widely demonstrated in classic pharmacology studies using CPP and SA in exploring dose-effect curves, as between-subjects (i.e., CPP) requires conditioning to be completed with no adjustment period versus within-subjects (i.e., SA), which allows for fine-tuning per session (Bardo & Bevins, 2000). To substantiate the claim that some female rodents find aCPP contexts rewarding and to continue to disentangle the sexual dimorphism of aggressive behavior, additional studies must be conducted that aim to minimize bias and account for both novelty and state changes.

Unfortunately, past aggression literature has centered around male interactions, with limited attention to female subjects (Newman et al., 2019; Oliveira & Bakker, 2022; Rieger et al., 2022). This is problematic, as sex differences have been identified across many neuropsychiatric disorders, with women having a higher predisposition to diagnoses of depression, anxiety, and post-traumatic stress disorder (Asher & Aderka, 2018; Boyd et al., 2015; Kessler, 1995; 2003; Salk, Hyde & Abramson, 2017), which is representative of an overwhelming mental and public health disparity. Furthermore, many of these disorders have features consistent with aggression (Baird et al., 2010; Fritz et al., 2020; Meeus et al., 2016), suggesting that women are an underserved population in public health.

In males, studies have investigated an association between mental health disorders and aberrant social behaviors. Much less has been investigated in females. For example, in males with borderline personality disorder (BPD), deficiencies in prefrontal cortical regions that are primarily responsible for emotional control suggest a mechanism by which aggressive outbursts may occur (Bertsch et al., 2019). Additionally, in a study of male twins, a gene-environment interaction between childhood and adulthood predicts antisocial behavior, resulting in psychopathy and criminality (Lyons et al., 1995). Other examples of this sex bias include studies on the interaction between benzodiazepines, a first-line medication indicated for anxiety disorders, and the motivation to engage in aggressive behavior (Albrecht et al., 2016; Gourley et al., 2005; Kršiak, Podhorná & Miczek, 1998); on how varying levels of anxiety impact aggression through selectively bred rats, and how the NAc may impact these behaviors (Beiderbeck et al., 2012); in substance use disorder (SUD) patients who underwent transcranial direct current stimulation (tDCS) to modulate empathy and reduce their violent tendencies (Sergiou et al., 2022); on the effect of a novel antipsychotic, Molindone, in attenuating impulsive aggression outbursts in attention-deficit/hyperactivity disorder (ADHD) (Ceresoli-Borroni et al., 2021); on the effects of both deep brain stimulation (DBS) and SSR125543, a CRF1 antagonist, as therapeutic treatment approaches in treatment-resistant depression (TRD) (Dournes et al., 2013); on intermittent explosive disorder (IED) and the propensity to retaliate against a fictitious opponent while undergoing functional magnetic resonance imaging (fMRI) (Gan et al., 2016), and on studies exploring an interaction between appetitive aggression and PTSD in child soldiers raised in and concurrently experiencing extreme acts of violence (Crombach & Elbert, 2014; Köbach et al., 2015; Nandi et al., 2020).

In contrast, few studies have explored the relationship between mental health disorders and agonistic behavior in females. This may be falsely attributed to the belief that females are only mildly aggressive and typically only engage in maternal, territorial, or rival aggression (Börchers et al., 2023; Oliveira & Bakker. 2022). Thankfully, partly due to changes in policies regarding NIH-funded research (NIH, 2015) and due to the emergence of the importance of sex as a biological variable (SABV) in biobehavioral research, studies incorporating females and minorities are now being conducted as these variables directly influence research design, methodology, and analysis (Arnegard et al., 2020). Furthermore, incorporating SABV into future aggression research will improve the generalizability of our results and provide us with a more robust understanding of the biobehavioral and neurobiological mechanisms to fill these wide gaps within the literature.

In commonly investigated female rodents, aggressive behavior serves a defensive function, particularly in protecting offspring during the postpartum phase (Lonstein and Gammie, 2002; de Almeida et al., 2014). This form of maternal aggression is displayed shortly before giving birth and while the female is lactating (Svare and Gandelman, 1976). This is considered a prosocial form of aggression, as it mainly exists to protect offspring (Oliveira & Bakker, 2022). However, non-defensive forms of aggressive behavior, removed from the immediate reproductive context, remain to be explored in females (Brain et al., 1992; Oliveira & Bakker, 2022).

Although recent research in females is slowly progressing from a maternal context to more instrumental forms of aggression, the evidence needs to be less sparse and more rigorous and consistent. For example, one study suggests that although intact female mice may engage in aggressive behaviors, they do not find it as reinforcing as males and do not glean an appetitive or hedonistic drive from such interactions, further confining them to the defensive characterization (Aubry et al., 2022). In contrast, intact but not ovariectomized female mice who underwent similar conditions were reinforced by the opportunity to fight a same-sex conspecific as males (Newman et al., 2019). The conflicting findings between these studies are not directly apparent but could be attributed to various factors, including the experimenter's sex or housing conditions (Georgiou et al., 2022; Faraji et al., 2022; Crawley, 2003(a), 2007(b)).

Although these studies were pivotal in understanding female aggression, there are two important caveats to discuss. First, Swiss Webster-derived (CFW) mice were used in both studies. CFWs are an outbred albino strain that, like others, have been selectively bred and display aggressive behaviors in both males and females (Jones & Brain, 1987; Miczek et al., 2001). Although CFWs were not originally bred for aggression, they are commonly used in aggression research due to their diverse genetic background, resulting in high variation in social behaviors (Maxson, de Boser & Sluyter, 2013; Miczek et al., 2001). As such, there is the possibility that selection bias has influenced the research design. Second, using nulliparous, intact female mice challenges the widely held belief that females only engage in maternal, rival, and gestational aggression (for a more comprehensive review of female aggression, see Oliveira & Bakker, 2022). Moreover, studying this population supports the growing body of literature that suggests that the estrous cycle has a minimal impact on social behaviors, including aggression, in female rodents (de Jong, Beiderbeck & Neumann, 2014; Morè, 2008; Zeng et al., 2023), although, in humans, some evidence for menstrual cycle effects have been demonstrated in proactive aggression in adolescents diagnosed with BPD, with reactive aggressive behaviors highest during the luteal phase, and proactive aggressive behaviors highest during the follicular and ovulatory stage (Peters et al., 2020).

