Effects of blocking estradiol during impulsive choice and timing in female rats

by

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Abstract

Previous research showed that female rats made more self-controlled choices and were more accurate and precise timing short delays during the proestrus phase of the estrous cycle (cycle of sexual fertility in rodents), which is when estradiol levels are highest compared to the other phases of the estrous cycle. The same pattern of decision making has occurred in human females based on self-reported menstrual cycles as well. These results suggest that estradiol may influence decision making in rats and humans. To test this hypothesis, rats received 0 mg/kg and 1 mg/kg of an estradiol antagonist before completing an impulsive choice task with timing assessments for multiple sessions. Overall, rats did not complete all trials, possibly due to satiety within the sessions, and the delay length and order of delays presented in the choice task may have negatively influenced decision-making above and beyond the effects of estrous cycle. On 0 mg/kg, rats in estrus made the most self-controlled choices, but there were no differences in self-controlled choices across estrous phases between 0 and 1 mg/kg. This suggests that natural increases in progesterone may result in increased self-control and estradiol may not reliably influence impulsive choice. However, blocking estradiol did affect temporal perception, although not in a systematic manner. The length of the timing interval and the reward values associated with each delay may have interacted with estradiol's effects on temporal perception. In sum, estrous cycle effects on decision-making and timing may be mediated by delay length, delay order, and associated reward values.

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Chapter 1 - **Introduction**

Sex differences are not new. Many scientific fields have sought to understand how females and males differ for decades. Converging evidence from geneticists, evolutionary biologists, psychologists, and related fields show that females and males differ. But, few studies examine females only with the intent to understand how female sex hormones influence the observed sex differences. In the decision-making field, researchers have investigated the differences in choice behavior between female and male rodents, but no studies have evaluated if female sex hormones influence impulsive decision-making.

Previously observed sex differences may stem from capturing the females' decisions during specific phases of the estrous cycle, the recurring cycle of sexual fertility in rodents. Multiple sex hormones fluctuate across the estrous cycle and these could potentially impact females' choices. The influence of these sex hormones on decision making may be an evolutionary advantage for females. The sex differences decision-making literature to date suggests females are more self-controlled than males. Self-control may be beneficial to the individual because waiting can result in a larger reward. Occasionally, the option available immediately is more advantageous than waiting for the other outcome. Specifically in unpredictable environments, the immediate outcome is the more certain option, but in stable or reliable environments, waiting for the larger reward is optimal. Females may be more self-controlled for a variety of reasons like reproduction where waiting between mating events increases reproductive success.

Understanding the mechanism of the effects of female sex hormones on the neuronal processes involved in decision-making begins to address a gap in knowledge surrounding female health. Until recently, sex as a biological variable has been largely ignored in animal studies. In

fact, much of neuroscience research is conducted in male animals only (Beery & Zucker, 2011). The lack of research including female animals results in limited information about the effects of female reproductive hormone cycling on the brain, cognition, and behavioral processes. The National Institutes of Health (NIH) released a statement outlining the importance of including female animals to further human health holistically (Clayton & Collins, 2014). A similar emphasis in NIH-funded clinical research occurred more than twenty years ago and resulted in new discoveries such as sex-specific dosing needed for drugs and different preventative effects of medication in males and females (Clayton & Collins, 2014). Elucidating the mechanism of the effects of female sex hormones on impulsive choice behavior will further current knowledge of sex differences in prevalence, progression, and treatment outcomes of disorders and maladaptive behaviors associated with impulsive decision-making and eventually contribute to new clinical therapies for sex-specific treatments of impulse control disorders. These outcomes underline the importance of sex as a biological variable in preclinical research.

To begin to understand sex hormone effects on impulsive decision-making, a preliminary study was conducted to measure estrous cycles every day during an impulsive choice task in a group of female rats (Panfil, Deavours, & Kirkpatrick, in preparation). When females were in proestrus, they made more self-controlled choices and had a steeper slope than rats in estrus or metestrus/diestrus (see *Estrous Effects on Behavior*). Altogether, this preliminary data suggests that female rats' decision-making may be influenced by the fluctuations in sex hormones across the estrous cycle. The proposed experiment seeks to target the mechanism through which these fluctuations in behavior may occur.

