Ovarian hormones act to differentially impact choice for sexual and feeding behavior at the same time in operant choice paradigm

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Abstract

Motivation is what drives animals to choose which stimuli to engage in out of the hundreds of choices they are faced with daily. In terms of sexual behavior, the motivation for females to engage in copulatory behavior apart from their ability to do so is not well studied. Further, the impacts of estradiol on the motivational aspects of sexual and feeding behavior have not been tested in a lab setting. Here, Becker Lab scientists have developed a novel paradigm to study the motivational aspects of female sexual motivation apart from the consummatory aspects of physical mating behaviors. Ovariectomized female rats were trained on an FI15s operant conditioning schedule to nose poke for palatable food pellets or access to a sexually receptive male within the same apparatus. Males were tethered to one side of the apparatus, allowing females to have free range and control the pace of mating interactions. Results showed that when primed with injections of estradiol benzoate and progesterone, females made more responses for access to a sexually receptive mate than for palatable food pellets. Oppositely, females made more responses for pellets when unprimed, and less responses for access to a mate. Females made the same amount of effort across all trials, indicating that circulating levels of estradiol increase the incentive value for cues associated with a male while at the same time decrease the incentive value for pellet cues.

Keywords: motivation, estradiol benzoate, progesterone, sexual behavior, operant conditioning

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Motivation makes it possible for animals to prioritize which behaviors to engage in out of the hundreds of choices they are faced with daily. Motivation is usually understood in pursuit of a reward, like food or sex. The rewarding value of a stimulus depends on the attributed amount of incentive salience, or how much an animal *wants* the stimulus, which influences what stimuli animals will seek out and how hard they will work to attain them (Berridge, 2013). The mesolimbic dopamine system controls an animal's assessment of the salience and value of a stimulus, and if it will seek out the stimulus again. Female mammals' adaptive behaviors are mediated by estradiol and progesterone, which work together to influence feeding behavior and sexual receptivity by decreasing motivation for food and increasing locomotor activity when estradiol levels are high. As recognized in male and female rats in pursuit of a food or sex reward, behavior driving animals toward these stimuli can be divided into consummatory and motivational aspects, which can also be measured differently. Consummatory aspects of sexual behavior pertain to the physical ability to copulate, while motivation refers to the drive to engage in sexual behavior. Fluctuating levels of estradiol have been found to influence a variety of behaviors, with profound effects on sexual and feeding behavior throughout the estrous cycle. Previous research has demonstrated an effect of estradiol and progesterone on the consummatory aspects of feeding and sexual behavior, but the dissociation of these types of behaviors is not largely studied. To this point, studies examining hormonal influences on sexual behavior largely focus around the physical ability and receptive behaviors exhibited by females, but this does not speak to how motivated a female is for engaging in sexual behavior. In addition, because the

effects of these hormones on pursuit of a food or mate choice reward simultaneously has not been studied, this study aims to determine whether hormones oppositely impact motivation for a food or sex reward when animals are given the choice between them at the same time.

What is Motivation?

Motivation is the intrinsic drive to engage in a behavior, usually for a reward (Berridge, 2004). Animals are faced with many choices daily, and motivation makes it possible for them to prioritize which behaviors to engage in. This is most understood in contexts in pursuit of food, water, or sex, where the drive to engage in these kinds of behaviors is imperative to an animal's survival and opportunity to reproduce (Becker, 2009). When it comes to mating, animals display specific behaviors to ensure their genes get successfully passed onto future generations. Male and female animals display different behaviors to ensure reproductive success (Becker, 2009). Among rats, females prefer to pace sexual encounters to ensure reproductive success. Pacing behavior is defined by a series of approaches and withdrawals from the male rat in which to control the timing of received mounts, intromissions, and ejaculations from a male rat (Brandling-Bennett, Blasberg, & Clark, 1999). This type of sexual behavior is rewarding to females, for it enhances reproductive success (Cummings & Becker, 2012). Reinforcement for a reward, like pacing behavior, is assessed by specific circuitry in the brain.

Reward Circuitry in the Brain

The learned valence of a stimulus is controlled by the mesolimbic dopamine system. The mesolimbic pathway is characterized by the ascending dopamine projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) within the ventral striatum area, ultimately projecting to the cerebral cortex (Davis & Benoit, 2013). The VTA is proposed to inform the organism whether a stimulus is salient, and the NAc influences motivation and

craving for the stimulus (Berridge, 2013). The ventral striatum mediates habitual and reflexive responding for rewards and reward cues (Tremblay, Worbe, & Hollerman, 2009). The prefrontal cortex, the final destination of the projection, manages choice of whether the organism will seek out a stimulus (Foy & Foy, 2009). Other areas within the brain such as the hippocampus, amygdala, hypothalamus, and locus coeruleus contribute to associating the experience of a reward with learning and memory, environmental cues, and coordinating the organism's interest in a stimulus with the organism's mood and physiological state (Adinoff, 2004). Altogether, these regions inform the animal of the degree to which the stimulus and its corresponding cues provided a rewarding or aversive experience for the animal and whether it should seek out the stimulus in the future.

Dopamine, a neurotransmitter, plays a crucial role in this circuitry. Neurotransmitter detection techniques have demonstrated that dopamine levels are increased when an animal is engaging in motivated behaviors, and it is also important for assigning motivational value to reward predictive cues (Roitman, Stuber, Phillips, Wightman, & Carelli, 2004). It functions to assign incentive salience for a specific cue, driving the animal to 'want' the stimulus (Berridge, 2013). Neurons that are activated by dopamine project throughout the amygdala, hypothalamus, and other brain regions to attribute goal directed behaviors with the incentive salience, allowing an animal to remember the stimulus and its corresponding cues and allocate the motor function to seek it out (Yoest, Cummings, & Becker, 2016). Within this system, the amount of dopamine projected to regions along the pathway can be influenced by the action of hormones.

Estradiol and Progesterone Within the Mesolimbic Dopamine System

Estradiol and progesterone affect the mesolimbic dopamine system in a marked number of ways. In testing with amphetamines, it has been found that estradiol, the steroid hormone

produced by the ovaries, acts on receptors within the NAc and the striatum to influence dopamine transmission (Becker, 2005). As viewed in ovariectomized female rats, exogenous injections of estradiol that work to mimic sexual receptivity indicate an increase in striatal release of dopamine in response to amphetamine administration compared to ovariectomized females without hormone treatments. As seen when given acutely, or 30 minutes prior to a behavioral test, estradiol has both rapid and long-term effects on these dopamine transmissions within the dorsal striatum: the effects of dopamine transmission induced by amphetamines are increased when given acutely and are more pronounced after prior treatments with estradiol (Becker, 2005). Similarly, injections of exogenous estradiol demonstrate rapid stimulation of dopamine transmission within the NAc. The effects on sexual behavior in these two areas differ: when targeted to the specific region and lesioned from the other, estradiol delivered to the striatum enhances integration of the sensorimotor aspects of a sexual encounter, whereas estradiol delivered to the NAc works to enhance motivational aspects of the behavior (Becker, 2005).