Hormonal changes, including gonadectomy, have been shown to influence interfemale aggression and other social behaviors in a variety of ways. For example, in ovariectomized rats implanted with both estrogen and testosterone tubes, hormone-implanted females were more aggressive than their counterparts in securing access to food and fighting against a novel conspecific (Albert, et al., 1988; 1990). Estrogen and progesterone may also exert effects within the amygdala on anxiety-like and fear behaviors, as demonstrated in ovariectomized rats who were given subcutaneous hormone therapy and subjected to open-field and elevated plus maze (EPM) measures of anxiety (Frye & Walf, 2004), and blocking estrogen receptor beta (ERß) within the CeA during a stress-induced context can prevent the formation of depressive and anxiety-like behaviors (Smiley et al., 2023). Furthermore, in studies on the influence of sex hormones in operantly reinforcing seeking behaviors, ovariectomized rats who received estrogen but not progesterone therapy lever-pressed at a higher rate for IV cocaine infusions (Anker et al., 2007)

Studies exploring the VMHvl and estrogen receptor alpha-expressing (ESR1+) cells provide the most convincing evidence for the role of estrogen in female aggression. In males, optogenetic activation of ESR1+ cells can elicit both aggression and mounting behavior, suggesting a modulatory role for both aggressive and sexual behaviors (Lee et al., 2014); in contrast, optogenetic activation of ESR1+ cells prompted aggression against male and juvenile conspecifics, and inhibition of these cells reduces aggression with no effect on sexual behavior (Hashikawa et al., 2017), suggesting a vital role for ESR1+ cells in modulating female aggressive behaviors. Additionally, in a more recent study in both males and females on the effect of ESR1+ and gene expression, sex-differential gene expression and modulation of social behaviors were identified within the VMHvl and bed nucleus of the stria terminalis (BNST), implying a sexually dimorphic cellular network that is driven by hormonal fluctuation, that is context-dependent (Knoedler et al., 2022). To our knowledge, few studies have been conducted on ovariectomized females and aberrant behaviors. Future studies must incorporate this population to build on the theory that sex hormones influence appetitive behavior.

Although advances are underway in the biobehavioral exploration of female aggression, the method by which an aggressive phenotype in females emerges and the motivation behind the choice to engage in such confrontations is still relatively unknown. Only one other study has investigated appetitive aggression within ovariectomized female mice (Newman et al., 2019). Thus, the primary aim of this study was to bridge the gap in the literature regarding female appetitive aggression, motivation, and reward contingencies. Based on our findings, future directions will investigate the neurobiological underpinning of appetitive aggression in intact and ovariectomized female mice and explore the role of gonadal hormones in motivated behavior.

Methodology

Animals and Housing

Animals ("residents") were pair-housed with a corresponding member of the opposite sex. The Swiss Webster-derived (CFW; Charles River Laboratories, Wilmington, MA) strain was used as residents (n=10 male, n=9 female). Murine models are practical for studying aggressive behavior in the laboratory, as the conditions in which they've been studied have been extensively documented and replicated across species (Kuske & Trainor, 2021). Furthermore, pair housing reduces isolation and stress within mice (Crawley, 2007). In line with past research, we used a validated outbred CFW strain commonly seen in general research models for aggression (Lynch, 1969).

Animals were cared for according to the National Research Council *Guide for the Care and Use of Laboratory Animals* (NIH 2011), and all procedures were approved by the Tufts University Institutional Animal Care and Use Committee (IACUC). Mice were housed in a temperature-controlled vivarium (21±0.2°C) and maintained on a 12-hour reverse light/dark cycle, with unrestricted access to water and rodent chow (Purina LabDiet, 5001).

In both cohorts, twelve-week-old CFW mice were housed in pairs in clear polycarbonate cages (18.9 X 29.7 X 12.8 cm) lined with pine shavings. Twelve-week-old intact male and female intruder C57BL/6J (B6) mice (Jackson Laboratories, Bar Harbor, ME) were group-housed in cages (25.7 X 48.3 X 15.2 cm; n=10/cage) on corncob bedding. Recombinant inbred strains such as C57BL/6J have been used to examine operant conditioning due to their mild and submissive behavior and because previous findings indicate that their social behavior leads to the formation of hierarchies, demonstrating their usefulness in stress, defeat, and ultimately aggressive behavior studies (Kudryavtseva et al., 1991, Miczek, 1999; Miczek et al., 2001; Falkner et al., 2016; Golden et al., 2019; Aleyasin, et al., 2018; Takahashi et al., 2011; 2013).

Screening

After an adaptation period of one week to the laboratory in group-housed cages, followed by one week of pair housing, both experimental resident cohorts were screened across 5-7 RI sessions for aggressive behavior in confrontations with group-housed, similar-aged, intact B6 male and female intruders in the resident's home cage. Only the residents who maintained aggression (i.e., 30 bites/2-minute confrontations) throughout the screening were considered for advancement to operant conditioning.

Operant Conditioning Procedure

The resident's corresponding partner was removed and placed into a separate clean cage while the resident remained in their home cage. To investigate the relationship between aggressive seeking and performance, all residents were trained to nose-poke via an operant conditioning response apparatus (MedPC Associates, Bennington, VT) in all experiments. A panel with two nose poke response units and cue lights (Miczek & de Almeida, 2001) was placed halfway into the resident's home cage. During the procedure, a green or yellow cue light designated the active nose poke hole. Once the resident reached the targeted FI, the cue light was turned off, an overhead white house light was illuminated, and an intruder was placed into the home cage.

Training began and gradually increased until the number of responses dependent on the reinforcement schedule (i.e., 10 minutes) was reached, which was maintained until the end of the experiment. The reinforcement schedule ranged from 1 to 10 minutes. It incrementally increased once the animal had reached three sessions within a minute and a half (i.e., to advance from an FI 4 to FI 5, the resident must have responded by reaching 4.5 for three days in a row). A threshold for reliability was determined to measure the rate at which the residents could successfully and accurately predict when they should nose-poke for access to an intruder. To assess this reliability, we used historical curvature measures as introduced by Fry et al. (1960), where as the conditioning procedure continues, the mean rate responses tend to trend positively in an average scalloped line over time. Positive curvature indices are aligned with more responses towards the end of the encounter, culminating in an asymptote that reflects the measured behavior. Our index of curvature (IOC) was 0.25, which aligned with historical data and past experiments within our lab.