Estrous Stages and Sex Hormones

Female rats progress through the estrous cycle every 4 to 5 days on average. However, for about 20-30% of females, the cycle lasts longer than 4 to 5 days with more time spent in estrus or diestrus (Hubscher, Brooks, & Johnson, 2005; Marcondes, Bianchi, & Tanno, 2002; Westwood, 2008). To track the estrous cycle, researchers collect vaginal samples daily from female rats for at least one to two cycles (Marcondes et al., 2002). The cycle is typically divided into four stages that repeat in a set order: proestrus → estrus → metestrus → diestrus (Marcondes et al., 2002; Westwood, 2008). Each stage is characterized by different cell types present. The changes in cell morphology reflect the fluctuations in estradiol (a form of estrogen) and progesterone released from the ovarian follicles (Cora, Kooistra, & Travlos, 2015). These hormones begin to fluctuate when females reach sexual maturity, which can begin as early as post-natal days (PND) 35-40 (Sengupta, 2013). Rats typically reach sexual maturity at 6 or 7 weeks old (Adams & Boice, 1983; Long & Evans, 1920). Regularity in cycle is typically established by PND 70 but can be affected by multiple environmental stimuli. For example, female mice and rats cycle more regularly when males are housed in the same room (Whitten, 1956). In addition, the estrous cycle is sensitive to the length of light exposure in the light:dark cycle. More light exposure lengthens cycles and causes irregularity, and continuous light exposure causes rats to remain in anestrus (no cycling; Cora et al., 2015; Sridaran & McCormack, 1979).

Proestrus is a short phase lasting 12-14 hours on average and is the period in which the vagina, uterus, and ovaries are preparing for implantation of a fertilized egg (Hubscher et al., 2005; Westwood, 2008). Proestrus is characterized by a large number of small nucleated epithelial cells and little to no leukocytes or non-nucleated epithelial cells (Figure 1.1A; Cora et

al., 2015; Hubscher et al., 2005; Westwood, 2008). Compared to other stages, proestrus contains low to moderate cell numbers per collection (i.e., low cell density). Cells in proestrus typically cluster together, so most of the cells collected in a sample are concentrated in one area of the slide. An example of this clustering is shown in Figure 1.1A. During proestrus, estradiol levels rise rapidly and peak before estrus (Figure 1.2; Yoest, Quigley, & Becker, 2018). While estradiol levels peak, progesterone levels are at the lowest across the cycle but begin to rise as estradiol levels drop off (Yoest et al., 2018). The peak and decline of estradiol in combination with progesterone rising causes a rapid rise in luteinizing hormone (LH; Staley & Scharfman, 2005).

The increase in LH causes ovulation to occur, which is the defining characteristic of the estrus stage (Hubscher et al., 2005; Marcondes et al., 2002; Westwood, 2008). This stage lasts 24 to 48 hr (Hubscher et al., 2005; Westwood, 2008). Progesterone levels peak and begin to decline during estrus while estradiol remains low throughout the phase (Figure 1.2; Staley & Scharfman, 2005; Yoest et al., 2018). The estrus stage has a relatively high cell density compared to other stages. Similar to proestrus, these cells are clustered together. Some small clusters are shown at the bottom of Figure 1.1B, but clusters are typically much larger, making individual cells hard to delineate. Non-nucleated keratinized epithelial cells are the predominant cell type, but a small amount of nucleated epithelial cells may be present (Figure 1.1B; Hubscher et al., 2005). Leukocytes may be observed during the later portion of the estrus stage (Marcondes et al., 2002). The presence of leukocytes indicates that the rat is transitioning into metestrus.

After the estrus stage, rats transition into metestrus (about 6 to 8 hours in length; Hubscher et al., 2005; Marcondes et al., 2002). Metestrus is characterized by the presence of

leukocytes, small and large nucleated epithelial cells, and non-nucleated keratinized epithelial cells (Marcondes et al., 2002). Like estrus, metestrus samples have a moderate to high cell density (Marcondes et al., 2002). Low to moderate concentrations of the small and large nucleated epithelial cells remain from the estrus stage. The numbers of leukocytes increase dramatically during metestrus until they predominate. This transition marks the beginning of diestrus. There are relatively low levels of hormones circulating during metestrus as this is the day after ovulation (Figure 1.2; Staley & Scharfman, 2005; Yoest et al., 2018). The brevity of metestrus makes it difficult to capture a sample that is not already in transition to diestrus. For this reason, metestrus and diestrus are often collapsed into a combined met/diestrus stage, which is characterized by the presence of some nucleated epithelial cells and a large number of leukocytes (Figure 1.1C).

Finally, diestrus follows metestrus and is distinguished by the large presence of leukocytes (Cora et al., 2015; Marcondes et al., 2002; Westwood, 2008) that outnumber all other cell types (Figure 1.1C). Usually, the diestrus stage lasts the longest, 48-72 hours in total (Cora et al., 2015; Marcondes et al., 2002). Like proestrus, there are moderate numbers of cells present. Estradiol increases steadily during this stage, and progesterone has a shallow rise and fall (Figure 1.2; Yoest et al., 2018). Following diestrus, the cycle begins again with proestrus and so on.