Behavioral testing with injections of progesterone shows that progesterone's effects on striatal transmission of dopamine is only viewed after pretreatments with estradiol, meaning that the two usually work synergistically. Progesterone receptors are a gene product of estradiol; therefore, the response to progesterone is indirectly regulated by estradiol and progesterone alone is insufficient to induce the sexually reflexive posture of lordosis in female rats on its own (Becker, Breedlove, & Crews, 1992). Progesterone in estradiol-primed rats can act to induce sexual receptivity, but with prolonged exposure, it can inhibit it. This is due to the biphasic effects of the hormone, in which levels are naturally cycled within the hypothalamus to down-regulate receptor concentrations once estradiol levels spike to induce sexual receptivity (Becker

et al., 1992). Due to its influence by estrogen receptors, its impacts on striatal dopamine transmission or the impact on motivation have not been largely studied on their own. Similarly, although doses of estradiol alone induce the lordosis response, they do not increase motivation to engage in sexual behavior. Here, progesterone does play an important role in inducing sexual proceptivity: when paired with doses of estradiol, progesterone has been found to induce motivation to engage in sexual behavior in females as viewed through the display of sexually proceptive behaviors of ear wiggling and hopping and darting- traditional, if not outdated measures of sexual motivation in female rats. This means that estradiol paired with progesterone is required in order for a sexual encounter to be rewarding to a female. Looking at characteristics of male and female sexual behavior can help further explain the effects of these hormones on motivation.

Dimensions of Sexual Behavior

Sexual behavior can be dissociated into motivational and consummatory aspects. Although males can engage in sexual behavior at any point, they do so most successfully when they are motivated to engage in sexual behavior and have the physical ability to do so. In studying male sexual behavior, Barry J. Everitt points out that the brain regions involved with male copulatory behavior of mounting, intromitting, and ejaculating are separate from the regions that mediate motivation to engage in sexual behavior (1990). Results these studies found that males were unable to engage in sexual behavior after lesioning of the medial preoptic area (mPOA) within the hypothalamus, but they did not show a change in appetitive sexual responses for a female (Everitt, 1990). This means that the desire to engage in sexual activity with a sexually receptive female was unchanged even when a male was physically unable to perform appropriate copulatory behavior, demonstrating that motivation and ability can be dissociated in

the male. This effect is also shown through failed attempts to mate with the female while displaying persistent efforts to gain access to and investigate the female (Hughes, Everitt, & Herbert, 1990). Furthermore, male rats showed similar behavioral effects after castration: males still showed a strong preference for a receptive female over an ovariectomized female in initial tests and made failed attempts to mount and intromit, demonstrating their motivation to access the female even when physically unable to engage in sexual behavior (Hughes et al., 1990). On the other hand, decreasing ventral-striatal dopamine projections through the use of selective dopamine receptor antagonists reduced the amount of responses a male rat made to gain access to a female, and delayed time in initiating mounts and intromissions (Pfaus & Phillips, 1989). The opposing effects of impacting motivation versus the ability to engage in sexual behavior when selectively inactivating the mPOA or the dopamine projections to the ventral striatum demonstrates that the two behaviors are separate, where the mPOA plays a role in maintaining sexual ability, and the dopamine projections in the ventral striatum impact motivation for sexual activity (Pfaus & Phillips, 1989). It is now known that hypothalamic regions, including the mPOA, are most important for mediating the consummatory aspects of sexual behavior, whereas the mesolimbic dopamine system is most important at mediating the effects of reinforcement involved with motivational behavior (Berridge, 2013). For this paper, a focus will be placed on measuring motivation through this neural framework.

Male and Female Typical Sexual Behavior

Male and female sexual behavior is mediated by gonadal hormone systems. Although both males and females have circulating levels of estradiol, sexually dimorphic organization of the nervous system due to the organizational and activational effects of hormones leads to differing circulating levels of estradiol (Becker, 2009). Male and female mammals also

demonstrate sexual receptivity in different ways due to these hormone differences and adaptive strategies to ensure reproductive success: males have hundreds of gametes and can engage in sexual behavior at any time, whereas females have much fewer gametes, and only engage in sexual behavior when they are ovulating (Becker, 2009). A rat's estrous cycle is characterized by four stages that occur every 4-5 days: proestrus, estrus, metestrus, and diestrus (Paccola, Resende, Stumpp, Miraglia, & Cipriano, 2013). Proestrus and estrus are the two shortest stages in the cycle and are when females are sexually receptive, coinciding with rising levels of estradiol and subsequent rising levels of progesterone. Females experience ovulation during the estrus portion (Paccola et al., 2013). While sexually receptive, females experience a behavioral shift in which locomotor activity is increased, and motivation to obtain food is decreased (Yoest, Cummings, & Becker, 2016). The increase in locomotor activity enables females to engage in behaviors that would make mating more likely, through actively seeking out a mate, spending more time with males, and engaging in copulatory behavior. Similarly, when females are not in estrus, the motivation to gain access to a sexually receptive male is low and the motivation to gain access to food is high (Cummings & Becker, 2012). This could be because it is more energy efficient for a female to spend her time in pursuit of a successful mate when she has the most opportunity to become pregnant (Yoest et al., 2016). Similarly, it is more efficient for her to spend her time and energy on other activities necessary for survival when she will not have as high of an opportunity to become pregnant (Yoest et al., 2016). Similar to male typical sexual behavior, female sexual ability and motivation to engage in copulation are also postulated to be mediated by different brain regions.

Dimensions of Female Sexual Motivation

Like males, female sexual behavior can be divided into consummatory and motivational components. It was previously postulated that a female's motivation for sexual behavior could be measured by her willingness to demonstrate the female copulatory behavior of hopping and darting and ear wiggling, paired with the reflexive behavior of lordosis (Becker, 2009). Cummings and Becker (2012) demonstrated that physical *ability* to engage in copulatory behavior and *motivation for* sexual behavior can be dissociated through their use of an operant conditioning paradigm in which females primed with vehicle injections or estradiol and progesterone had to nose-poke to gain access to a male. They found that while hormone primed, females made significantly more operant responses to gain access to a sexually experienced male. In addition, their latency to enter the male's side of a two-chambered box after the door opened was significantly decreased. When primed with a vehicle injection, females still worked to open the door and gain access to the male side, albeit to a lesser extent. Additionally, they spent significantly more time in the doorway (out of reach of the male) and allowed the door to close without engaging in sexual behavior. These behavioral results suggest that after being primed with the vehicle injection, females could simply enjoy the social interaction while the door is open, refuting the hypothesis that proximity to a male and propensity to demonstrate copulatory and reflexive behavior determines motivation for sexual behavior (Cummings & Becker, 2012).

Hormones and Feeding Behavior

Like sexual behavior, studies have uncovered the influences of gonadal hormones on feeding behavior. Summarized by Eckel, various studies looking at meal size during estrus in female rats has found that when levels of estradiol are low, food intake is high (2011). The same is true for the opposite: when estradiol levels are high, food intake is also low. She recognizes

this difference in terms of meal sizes during both naturally cycling estrus and hormonally induced estrus in ovariectomized female rats (Blaustein & Wade, 1976). Progesterone alone was found to not influence feeding behavior in the same way as estradiol, but was found to inhibit estradiol's anorexigenic effect at large doses in addition to estradiol (Eckel, 2011). Although alone these results work to demonstrate that consummatory behavior is oppositely influenced by levels of estradiol, they do not speak how motivated a female rat is for food given different levels of estradiol and progesterone. As a part of my earlier research in the Becker Lab, we used the paradigm previously used to dissociate the effects of estradiol and progesterone on sexual behavior to determine whether there is a similar dissociation of estradiol's effects on feeding behavior, detailed in the next section.