Lastly, the resident's behavior was monitored for the initiation of aggression. Historically, aggressive behaviors refer to biting, chasing, sideways threats, or piloerections (Miczek & O'Donnell, 1978). After the first bite, the resident was observed for up to three minutes unless a finite number of bites (i.e., 30) were reached. Intruders were removed after the three-minute aggressive encounter or at fifteen minutes if no aggression was observed. Two trained experimenters observed and counted all outcome measures using a stopwatch and manual clicker. Both residents and intruders were promptly returned to their home cages after the confrontation. If an intruder accrued thirty bites during the encounter, their tail was marked with a permanent black marker indicating that they would not be reused in a subsequent encounter; otherwise, a new intruder was used for every session.

Statistical Analyses

Statistical analyses were performed using Prism software, version 10.0 (GraphPad, San Diego, CA). As individual responses can vary widely and our behavioral data was not normally distributed, non-parametric statistical analyses were used to compare behavioral seeking and aggressive performance measures between sexes. A between-groups Mann-Whitney U Test was used to investigate whether differences existed within seeking behavior (e.g., cumulative FI responses and time, index of curvature) between sexes. To examine whether there were sex differences in aggressive performance behaviors (i.e., the number of bites, latency to attack, and the total fight duration), additional between-groups Mann-Whitney U Tests were performed. We conducted non-parametric Spearman correlations to investigate whether a relationship existed between the variables of seeking and consummatory components (e.g., FI responding vs. aggressive performance measures and between aggressive performance measures) in each sex separately and then in total within-group via a Spearman correlation matrix. When a significant correlation was identified, a simple linear regression was also performed to produce a line of best fit. The significance level was set at $p < 0.05$. All data are represented as medians with interquartile ranges (IQR) to account for non-normal distributions.

Results

FI as a Measure of the Motivation to Fight

Ovariectomized female mice, like males, accelerate their responses towards the end of the FI when they are reinforced by the opportunity to confront a same-sex conspecific (Figure 1). As expected, males readily responded to the opportunity to fight a conspecific. This suggests that both males and ovariectomized females are sensitive to the FI procedure and are reinforced by the opportunity to attack independently of sex. The datum for these comparisons was taken per cohort exactly thirty-three days from when the resident began at an FI-10, with the index of curvature (e.g., our reliability threshold) of 0.25, indicating a stable baseline. Our primary outcome measures of motivation, termed cumulative FI responses per animal and the index of curvature, were measured as the cumulative number of nose-pokes over ten minutes during a single session of FI and 0.25 and did not significantly differ between sexes. A Mann-Whitney U test revealed no significant difference in responding between males (median = 40) and females (median = 31), U=31, p = 0.27, and no significant difference in the index of curvature between males (median =0.21) and females (median=0.19), U=40.5, p = 0.73).

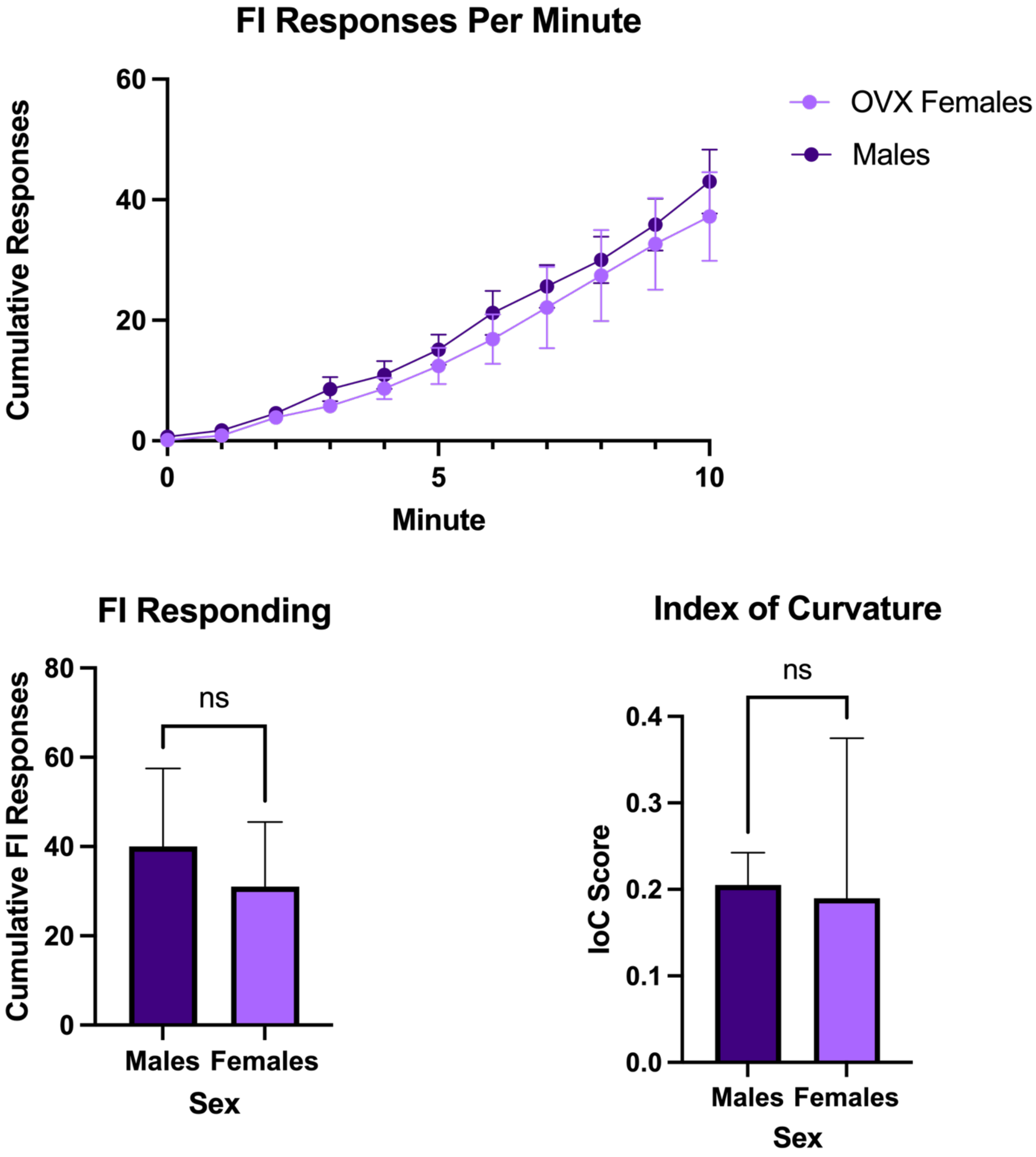


Figure 1. *Male and female mice show no differences in aggression-seeking*

Top: Male (n=10) and female CFWs (n=9) cumulative responses, as measured across time, indicate a scalloped pattern of FI responding that increases over the interval. Comparisons of both FI curvilinear relationships depict similar scheduled-induced responding patterns for access to an intruder.