Tracking the estrous cycle for extended periods can be time-intensive, so scientists have devised some alternatives to manipulate the estrous cycle. One common option is to ovariectomize the females and deliver hormones via injections at key time points to mimic the estrous cycle (e.g., Sell, Scalzitti, Thomas, & Cunningham, 2000; Sircar & Kim, 1999). This approach allows researchers to investigate acute effects of hormones. For example, the effects of

estradiol and progesterone can be dissociated by administering estradiol and progesterone to one group and estradiol only to another group. In addition, this approach can be used to understand receptor-specific effects. There are two estrogen receptors (ER), both of which can be targeted with agonists that reach peak plasma levels 30 min after administration (Lund, Rovis, Chung, & Handa, 2005; Sepehr et al., 2012). However, this approach does not ensure that all sources of estradiol are removed. For example, estradiol can be synthesized relatively quickly in the brain (Rudolph et al., 2016).

Another possibility is to ovariectomize and implant devices that administer hormones gradually over weeks (Mannino, South, Inturrisi, & Quinones-Jenab, 2005; Mosquera et al., 2015). This option does not mimic the estrous cycle entirely but isolates one hormone for an extended period. For example, the hormonal implant may deliver estradiol at low levels at a continuous rate. This procedure can be paired with injections to study acute exposure as well as a complete hormonal profile of a specific phase of the estrous cycle. In order to study the estrus phase, for example, injections of progesterone would need to be administered to accurately mimic all hormonal activity in that phase (Figure 1.2). This approach offers a feasible way to avoid compensatory mechanisms because removing all sources of estradiol may induce compensatory changes in estrogen receptors or production. Continuous administration of low levels of estradiol more closely mimics the endogenous state during phases when estradiol is lowest (metestrus and estrus; Figure 1.2). While this approach is the most like endogenous conditions, it does require anesthesia, surgeries, and extra animals in case of surgical complications. Invasive procedures that require surgery such as these can change behavioral and cognitive performance. For example, isoflurane, a common anesthetic, can induce deficits in spatial memory in rats (Callaway, Jones, & Royse, 2012; Culley, Baxter, Crosby, Yukhananov,

& Crosby, 2004; Culley, Baxter, Yukhananov, & Crosby, 2004). While these effects have not been investigated in impulsive choice and temporal precision tasks specifically, there are other non-invasive methods that can be used.

Selective agonists or antagonists may be used to target specific hormones or receptors in naturally cycling animals without requiring invasive surgeries. For example, an estrogen receptor antagonist such as antiestrogen ICI 182,780 takes effect within 30 min and binds to all estrogen receptor types for up to 24 hr (Alfinito, Chen, Atherton, Cosmi, & Deecher, 2008). This antagonist can be administered systemically or intracranially to a specific brain region to block the effects of estrogen (e.g., Alfinito et al., 2008; Song, Yang, Peckham, & Becker, 2019; Xiao & Becker, 1997; Xiao, Jackson, & Becker, 2003). Organizing experiments around naturally cycling animals is a caveat to vaginal lavage with pharmacology because females cycle independently from each other. Experimental manipulations would have to occur at specific times for each rat based on the naturally occurring cycles. However, this issue can be minimized with agonists or antagonists that induce the natural estrous cycle. Gonadotropin-releasing hormone receptor agonist can be used to synchronize females' cycles by inducing a sharp rise in LH, which results in ovulation (Rudolph et al., 2016). Two injections of this agonist at key time points induces the metestrus stage two days after injection (Rivier & Vale, 1990; A. J. Roberts, Smith, Weiss, Rivier, & Koob, 1998). This can be used to synchronize females for at least one full estrous cycle so that all animals can be exposed to a common set of experimental conditions at the same time (Rivier & Vale, 1990; A. J. Roberts et al., 1998). The proposed experiment will synchronize cycles and then use an estrogen receptor antagonist to study the effects of blocking estrogen on impulsive choice and timing.