Studies of Motivation for Food and Sex

Two types of studies to measure motivation for feeding behavior and sexual behavior separately have been completed within the Becker Lab utilizing an operant response paradigm to examine motivation for reward. Here, female rats are trained to nose poke for rewards in active holes paired with a light-cue, which measures how much effort rats are willing to put forth in order to earn the reward. In the first study, we hypothesized that estradiol acts to attenuate the incentive motivation for food-paired cues (Yoest, Shashlo, Cummings, & Becker, 2017, June). In this study, ovariectomized female rats were trained to nose poke for palatable food pellets on various schedules of reinforcement in operant conditioning chambers detailed in the methods section below. Females were tested both while unprimed and primed with estradiol benzoate (EB) and progesterone (P) to induce sexual receptivity. We found results consistent with the hypothesis: while EB and P priming successfully induced sexual receptivity indicated through

lavage samples, it also decreased motivation for food rewards indicated through a decreased amount of nose pokes on fixed interval and progressive ratio schedules (Yoest et al., 2017, June).

In addition to testing motivation for palatable pellets, Cummings and Becker (2012) hypothesized that hormone priming with EB and P would increase incentive motivation for mate-paired cues. They developed a dual chambered apparatus (*Figure 2*) utilizing nose-poke holes to test how much effort ovariectomized, female rats were willing to make to gain access to a sexually receptive male. Females were trained on various schedules of reinforcement to make responses in nose-poke holes paired with light cues to open a sliding door, in order to gain access to a male. The male was tethered to the far side of the apparatus, ensuring the female had free range of both chambers while the male could only move around one side, deemed the male side of the apparatus. The most interesting results researchers found where that females worked harder to attain access to the male when hormone primed (Cummings & Becker, 2012). They also found that when unprimed, females put forth the effort to nose poke to open the sliding door between the chambers, and spent more time in view of, but just out of reach of the male. This suggests that females could be more interested in the social interaction, rather than engaging in sexual behavior when unprimed, refuting a previous hypothesis that proximity to a male influences motivation to engage in sexual behavior.

This current study aims to combine previous paradigms developed by Cummings and Becker (2012) to determine the effects of EB and P on the motivational and consummatory aspects of both feeding and sexual behavior, but within the same apparatus. Both previous studies completed in the lab found that EB and P oppositely impact the motivational and consummatory aspects for feeding and sexual behavior while in separate paradigms, so researchers now look to combine these paradigms in order to replicate the choices that animals

have within a natural setting. We hypothesize that, when given the choice between palatable food pellets and access to a sexually receptive male, ovariectomized, female rats hormone primed with injections of EB and P will increase incentive value for mate-paired cues *while at the same time* decreasing the value of the food-paired cues when both response options are available.

Method

Animals

Experimental animals used were 10 female rats, 55-60 days of age upon arrival, obtained from Charles River (Portage, MI). Fifteen proven-breeder, male Long-Evans rats, also obtained from Charles River (Portage, MI) were used as the stimulus males. Animals were pair-housed by sex in Allentown, Inc. NexGen laboratory cages maintained on a 14:10 light: dark cycle, in which lights would go off at 1:00 pm. Animals had free access to rat chow and water. Females were food restricted four hours prior to testing, and food hoppers were returned immediately after testing.

Ovariectomy

Females were bilaterally ovariectomized two weeks before training. Surgeries were performed with a single dorsal incision using 5% isoflurane anesthesia via inhalation. Vaginal lavage samples were collected daily beginning 10 days after surgery using saline solution to ensure surgeries were effective. Females were cycled using hormone priming to induce sexual receptivity. Beginning 48 hours before testing, hormone injections consisted of two injections of 5μg of 17 β-estradiol Benzoate (EB) in 0.1 ml peanut oil administered subcutaneously 24 hours apart, followed by an injection of 500 μg progesterone (P) in 0.1 ml peanut oil, occurring 4-6 hours before testing. Hormone injections were administered during week 2 of pellet training and continued throughout the training and testing schedule.

Apparatus

Two separate types of apparatuses were utilized for training in this experiment, and a third apparatus that combines each training apparatus was utilized for testing. Each apparatus was designed and fabricated within our laboratory using standard components form MedPC (Fairfax, VT) and AnyMaze (Stoelting Co, Inc; Wood Dale, IL).

The first apparatus was utilized for training in responding for palatable food pellets (pellet chamber, Figure 1). A single, square, acrylic chamber was set up containing two nose poke holes with a food dish fixed between them on one wall of the chamber. One nose poke hole was designated as the inactive hole, and the other was designated as the active hole that controlled the dispersal of a pellet into the dish. A light cue, located directly above the active nose poke hole, would illuminate for 1 second after the active hole is triggered, indicated through a break in an infrared beam running across the active nose-poke hoe. During active sessions, a house light would illuminate the entire chamber. During 15 second time-out sessions activated after an animal failed or completed a trial, the flood light would go off. Females were trained to nose poke in the active hole for a pellet reward on increasing schedules of reinforcement, detailed in the next section.

The second apparatus was utilized for training in responding for a mate. This apparatus is the same one utilized by Cummings and Becker in their 2012 experiment, which was designed utilizing two chambers separated by a sliding door (mate chamber, Figure 2). Similar to the first apparatus, one chamber was designated as the female side of the apparatus where a set of nose-poke holes were located. Nose poke holes were positioned on the wall adjacent to the sliding door, 3 inches from the bottom of the cage and 6 inches apart from each other. One inch above each nose poke hole is a light that is triggered for 1 second as either nose poke hole is activated,

indicated by a break in an infrared beam. The other compartment, separated by the sliding door, was designated as the male side, where a male rat was tethered to the far side of the cage using a stainless-steel wire connected to a felt vest worn by the male. The female was trained to nose poke in the active hole to open the sliding door between the two chambers, where she had free access to move between them at her own pace. The sliding door would automatically close after she returned back to the female side and remained there for at least 2 seconds. Animal tracking was recorded by AnyMaze software (Wood Dale, IL).

In the final apparatus designed for testing, a dual chambered apparatus similar to the second apparatus was utilized (choice chamber, Figure 3). This third apparatus was also divided into two chambers separated by a sliding door operated by nose-poke holes. The female side of this apparatus featured two sets of nose-poke holes positioned on adjacent walls of the chamber: one set featured an active and inactive hole with corresponding light cues that triggered the disbursal of a food pellet into a food dish, and the other set featured an active and inactive hole with corresponding light cues that controlled the sliding door between the male and female side of the chambers. The nose-poke holes and cue lights were oriented in the same positions as in the training chambers. After training in each of the first two chambers separately, the female was briefly trained, and then tested in this third chamber, where she was given the choice between receiving a pellet or mate reward within the same apparatus.

Training

Following surgery, females were trained to nose poke for food pellets in the pellet chamber on various fixed ratio (FR) and fixed interval (FI) schedules in 30-minute sessions. While training for pellets, females were also trained to nose poke in the pacing chambers for access to a sexually receptive male. Training lasted for a total duration of five weeks, in which

each female was given two days of pellet training in the pellet chambers and one day of pacing training in the pacing chamber during a 5-day training week (Figure 4). Females were given one day of pellet training when primed with an acute injection of estradiol, and a day when unprimed. Females trained in the pacing chambers when primed with EB and P. After being trained when primed, females were given at least 72 hours until next dose of EB.