Bottom: Comparisons between males and females with the median cumulative FI responses (left) and of the IOC (right) show no significant differences in either responding for access to an intruder or the ability to correctly predict the reinforcement's timing. All data are shown as median and the interquartile range (IQR).

Aggressive Behavior as a Measure of Performance

Not only will ovariectomized female mice work for the opportunity to fight a same-sex conspecific as males, but there were no statistically significant sex differences in consummatory performance outcome measures (Figure 2). This suggests that both sexes aggress similarly when confronted with an unfamiliar conspecific. Our outcome measure for reward, termed aggressive performance, was measured as the number of bites, latency to attack (LTA, measured in seconds), and the total fight duration (measured in seconds). Mann-Whitney U tests revealed no significant difference in the number of bites between males (median = 12) and females (median = 18), U=34, p = 0.38, the latency to attack (males, median = 23.5; females, median = 6), U= 38, p = 0.58, or in the total fight duration (males, median = 180; females, median = 180), U = 36, p = 0.34.

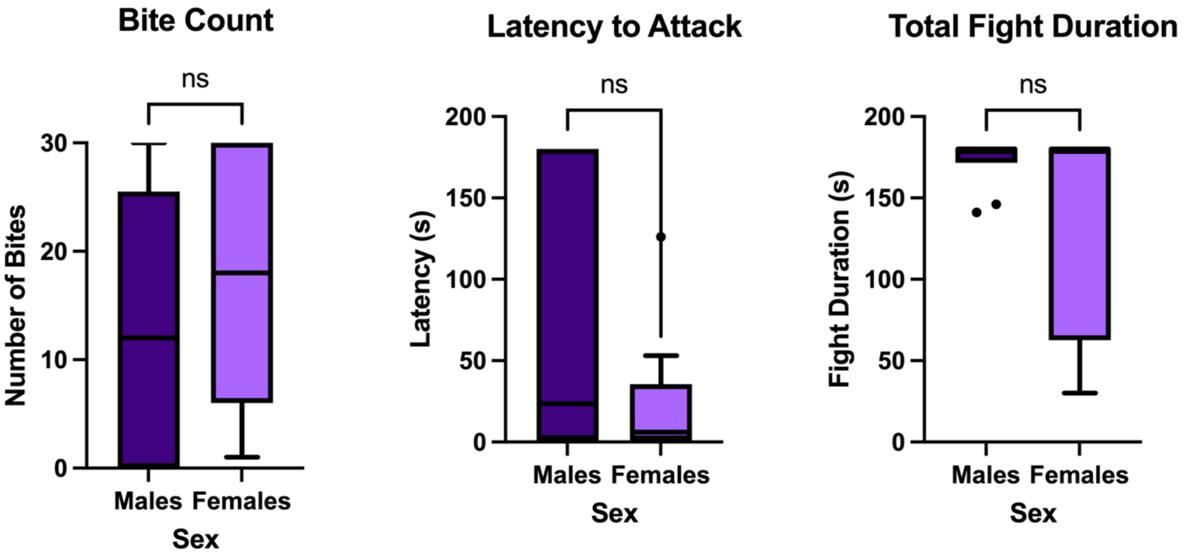


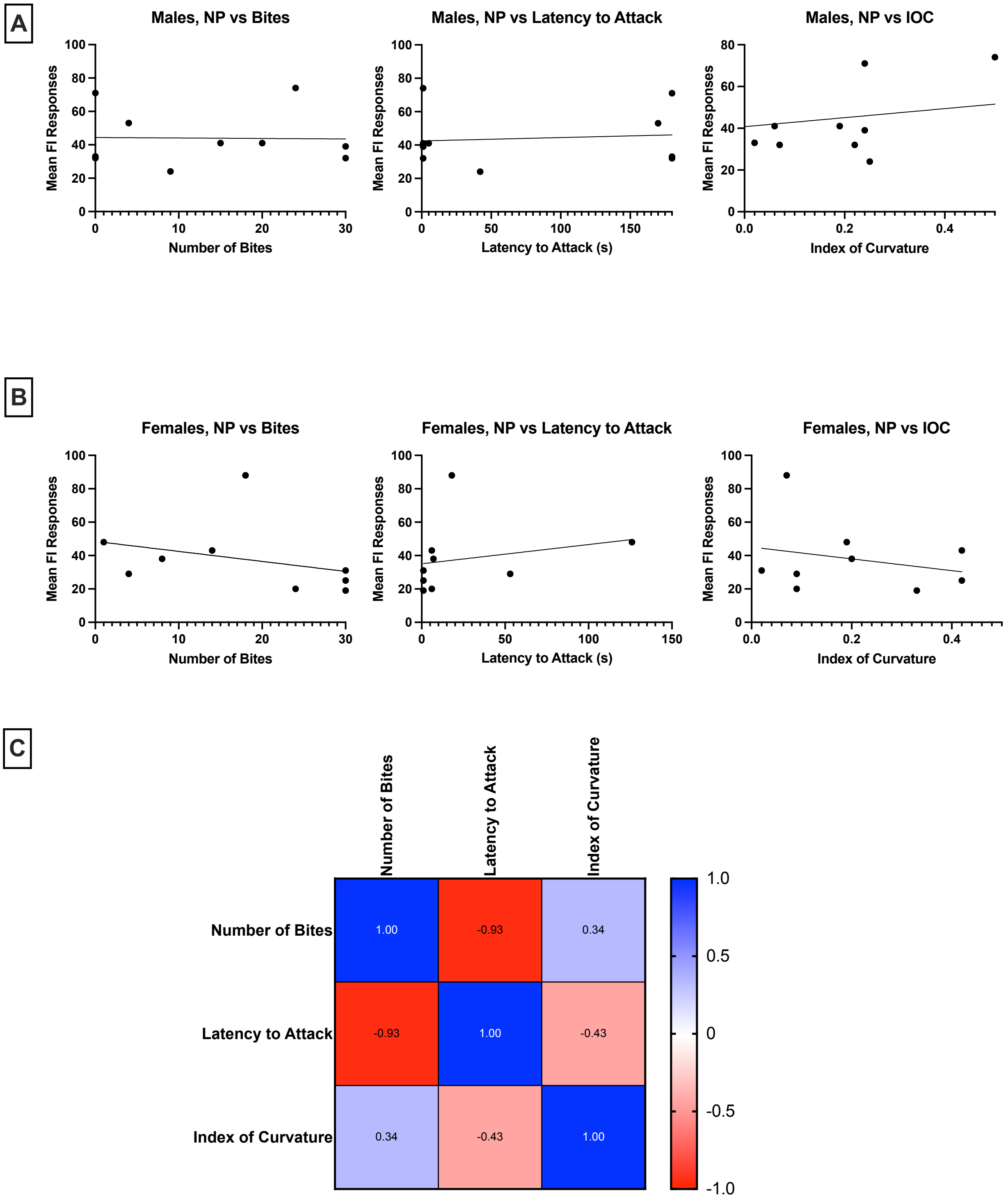
Figure 2. *Male and female mice show no differences in aggressive performance*

(From left to right): Comparisons of aggressive performance were measured in male (n=10) and female (n=9) CFWs via the number of bites, the latency to attack (measured in seconds), and the total fight duration (measured in seconds). All data are shown as median and the interquartile range (IQR).