Females began week 1 pellet training on an FR1 schedule, in which they were required to make a single nose poke in the active hole to receive a pellet, followed by a 15 second time out period where behavior is recorded but no pellets are delivered. After two days, animals who mastered the FR1 schedule, deemed as receiving more than 20 rewards in a session, began week 2 training on an FR5 schedule where they had to make five nose pokes in the active hole in order to receive a reward, followed by the 15 second time out period. After mastering the FR5 schedule indicated by receiving 20 or more rewards, females were then moved up to a fixed Interval 15 second (FI15) schedule, which is a schedule of fifteen second active intervals followed by fifteen seconds of an inactive time out where behavior is recorded but no rewards are delivered. Females were trained to make an initial nose poke to begin the fifteen second interval. Within that active interval, the female is allowed to make as many nose pokes as she desires, and all nose pokes are counted and result in presentation of the cue light. The female would only receive a pellet if she made at least one nose poke within a 5 second latency period initiated after the 15 second interval ends. Motivation for pellets was measured through number of rewards, or food pellets, achieved during the 30-minute fixed interval sessions. Motivation was also measured in terms of number of responses recorded during each interval, and responses on the inactive nose poke hole were measured to account for variation in locomotor behavior.

While training for pellets, females were also trained to nose poke in the pacing chambers for access to a male. Training for pacing slightly differed from pellet training during the first two weeks: females began Pavlovian conditioning in week 1, where the light cue would signal and the sliding door would open automatically to give females sexual experience and orient them to the action of the sliding door paired with the light cue. During week 2, females started out the session with shaping, in which experimenters would manually signal the light cue and open the door until the female made a nose poke in the active hole to open the door herself. After this, the session would continue on an FR1 schedule controlled by the female. During week 3, depending on their mastery of the FR schedules and timing during the week of pellet training, females were either trained on an FR5 or an FI15s schedule. In the remaining two weeks, females were trained on the FI15s interval. In this, the female was required to make a nose poke in the 5 second delay following the 15 second interval in order to open the sliding door. The female was then allowed to roam freely through both chambers, gaining access to the tethered male on the other side. After the female moved back and stayed on the female side for at least 2 seconds, the sliding door would close and begin a new trial. Trials were defined by the start of the session, and after each time the door closes during the 30-minute session. It is important to note that males were rotated so that each female was paired with a different male each session for training and was also tested with a novel male to minimize confounding effects of partner preference.

After the 5 weeks of training and mastering an FI15s schedule in both chambers, females were moved to the final, choice chambers, where they would spend 3 weeks being trained then tested for a choice between a pellet or mate reward. Females were given a week of orientation in this novel chamber as a refresher with either set of nose poke holes. During this week of orientation females did not respond for pellets or interact with the pellet nose poke holes, so an

acute session of pellet training was implemented in the following week to remind females of its action. During week 2, females were trained twice, once after hormone priming and once when unprimed. When females were not hormone primed, the testing session started with a 10-minute session in the pellet-only chamber, followed by 10 minutes in the choice chamber with a piece of acrylic covering the nose-poke holes used to respond for access to the mate in order to ensure animals were familiar with responding for pellets in these novel chambers. After this initial period the acrylic was removed, and animals were allowed to respond for either reward. After re-associating both nose poke holes with their rewards, females were tested twice in week 3, once when primed and unprimed, in the full 30-minute session with both sets of nose poke holes and rewards available the whole time. This week of testing was recorded and analyzed for the results of the study.

Statistical Analyses

Two animals were excluded from the study due to failure to master operant conditioning tasks, leaving a final N= 8 females who were used for statistical analysis. Using the results from these females, two degrees of analysis were employed in order to examine the effects of hormone priming within the choice paradigm for (1) choice and (2) motivation for each trial type. First, choice was divided into three measures assessing whether females demonstrate a preference for either mate or pellet trials when primed and unprimed. The average percent of either mate or pellet trials initiated within each 30-minute choice test were collected for analysis using a 2-way repeated measures ANOVA of trials initiated with two within subjects factors of treatment (primed or unprimed) and trial type (mate or pellet). Furthermore, the average percent of each trial type completed was measured using a paired t-test to compare the amount of each trial type (mate and pellet) completed or failed when primed and unprimed.

Along with understanding how hormones impact trial choice, measures were utilized to determine the effect of priming on motivation for a pellet or sex reward. In the first of these two measures, a two-way repeated measures ANOVA was utilized to assess the average number of responses per interval for each type of interval (mate or pellet) and treatment (primed or unprimed).

In addition, the amount of time the animal spent in each area of the chamber was quantified to determine the effect of hormone priming on social behavior. The duration of time spent was measured on 3 different areas of the apparatus- the female side, male side, and time spent in the doorway between each side in view, but just out of reach of the male. The amount of time spent in each of the sides was not normally distributed, therefore non-parametric tests were used to analyze duration results. The total time (seconds) that female rats spent on each of the three areas of the cage (female side, door, and male side) was compared with a paired Wilcoxon Sign Rank test based on treatment (primed or unprimed).

Results

Effect of Hormone Priming on Choice Between Food and Sex

The results of the analysis show that there is a significant effect of hormone priming on the trial types initiated and completed. There was no significant main effect of either hormone priming or trials initiated, however, there was a significant interaction between hormone priming and the type of trial initiated ($F(14,14) = 11.42$, $p < 0.001$) showing that females initiate significantly more mate trials than pellet trials when hormone primed ($p = 0.01$) and oppositely, initiate significantly more pellet trials than mate trials when unprimed ($p = 0.01$) (Figure 5).

There is a significant effect of hormone priming on percentage of trials completed for both mate ($t(7) = 4.21$, $p < 0.005 = 4.21$) and pellet ($t(7) = 3.95$, $p = 0.005$) trials. These data

show that, in addition to initiating more mate trials overall, primed females completed significantly more mate trials than failed mate trials and completed significantly more pellet trials than failed pellet trials when unprimed (Figure 6 a & b).

Effect of Hormone Priming on Motivation for Food or Sex

Similarly, while there was no significant main effect of either hormone priming or trial type, the results demonstrate that there is a significant interaction between hormone priming and trial type on responses per interval of each trial type ($F(1,7) = 27.63$, $p = 0.001$). Further analysis revealed that hormone primed females made significantly more responses during mate trials ($p = 0.046$) and less responses during pellet trials ($p = 0.006$). Unprimed females also made significantly more responses during pellet trials compared to mate trials ($p < 0.0001$), but there was no significant difference in responding during pellet and mate trials when females were hormone primed ($p = 0.31$), indicating that the shift in motivation during sexual receptivity is driven by a decrease in motivation for pellets but an increase in motivation for access to a mate (Figure 7).

Furthermore, although time spent on both the female and male side of the apparatus did not reach statistical significance, time spent on the male side of the cage did indicate a trend toward significance. Time spent on the female side was roughly the same between treatment groups ($p = .98$). Time spent in the male side demonstrated an increasing trend for females to spend more time on the male side when primed than when unprimed ($p = 0.55$). Time spent in the door was significant between treatment groups ($p = 0.039$), in which unprimed females spent significantly more time in the doorway, in view of but just out of reach of the male, than when primed (Figure 8 a, b, & c). These data help further explain the interception of social and sexual motivation that influences choice, as discussed further in the next section

Discussion

Due to the scarcity of research investigating female sexual motivation apart from physical ability, this study introduces a novel paradigm that enables assessment of motivation for an incentive cue and paired reward as influenced by hormone treatment in an apparatus that reflects adaptive choice behavior in a natural environment. Shifts in motivation in responding for a food reward or mate reward have been tested separately in a single-task apparatus and have been found to be inversely proportional to each other: as estradiol and progesterone levels increase, motivation in responding for food decreases and responding for access to a sexually receptive mate increases, and vice versa. This study supports the hypothesis that estradiol and progesterone act to increase sexual motivation when ovulating *while at the same time* decreasing motivation for feeding behavior as a function of evolutionary adaptation when given the choice between stimuli.

Results of priming on choice between food and sex reveal that females initiate an equal number of trials regardless of priming. The major differences within trials initiated were the trial type based on priming. This is a significant finding for the basis of the other measures, for it suggests that overall effort is unchanged in primed and unprimed animals, indicating that this paradigm provides a way in which to assay a proportional, inverse shift in motivation for specific trial type, rather than global changes in motivation. Females made a greater amount of nose pokes for the mate and made less nose pokes for pellets when primed and did the opposite when unprimed. Due to the nature of the fixed interval operant conditioning schedule allowing females to make a variable amount of nose pokes within the 15s timeframe, animals make more nose pokes when more motivated for a reward. Moving a step further, primed females completed a greater amount of mate trials than failed mate trials, and the same is true for

unprimed females in responding for pellets. This suggests that primed females are more motivated to sustain consistent responding until the full completion of the interval, and unprimed females are more motivated to complete pellet intervals until receipt of a pellet. As shown through the number of responses made per interval, females responded more, and therefore worked harder, for access to the mate when primed and for pellets when unprimed. We can also deduce that this is separate from locomotor behavior: although locomotor behavior is an observed characteristic of sexual receptivity, the data do not show an increase in responding for both mate and pellet trials when primed. Because primed females did not demonstrate an overall increase in responding compared to unprimed females, measuring both how hard a female will work and how much she is able to sustain her responding to access a reward is an accurate measure of motivation in this form.

Time spent in each area of the cage demonstrates an interesting interplay of social and sexual motivation. The first measure, time spent on the female side, was roughly equal among both treatment groups. This is because both sets of nose-poke holes are located on the female side of the apparatus and therefore where each trial type is initiated. This is also consistent with above results indicating that there was no significant difference in the amount of trials initiated between treatment groups. Time spent in door was found to be significantly greater for the unprimed females. As discussed in Cummings and Becker (2012), unreceptive, unprimed females are still motivated by social behavior to respond for access to the male. However, instead of moving over to the male side or engaging in sexual interaction with the male, unprimed females will more often sit in the doorframe, out of reach of the male. This allows her to fulfill a social desire to see the rat on the other side of the door. Because females are unreceptive and do not interact with the male in these trials, it also shows that the desire to see

the male is not due to sexual motivation. Lastly, the time spent on the male side of the apparatus followed an increasing trend for the primed females. When females are primed, mimicking the hormonal surges associated with ovulation in intact females, they are motivated to become pregnant in this time, and will ensure a sufficient number of mounts and intromissions is received during trials with respect to pacing behavior. This demonstrates how sexual receptivity can influence females to spend more time in direct contact with a male. Possible reasons the metric did not reach statistical significance regard individual differences in the stimulus males uses and the specific male paired with the female on test day.

Some limitations of the study design regard the stimulus male used and the shifting of paradigms throughout training and testing. First, it was uncovered during training that some of the males would fail to mount the sexually receptive female at all during a session, even after given sex experience with stimulus females. If males failed to mount the female within 10 minutes of a training session in which females initiated mate trials, the male was removed and replaced with a different one. Even in the case that a male would show consistent mounts and intromissions across sessions, there were sessions in which males refused to mount, but would resume normal mating behavior in a following session, although this occurred very infrequently. Upon the week of testing in the choice chambers, we were left using 7 of the 15 males. Further, some of the males were more aggressive when approaching a female, and others were subdued. Although extremities were excluded from the study and males were rotated so that females were exposed to a variety of mating behavior, individual differences exhibited by the males and partner preference by females must still be considered when quantifying the female's motivation to work for access to the male, approaches during mate trials, and behavior while interacting with the male.

Furthermore, variances in cage design also seemed to have an initial impact on behavior, most notably between the pellet and choice chambers. The choice and pacing chambers were most similar, as both were the same body design with a male on one side, whereas the female was alone in the single-chambered pellet apparatus. We worked to mitigate environmental impacts on nose poking trends by resuming training in pellet chambers before introducing both reward options within the same paradigm. When given the choice of both options in the choice chambers, we manually disbursed a pellet into the pellet dish at the beginning of the session to remind females of its function. This was effective in reinitiating responding for pellets by the week of testing but took a few weeks of additional training to achieve.

Another difference between the pellet and choice chambers regarded the house light and white noise. The pellet chambers contained white noise would signal the beginning and end of the 30-minute session and would stay on throughout the entire session. They also contained a light that would illuminate the entire apparatus during a 30-minute training session and would go off during the 15 second latency period when an interval was been completed or failed. This worked to notify animals when they had failed an interval and when they could resume nose poking, mitigating confusion. The pacing and choice chambers did not contain either cues. Due to this, females would sometimes become confused why the nose-poke light cue would not illuminate when poking in the paired nose-poke hole due to a lack of signaling that it was inactive, or when multiple failed trials resulted in lack of reward even after a large number of responses had been made. Some females would give up responding on either of the tasks for a duration of time after assessing that it does not work. Incorporating better cues to indicate failure in the pacing and choice chambers could further exclude confusion with the status of active and inactive intervals and poke-holes, helping aid in learning consistent behavior.

Further studies to be completed in our lab will utilize the same cohort of animals and paradigm to examine DA projections in the NAc shell *in vivo* using Fast-Scan Cyclic Voltammetry (FSCV) to help us understand how DA encodes the incentive value associated with each stimulus as compared to motivation. Voltammetry is used as a way to measure neurotransmission in response to an event occurring on a sub-second timescale (Roitman et al., 2004). This will provide valuable insight in how changes in DA transmission are linked to specific behaviors that are occurring in real time. Because DA release and reuptake is associated with the motivational value associated with a stimulus, tracking changes in DA projection allows us to measure the degree of positive valence that is attributed to the mate-paired and pellet-paired cues present in the choice chamber as affected by priming. Consistent with our results from this study, we expect to see a greater increase in DA release in the NAc upon presentation of the mate-paired cues than food-paired when females are primed, and similarly, we expect to see a greater increase of DA projection upon presentation of food-paired cues than mate-paired cues when females are unprimed.

Altogether, this study presents a novel approach in understanding how estradiol and progesterone affect motivation for food and sex when given a choice. The results of this study and further research with this paradigm contribute to the growing body of work surrounding understanding how hormones influence motivation. Understanding how females approach sexual interaction in a lab setting can provide a framework for measuring partner preference and social motivation apart from sexual motivation. The paradigm can also be applied broadly due to its ability to measure motivation separate from consummatory aspects of sexual behavior, which gives it considerable promise to uncover longstanding questions surrounding disorders in sexual dysfunction in both males and females.