Comparison of Motivated & Aggressive Behaviors Between Sex

Overall, our results suggest that aggression-seeking does not predict aggressive performance. Both male and ovariectomized female mice show a similar pattern of correlations between seeking and consummatory performance behaviors, with a few exceptions. A Spearman's rank-order correlation examined the relationships between the number of nose-pokes (e.g., cumulative FI responses) and the number of bites, latency to attack (measured in seconds), and the index of curvature per sex. The results showed no significant correlations between these outcome measures in males (Figure 3a, (r(8)=0.02, p, =0.96; r(8)=-0.10, p =0.76; r(8)=0.28,

$p=0.46$)) or in females (Figure 3b, ($r(6)=-0.58$, $p = 0.11$; $r(6) =0.62$, $p =0.08$, $r(6)=-0.22$, $p =0.57$)).



	Number of Bites	Latency to Attack	Index of Curvature
Number of Bites	1.00	-0.93	0.34
Latency to Attack	-0.93	1.00	-0.43
Index of Curvature	0.34	-0.43	1.00

Figure 3. *Seeking and consummatory performance in male and female mice have dissociable states*

In male **(A)** and ovariectomized female CFWs **(B)**, no relationship was found between the number of nose-pokes (measured as the cumulative number of FI responses) and aggressive performance (measured via the number of bites, LTA, and IOC). In comparisons of seeking behavior and aggressive performance within all animals (n=19) **(C)**, there was a significant negative correlation between the latency to attack (measured in seconds) and the number of bites, but no significant correlations between seeking and consummatory performance.

Additionally, we created a Spearman correlation matrix to explore the strength and direction of a relationship between seeking and aggressive performance for all animals (n=19). We found one significant negative correlation between our outcome measures of aggressive performance (LTA and number of bites). Both males and females displayed a significant negative correlation between the LTA and the number of bites ($r(17) = -0.93$, $p < 0.001$)). However, we did not find significant correlations between seeking behavior (i.e., total responses) and aggressive performance (LTA, IOC, and number of bites). Furthermore, there were nonsignificant, but moderate to weak correlations between our other outcome measures (number of bites, LTA and IOC) (Figure 3c).

Lastly, males had a significant negative correlation and significant simple linear regression between the latency to attack and the number of bites ($r(8)= -0.70$, $p < 0.001$, [$F(1, 8)=35.04$, $p < 0.05$])) Figure 4a)). Surprisingly, females also had a significant negative correlation and significant simple linear regression in the latency to attack and the number of bites ($r(6) = -0.94$, $p < 0.001$, [$F(1,7)=8.364$, $p < 0.05$])). This is also visualized within all animals in our Spearman correlation matrix heatmap (Figure 3c). Furthermore, a significant positive correlation, but not a significant simple linear regression, between the latency to attack and the total fight duration (r(6) =0.82, p <0.05), Figure 4b)) was identified only in the females.