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Figures

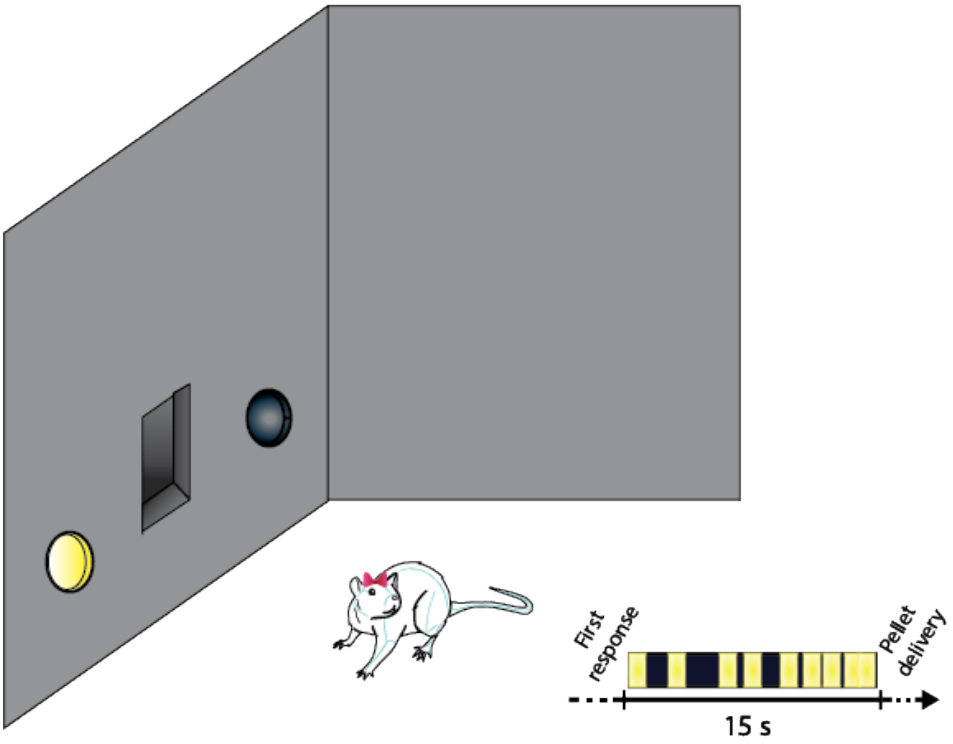

Figure 1. the pellet chamber features a set of nose poke holes affixed to one side of the square apparatus. The female was trained to poke on an FI15s schedule in which she could make any number of pokes between the time frame but would only receive a reward if she made a response during a 5s latency period beginning after the 15s interval.

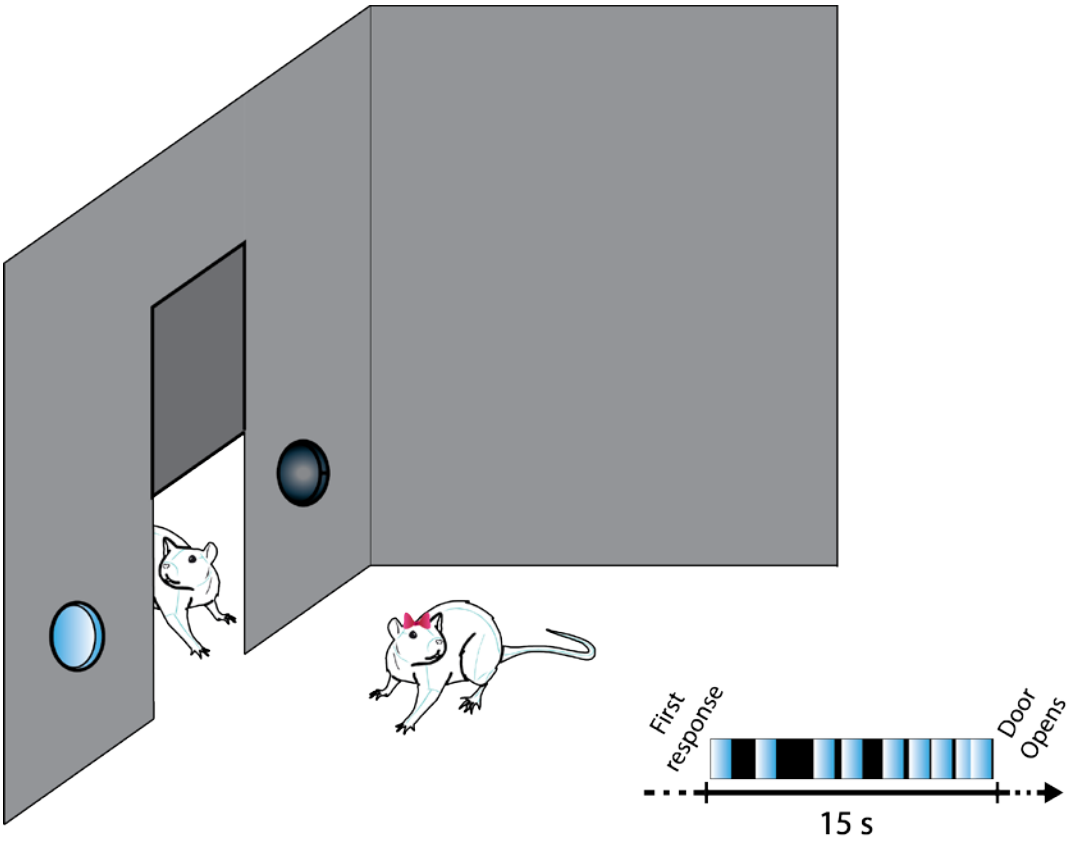

Figure 2. The pacing chamber features a set of nose poke holes that operates a sliding door for access to a male. The females were trained to make responses on the same FI15s as the pellet chamber.