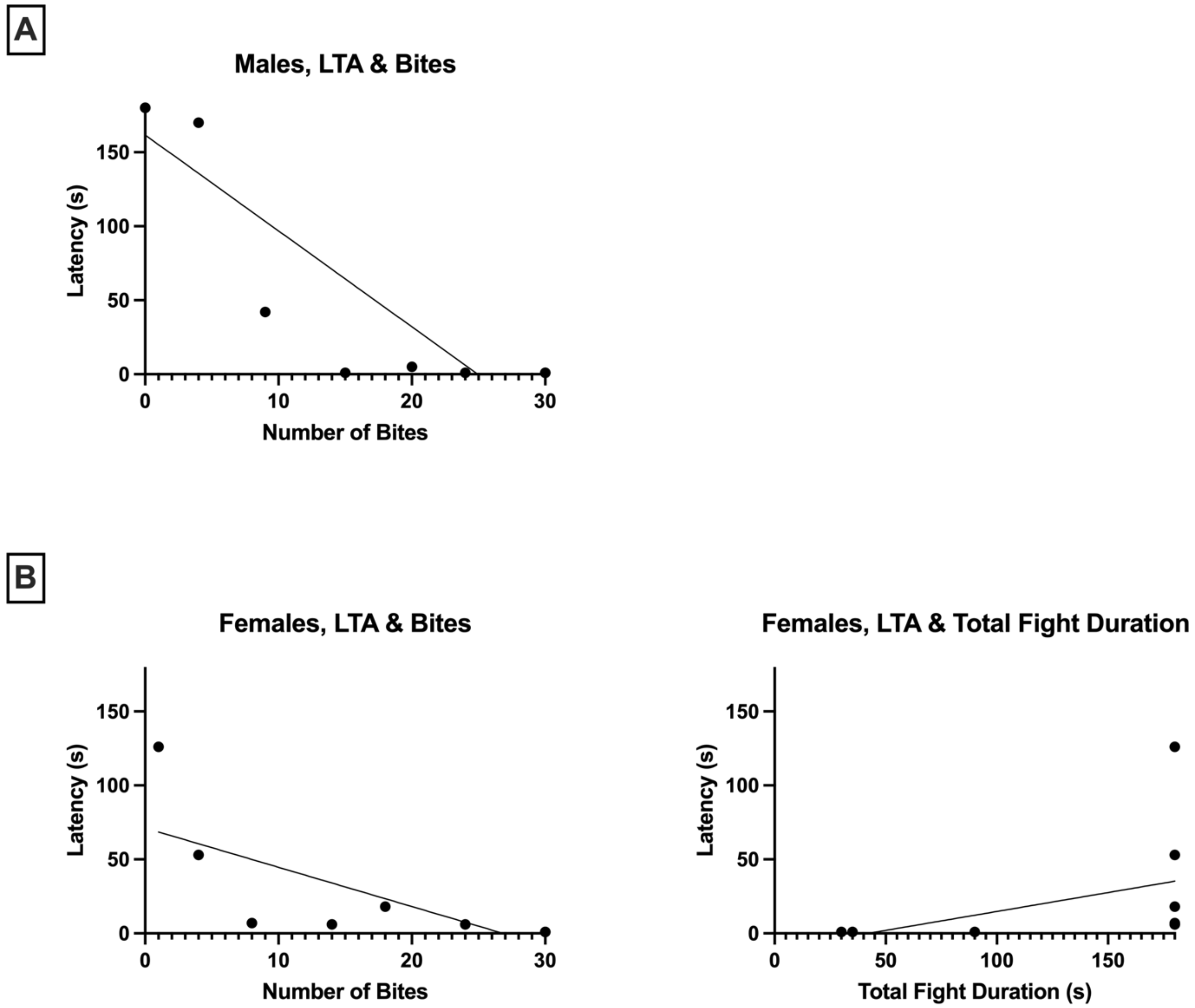


***Figure 4**. Male and female mice show similar relationships between measures of aggressive performance*

In male **(A)** and ovariectomized female CFWs **(B)**, a similar relationship was found between the latency to attack (measured in seconds) and the number of bites. In contrast, a significant positive relationship exists between the latency to attack (measured in seconds) and the total fight duration in females only.

Discussion

In contrast to the past literature exploring appetitive aggression in female mice (Aubry et al., 2022; Newman et al., 2019), our findings suggest that nulliparous, ovariectomized females will work for the opportunity to fight and engage in aggressive confrontations like that of males against similar-aged same-sex conspecific intruders under operant conditions, which implies that the act is reinforcing. In support of our original hypothesis, no statistically significant sex differences were observed in either the seeking or consummatory performance behaviors of aggression, suggesting that operant conditioning can reliably produce scheduled-induced aggression in both sexes. Our primary measures of motivation, operationally quantified as response rates (i.e., “nose-pokes”) and the IOC, and aggressive performance, quantified as the LTA, number of bites, and the total fight duration, were the same between the sexes.

As detailed in the original introduction of responding under the control of an FI schedule (Ferster & Skinner, 1957), responding accelerated towards the end of the FI, suggesting a curvilinear relationship between the number of responses and minutes of FI, symbolic of arousal and anticipation. Therefore, FI schedules of reinforcement are a valid tool in capturing an individual’s motivation to pursue a reward as it directly measures a time-locked schedule for aggressive performance, which enables the anticipation for a fight to be quantitatively and qualitatively defined (Covington et al., 2019). Furthermore, the accelerated response rate as the interval nears its end is an index for measuring incentivized motivation. Evidence for this rationale has been previously demonstrated in male mice who were operantly reinforced to nose-poke for access to an intruder on an FI/FR schedule and then underwent a contingency reversal to gain access to an intruder, suggesting that mice will work for access to an intruder as a reward, even when the reward contingency has changed (Fish et al., 2002). Additionally, this was demonstrated again in male mice who were reinforced by an FI-10 schedule for access to an intruder and engaged in escalated, species-atypical attacks compared to controls who did not complete an FI schedule (Fish et al., 2005). Lastly, the IOC directly substantiates our belief that FI schedules capture motivation accurately. A positive IOC (in our case, 0.25) is denoted by a steady and rapid temporal increase in responses towards the end of the interval, which can be interpreted as a measure of anticipation (Fry et al., 1960).

Our findings also support this theory of anticipation, as female residents learned that when they respond via a nose-poke on the designated hole, they will be confronted with an intruder as their reward, as previously demonstrated in prior literature with males. Moreover, we found no significant correlations between FI nose-poking and aggressive performance, suggesting that motivation and aggressive behavior have dissociable components. If a relationship existed between these elements - for example, between the response rate and the number of bites - this would suggest that the motivation to engage in a confrontation directly impacts the behavioral output, which we did not find. FI response patterns are typically stable, and deviations can indicate changes in motivational states that affect the prediction for reward (Covington et al., 2019). This is in line with past research demonstrating that male mice repeatedly exposed to ethanol experience changes in their motivation to fight but undergo sensitization before recovering to a baseline while fighting behavior is continuously disrupted (Covington et al., 2018).

The rationale behind the decision to engage in a confrontation, even if it is disadvantageous, is complex. Although several theories have been suggested, it is essential to highlight the impact of stress, the winner effect, and frustration on the behavior of residents and intruders. As the fixed interval continues, increased arousal and anticipation may culminate in a heightened emotional and physical state. Once the FI has been reached, the resident may be "lashing out," possibly due to frustration or excitement (Covington et al., 2019). Evidence for the role of heightened arousal comes from a study of male mice who underwent an FI-10 schedule and had corticosterone (CORT), a steroid hormone secreted during arousing or stressful experiences, measured before and after the reinforced encounter. CORT measurements doubled after an FI-10, and when inhibited by metyrapone, both instrumental responding and aggressive behavior were abolished (Fish et al., 2005). Additionally, female California mice subjected to an RI procedure display a significant increase in CORT levels compared to males and increased plasma OXT within the paraventricular nucleus (PVN) (Trainor et al., 2010). However, this relationship between CORT and female aggression remains unclear and warrants further studies exploring its role in modulating instrumental aggression.

Stressful experiences and prolonged heightened arousal can impact an individual's motivation and consummatory behaviors, leading to the development of psychopathologies and increasing the risk of mortality (Rosenbaum et al., 2015). Stress affects neural regions associated with emotional regulation and motivation, notably the amygdala, PFC, anterior cingulate cortex (ACC), and BNST (van de Poll et al., 2023). This has been demonstrated through increased activation and reduced responsivity via functional magnetic resonance imaging (fMRI) during FI in fear conditioning and extinction paradigms and has been extensively modeled in preclinical animal models (Shin & Liberzon, 2010). Furthermore, in intruder male mice who underwent ten daily social defeat episodes by aggressive residents, hippocampal cell proliferation was suppressed in the dentate gyrus (DG), suggesting that stress disrupts learning and memory processing and may contribute to the development of clinical depression (Yap et al., 2006). Furthermore, stress from an aggressive opponent has been shown to increase operantly reinforced lever pressing for IV cocaine in males (Miczek & Mutschler, 1996). In perinatal females, chronic stress through interactions with an aggressive male produces an avoider phenotype with depressive-like behavior (Ito et al., 2024), while women who have experienced intimate partner violence not only have a higher risk of developing PTSD but also SUD due to increased arousal and subsequently drug use to cope with symptoms (Sullivan & Holt, 2008). These examples highlight the importance of developing novel therapeutic strategies that should be explored in future studies centered around stress-related psychopathologies, especially in women, who are disproportionately affected (Bromet, Sonnega & Kessler, 1998; Ravi, Stevens & Michopoulos, 2019; Ramikie & Ressler, 2018).

Escalated aggression is also associated with the "priming effect," whereby attacking a conspecific is often followed by an even quicker attack when the individual is presented with the opportunity again (Potegal & Nordman, 2023). In humans, a similar effect can be seen in combat veterans. In a population of paramilitary group members, experiencing and perpetrating violent acts is strongly associated with appetitive aggression and steadily predicts future engagement in confrontation (Köbach, Schaal & Elbert, 2015). Individuals who have won a previous brawl will engage in aggression when the opportunity is offered again, also known as the "winner effect" (Hsu et al., 2005; Kloke et al., 2011). This is a well-documented phenomenon in aggression research, with a large body of literature investigating the neurobiological mechanisms underlying this effect. Recently, in male mice, the theory of the winner effect was supported by optogenetic activation and inhibition of the posterior amygdala (PA)-VMHvl pathway, eliciting either initiation or elimination of plasticity events during an RI paradigm (Yan et al., 2024).

Alternatively, perceived resource-holding potential ("RHP", an individual's estimate of winning a proposed fight) has also been studied for its effects on rostral and caudal medial pre-optic area (MPOA) ESR1 cells and their influence on aggressive behavior in male mice. When exposed to their oppressor, defeated males showed higher cFos activation of cMPOA^{ESR1} cells, suggesting that they recognize their opponent can beat them, and inactivation of the cMPOA^{ESR1}-VMHvl pathway increased aggression in these individuals, suggesting a neural mechanism that helps to encode and prevent an individual from participating in a costly behavior (Wei et al., 2023). Further evidence of the effect of repetitive aggressive encounters influencing the motivation to engage in future confrontations includes tests on male mice placed into a shared cage with a conspecific (i.e., the "partition" test, which uses a transparent divider and is often used as a measure of anxiety). In this paradigm, time spent close to the partition indicates a greater motivation to intimidate and engage in agonistic behavior against a subordinate; originally aggressively naïve CBA/Lac mice who aggressed spent a more significant proportion of time near the partition opposite a conspecific (Kudryavtseva, Bondar & Avgustinovich, 2004).

Unfortunately, these effects are relatively understudied in female populations and have been even less studied in nulliparous females. In female animal models, this has been examined exclusively in California mice, rats, and pigs. Females display higher OXT and CORT levels after winning a fight (Trainor et al., 2010) yet are sensitive to the stressful experience of fighting, even when they have won, as measured through cFos co-localization of AVP and OXT cells within the PVN (Kuske et al., 2024). In female pigs, baseline aggressiveness and prior contest experiences influence future agonistic behavior in RI tests (Oldham et al., 2020).

In humans, winning a contest increases testosterone concentrations in males but not females, with males expressing aggression more often (Carré et al., 2013); this has also been modeled in rats, with testosterone being a critical indicator in winner-loser effects, and with females aggressing less (van de Poll et al., 1982). In the present study, our results suggest that ovariectomized female mice who undergo serial sessions of aggressive confrontations are inclined to aggress again in the future, which is in line with historical data supporting this effect in males (Kudryavtseva, Smagin & Bondar, 2011; Oyegbile & Marler, 2005). However, to our knowledge, no studies have been conducted on the influence of the winner effect in predicting the motivation to aggress in female mice, nor on the impact of removing gonadal hormones known to influence aggression. As our experiment used operant conditioning, we cannot rule out the possibility that our females may have experienced escalated aggression because of repetitive encounters against an unfamiliar conspecific, which is a limitation of this methodology. Conducting future studies will yield promising results regarding the reinforcing effect of winner-loser behavior on maladaptive behaviors in this population and help bridge the gap surrounding sex differences under this theory.

It is also essential to touch on frustration in aggressive behavior. Frustration can motivate an individual, and frustrative non-reward (FNR) describes a process by which a behavior (e.g., aggression) is escalated when a perceived reward is withheld after the individual has associatively learned the cue for that reward (Amsel & Roussel, 1952). Additionally, reward omission can lead to robust response patterns (Potegal, 2023). Given that FI schedules of reinforcement require that the individual must learn to associate some cue with a reward, it is plausible that the anticipation of access to the learned reward (i.e., the intruder) prompts an escalated aggressive outburst, as modeled in studies of escalated aggression (Fish et al., 2002; 2005; Miczek, Fish & De Bold, 2003). Additionally, reductions in frustration-heightened aggression have been demonstrated in male mice using anpirtoline, a 5-HT$_{1b}$ receptor agonist, during the RI paradigm (de Almeida, 2002), suggesting that patterns of frustrative-escalated aggression can be modulated.

Interestingly, a recent study in humans exploring tDCS within the ventrolateral PFC (vlPFC) suggests that males and females experience similar levels of aggression when the left vlPFC is stimulated, an area known for frustration-induced escalated aggression (Gallucci et al., 2019). Furthermore, humans and animals both experience FNR to a similar extent, thus having a high potential for translational studies with greater face validity (Potegal, 2023). Given the evidence, it is plausible to assume that in the present study, our ovariectomized females, under a heightened arousal state, could have been motivated to attack an intruder as a measure of frustration, although no reward was omitted. Future studies exploring paradigms of schedule-controlled FNR will add to a growing body of literature supporting the role of FNR in aberrant aggression.

We identified similar significant correlations between components of the fight within males and females and a significant positive correlation between the LTA and fight duration only in females. Significant correlations between the different fighting elements demonstrate a relationship between the fight and both latency and duration of time. This suggests that in females, it takes time to accumulate the number of bites, which further substantiates that an FI schedule can reliably measure both motivation and aggressive performance. The quality of the experience seems different between males and females, but quantitatively, we found no differences between their components, as demonstrated in our aggressive performance measures. This aligns with the consensus that females have qualitatively different experiences, both physiologically and behaviorally, from males (Kuske et al., 2024) and that females display aggression in a context-dependent manner that is dissociable between reproductive states (Oliveira & Bakkar, 2022). Our findings confirm that no quantitative differences were observed.

However, future studies must address these effects and manipulate contexts to substantiate this claim.