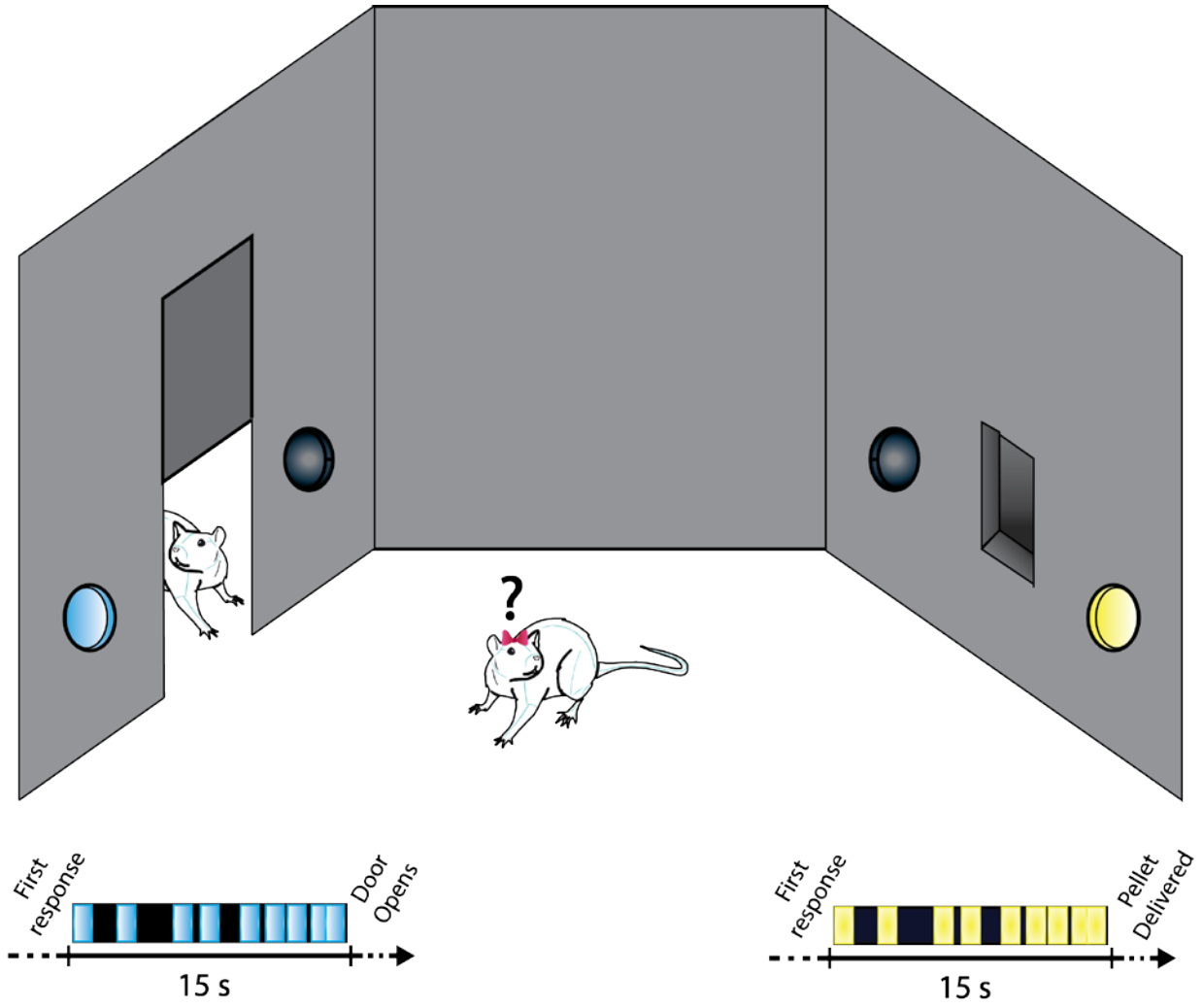

Figure 3. The choice chamber features two sets of nose poke holes affixed to different walls of the chamber. Both sets of nose poke holes feature an active hole that controls the associated reward, and one inactive hole that does not. One set of nose poke holes elicits a pellet reward, the other set operates a sliding door for access to a sexually receptive male. A cue light is associated with each active nose poke hole that will illuminate for 1 second after the rat has poked in it. An active nose poke is indicated by the break in an infrared beam running across the nose poke hole.

Pellet & Pacing Training Schedule	
Week 1	• Pellet: FR1 (2 days) • Pacing: Pavlovian Conditioning (1 day)
Week 2	• Pellet: FR5 (2 days) • Pacing: Shaping & FR1 (1 day)
Week 3	• Pellet: FR5/FI15 (2 days) • Pacing: FR5 (1 day)
Week 4	• Pellet: FI15 (2 days) • Pacing: FI15 (1 day)
Week 5	• Pellet: FI15 (2 days) • Pacing: FI15 (1 day)
Choice Training & Testing Schedule	
Week 1	• Primed: 30 min choice session (1 day)
Week 2	• Primed: 30 min full choice session (1 day) • Unprimed: 10 mins pellet in pellet chamber, 15 mins pellet only in choice chamber, 30 mins full choice session (1 day)
Week 3	• Primed: full 30 min choice test (1 day) • Unprimed: full 30 min choice test (1 day)

Figure 4. The total 5-week training schedule within pellet and pacing chambers included 2 days of pellet training and 1 day of pacing training each day per week. Training for pellet or pacing was offset per day to align with hormone priming injections and scheduled either pacing or pellet training on different days of the week, giving each animal a day of training for pellet when both primed and unprimed, and trained for pacing only when primed. During choice training and testing, animals were trained for 2 weeks: animals were trained on the full 30 min test during week 1 and were trained between the pellet and choice chambers on week 2. Animals were tested on week 3 in the full 30 min choice test, once when primed and unprimed, in which data was collected for analysis.

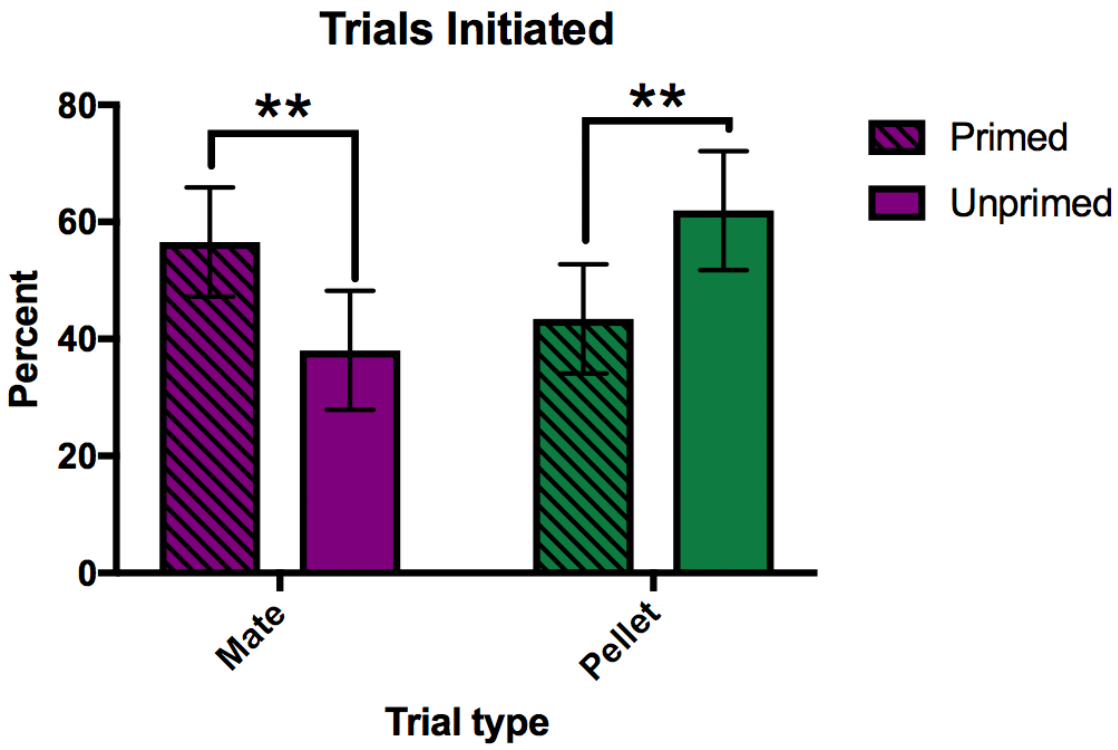

Figure 5. Percent of trials initiated: females initiated significantly more mate trials when primed (striped bars) than when unprimed (solid bars) and initiated significantly more pellet trials when unprimed than when primed. Because females initiated an equal number of trials overall, the difference in trial type initiated when primed and unprimed provides a baseline in which to assay a proportional shift in motivation, where females were more motivated to initiate mate trials when primed and more motivated to initiate pellet trials when unprimed.