Perhaps the most crucial element of this study was that our females were ovariectomized. The previous research on schedule-induced aggression in ovariectomized females has suggested that not only do these females not find the encounter reinforcing (Newman et al., 2019), but that the presence of sex hormones, such as estrogen, is directly responsible for modulating social behaviors, including aggression (French et al., 2013; Frye & Walf, 2004; Frye et al., 2008; Lisk, 1988). This is most evident in studies involving lactating or pregnant females that readily express aggression in the presence of an unfamiliar conspecific as compared to virgins (Abellán-Álvaro et al., 2022; Bosch, 2013; Kinsley et al., 2008; Noirot, Goyens & Buhot, 1975).

Limitations

Importantly, the nature of behavioral studies is highly variable due to a wide range of conditions that must be met in animal work; these include conducting serial operant reinforcement under optimal conditions (by accounting for environmental, endocrine, and social contexts) and consideration of factors like the age of the female aggressors, the amount of prior aggressive experiences, and how the schedule of training impacts how well aggression in females is maintained throughout FI duration. Individual differences in residents can produce highly variable data. However, aggressive behavior patterns are generally consistent, and latencies are typically stable. Furthermore, this pattern of consistency is usually valid for attack bites. To account for these variables, we kept pristine housing and experimental conditions and limited noisy distractions.

Additionally, we utilized the same animal handlers daily for all experiments. These strict conditions have previously been defined as the best contexts for producing replicable behavioral data (Crawley, 2003). Furthermore, isolation can increase aggressive behavior across the lifespan (Biro et al., 2023; Li et al., 2022; Novoa et al., 2021), and our females were not isolated but instead cohabitated with a member of the opposite sex, suggesting that social contact with an unfamiliar intruder was not the reinforcer.

Future Directions

To our knowledge, this study is the first to successfully measure instrumental aggression in ovariectomized female mice. Two other studies have successfully utilized similar behavioral paradigms (e.g., aCPP or RI) in intact and ovariectomized female rats (Börchers et al., 2023; DeBold & Miczek, 1984). Our findings suggest that females will engage in disadvantageous and costly confrontations beyond the adaptive context of gestational or territorial aggression, often seen without a necessary endocrine influence. Interestingly, in a study on aggression in virgin female rats, increased OXT and AVP within the ventral and dorsal lateral septum (LS, respectively) is necessary for aggression during a female intruder test (FIT) and in the CeA via infusions of synthetic AVP/OXT (Oliveira et al., 2021; Oliveira, de Jong & Neumann, 2022). Future behavioral studies (i.e., FI, aCPP, RI) in this population will strengthen the claim that nulliparous females will aggress independently from gonadal influence.

The urge to fight has been historically linked to the hypothalamus (for reviews, see Haller, 2022; Golden et al., 2018). Within the hypothalamus is a region referred to as the hypothalamic attack area (HAA), which can produce indiscriminate attack when stimulated (Roberts & Bergquist, 1968). Within this region lies the VMH, an area extensively investigated for its role in modulating aggression and motivation (for review, see Hashikawa et al., 2017). Recent research on this area suggests that the VMH modulates aggression in a sexually dimorphic manner (Lin et al., 2011; Falkner et al., 2016; Hashikawa et al., 2018; Zhu et al., 2024). A significant secondary component of anticipatory arousal may involve corticotrophin-releasing factor (CRF), a neuropeptide known for mediating stressful experiences through the HPA axis (Chen et al., 2008; Newman et al., 2018; Menzaghi et al., 1993; Zorilla & Koob, 2013. Elevated stress hormones, including CRF, may modulate aggression by acting on various hypothalamic and extra-hypothalamic nuclei (Backström, Thörnqvist & Winberg, 2021; Backström & Winberg, 2013).

CRF modulates endocrine stress responses through the CRF1 receptor (Vale, Rivier & Rivier, 1981). CRF1 antagonists, such as CP-376395, can block these stress responses and alter social behaviors (Chen et al., 2008). Past aggression research involving the CRF1 system has primarily been studied in females under a reproductive context, such as in maternal aggression or defense (Gammie et al., 2004; Gammie, Seasholtz & Stevenson, 2008), and not in the context of appetitive behavior. In contrast, male studies involving CRF1 and aggression suggest that the deletion of CRF protein affects confrontation and anxiolytic behavior (Gammie & Stevenson, 2006; Gammie, Seasholtz & Stevenson, 2008). However, much less is known regarding CRF1 signaling in female aggressive confrontations. To date, no such studies involving CRF1 antagonism in female appetitive aggression have been explored.

A future aim of this experiment is to explore a relationship between CRF1 antagonism and appetitive aggression in ovariectomized females. Previously, in our lab, we have preliminarily demonstrated that CRF1 antagonism attenuates schedule-induced responses for aggression in a dose-dependent manner in males. Given our current findings, it is feasible to suggest that the CRF system may be involved in the motivation to seek aggressive confrontations and may influence the behavioral patterns observed in reinforced aggressive encounters. By replicating these findings in ovariectomized female mice, we can establish a reliable response pattern that is operantly reinforced while further exploring dissociable states between motivation and performance. Similarly, we would expect that there would be no sex differences. As HPA axis activation is contingent upon CRF release, blocking CRF1 receptors may reduce the components of anticipatory arousal. This would demonstrate that CRF, an immediate and fundamental step in activating arousal within the HPA axis, can be manipulated pharmacologically with a CRF1 antagonist. A follow-up assessment should include drug testing and video analysis on a cohort of non-aggressive females to investigate the effects of CRF1 antagonism on prosocial behaviors. Moreover, given that CRF influences anxiety-like behaviors (Takahashi, 2001; Weera et al., 2022), future studies must explore conditioned place aversion (CPA) in conjunction with CRF1 antagonism and its effects on FI responding and aggressive behavior.

Future experiments using this methodology will strengthen the claim that fighting is rewarding and reinforcing in ovariectomized females. Operant conditioning allows aggressive behaviors to be quantified across various responses from the beginning to the end of the interval and stabilizes across sessions. As such, it is a reliable measure of appetitive aggression. Our results corroborate the belief that motivation and performance are dissociable components within aggressive behavior, which should be further investigated. This line of research represents both advancements in understanding the biobehavioral mechanisms of appetitive aggression and the underpinnings of motivational behavior in females. This methodology represents an ethologically relevant model and is translationally applicable to other domains, most notably neuropsychiatric disease and substance use disorder. Furthermore, future studies on the neurobiology of appetitive aggression in females will aid in the development of novel biological targets that are imperative in preventing and treating aggression and comorbid addiction-related disorders.

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