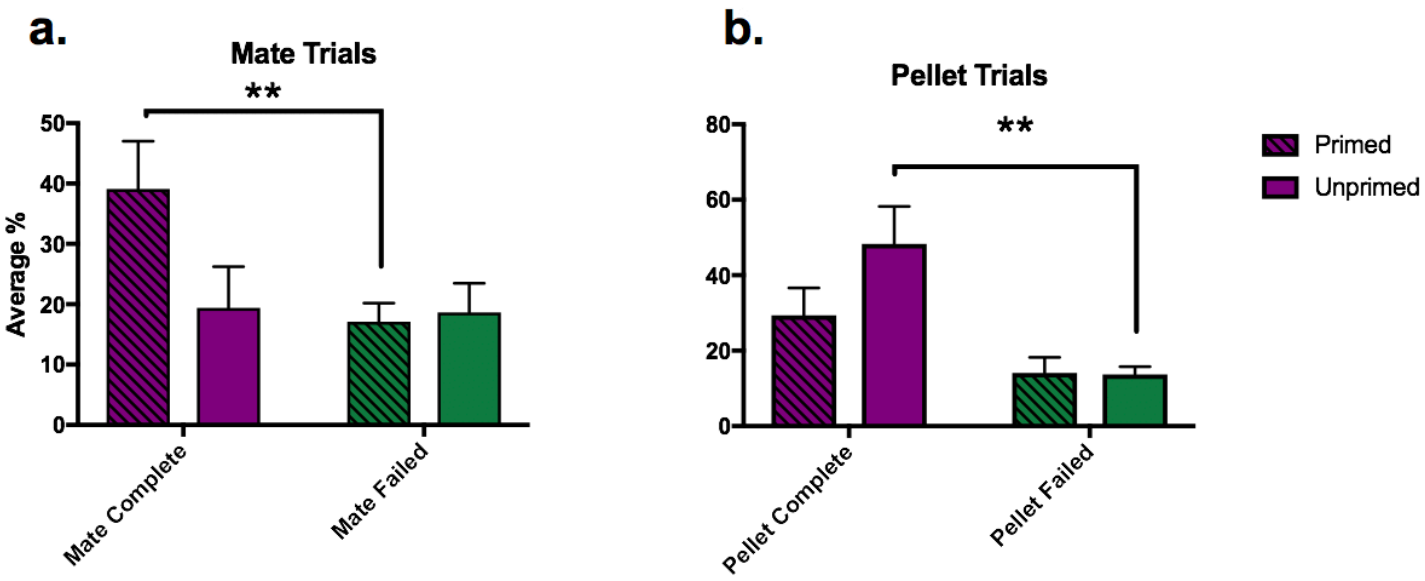

Figure 6. Average percent of trials completed and failed: a.) females completed more mate trials than failed mate trials when hormone primed (striped bars). In *b).*, the same is true for the opposite: females complete more pellet trials than failed pellet trials when unprimed (solid bars), showing that females are more motivated to sustain nose poking in order to complete mate trials when primed and pellet trials when unprimed for successful receipt of a reward.

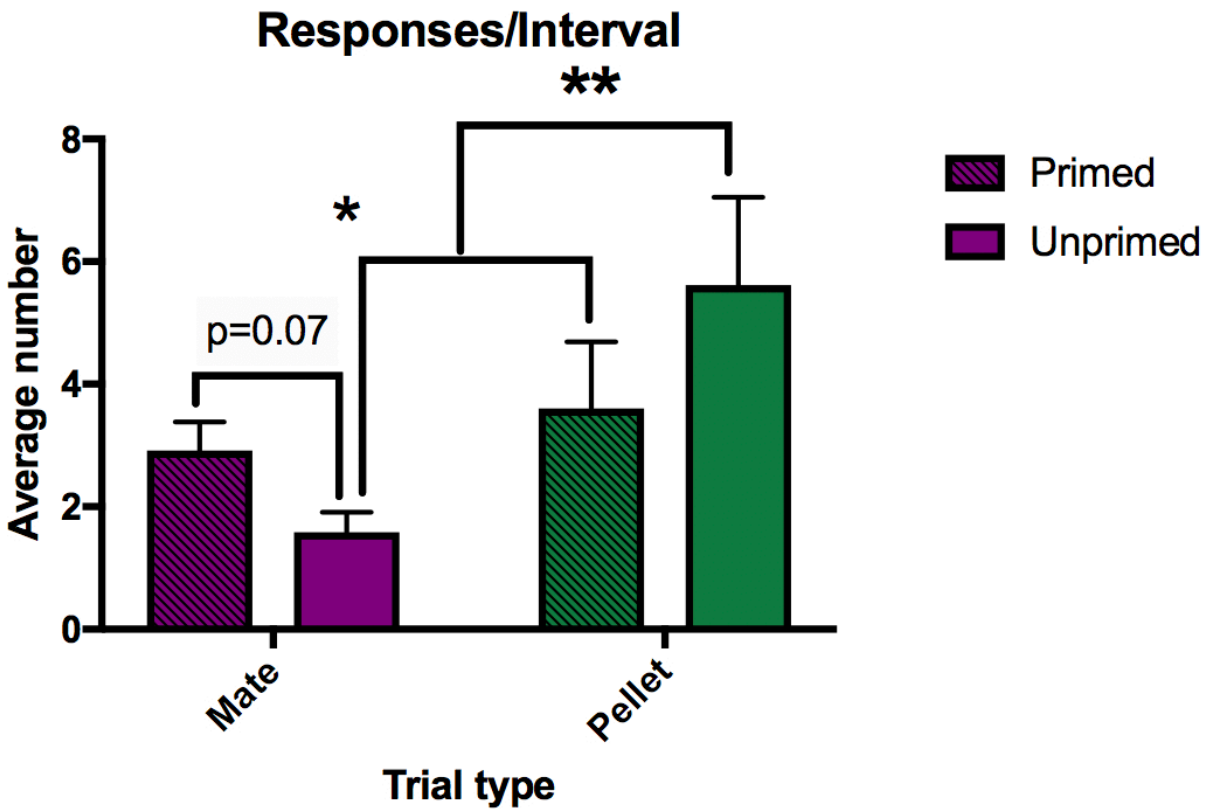

Figure 7. Average number of responses per interval: Females made more responses for pellets when unprimed (solid bars) than when primed (striped bars). Females also made more responses for pellet trials than mate trials when unprimed. Further, females also made more responses for pellets when primed than for males when unprimed. Finally, females made more responses for pellet trials when unprimed than for mate trials when primed. This demonstrates that priming impacts how hard a female will work for each stimulus as seen through average number of nose pokes per interval, most notably between pellet trials when unprimed and mate when primed.

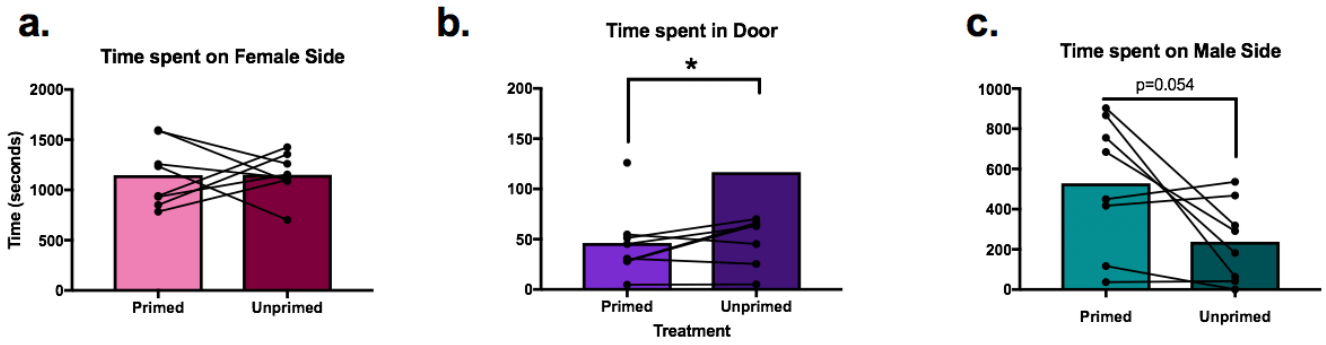

Figure 8. Time spent in each area of the apparatus: a.) females spent roughly the same amount of time on the female side when primed and unprimed. b.) females spent more time in the door frame, out of reach of the male but within his view when unprimed than when primed. c.) females showed a trend toward significance of spending more time on the male side when primed than when unprimed. Individual differences and partner preference are discussed as possibilities for this trend.