Estrous Cycle Effects on Behavior

Previous research across fields has shown that behaviors, specifically those related to reward processing, vary across the estrous cycle in female rats. For example, psychostimulants and the effects they produce interact with estrous cycle. Female rats are more responsive to cocaine during specific stages of the estrous cycle as evident in greater horizontal and vertical activity during normal cycling and manipulated cycling (Sell et al., 2000). Testing in ovariectomized rats suggests that the administration of estrogen and progesterone increases behavioral sensitivity to cocaine, which mimics the hormonal activity present during proestrus before ovulation (Hu & Becker, 2003; Sircar & Kim, 1999). Similarly, female rats self-administered significantly more cocaine during ovulation than during other phases of the estrous cycle (D. Roberts, Bennett, & Vickers, 1989). On a two-lever drug self-administration procedure for cocaine, females responded most on the lever associated with increases in cocaine (Lynch, Arizzi, & Carroll, 2000). These results suggest that the estrous cycle influences females' responsiveness and motivation to self-administer cocaine, possibly altering the incentive value of rewards. Psychostimulants such as cocaine act directly on the reward pathway in the brain, which is the same pathway involved in impulsive choices.

Impulsive choices are the desire to choose a smaller reward occurring sooner (smaller-sooner; SS) over a larger reward occurring after a delay (larger-later; LL). Consistently making impulsive choices is indicative of high levels of impulsivity. Impulsivity is typically a maladaptive behavior, which underlies many different disorders such as Attention-Deficit/Hyperactivity Disorder (Antrop et al., 2006; A. T. Fox, Hand, & Reilly, 2008; Marco et al., 2009), obesity (Rasmussen, Lawyer, & Reilly, 2010; Weller, Cook, Avsar, & Cox, 2008), and pathological gambling (Dixon, Marley, & Jacobs, 2003). One of the proposed processes

leading to impulsive choice is delay discounting, which is the gradual reduction of reward value as a function of time (Baumann & Odum, 2012; Mazur, 2000). Delay discounting is potentially a product of errors in temporal perception (Kirkpatrick, Marshall, & Smith, 2015; Litrownik, Franzini, Geller, & Geller, 1977; Marshall, Smith, & Kirkpatrick, 2014; Takahashi, 2005; Wittmann & Paulus, 2008) or a strong aversion to waiting also known as delay aversion (Kirkpatrick et al., 2015; Marshall et al., 2014; Sonuga-Barke, Taylor, Sembi, & Smith, 1992; Winstanley, Eagle, & Robbins, 2006). Delay aversion may lead to temporal processing deficits (or vice versa) because avoiding delays limits experience with longer time periods. This decreases exposure to long delays, limiting the ability to learn those delays. Impulsive choices may be a result of errors in temporal perception and/or delay aversion.

Previous literature comparing males and females' impulsive choice behavior suggests that female sex hormones may influence temporal perception and/or delay aversion processes. Women discount more rapidly with hypothetical rewards compared to men, but men discount real money faster than women (Weafer & de Wit, 2014). Female and male mice and rats discounted differently based on delays (Koot, van den Bos, Adriani, & Laviola, 2009; van Haaren, van Hest, & van de Poll, 1988). More specifically, Koot et al. (2009) grouped mice into steep and flat discounters during analysis. Females with steep discounting functions made more impulsive choices at long delays than males with steep discounting functions (Koot et al., 2009). Recently, two cohorts of male and female rats completed multiple impulsive choice tasks after a behavioral intervention to improve self-control. Females were less impulsive than males, but the effects appeared to be task-dependent (Panfil, Bailey, Davis, Mains, & Kirkpatrick, 2020). These differences may potentially be a result of the estrous cycle. Perhaps the stage in which the task begins affects females' ability to time or tolerate the delays associated with each choice.

Multiple studies have measured impulsivity in male and female rats (see Weafer & de Wit, 2014 for a review), but none have tracked estrous cycles and mapped the phases onto changes in behavior except for our preliminary study.

Preliminary research suggests that impulsive choice behavior may be affected by the estrous cycle. Recently, Panfil et al. (in preparation) tracked estrous cycles every day for 30 days while female rats completed an impulsive choice task. Females made more LL choices during proestrus when estradiol levels were highest compared to the other estrous stages when estradiol was lower. To test the rats' preference for immediate rewards and reward magnitude discrimination ability, statistical models conducted on the data assessed hypothetical choices between a 0-s delay for one food reward and a 30-s delay for two food rewards as well as a 30-s delay for one food reward and a 30-s delay for two food rewards. There were no differences in the rats' biases for immediate rewards across estrous cycle, meaning that the rats made similar amounts of impulsive choices across estrous cycle. Interestingly, there were differences between magnitude discrimination ability based on estrous stage. Females preferred the larger magnitude reward even though they had to wait for it when they were in proestrus, $t = 2.68$, $b = 0.4$, $p < .01$, (Figure 1.3; Panfil et al., in preparation). In addition, rats in proestrus had a steeper delay slope than rats in estrus or met/diestrus, $t = 2.30$, $b = 0.57$, $p < .05$, suggesting that rats in proestrus were more sensitive to changes in delay (Figure 1.3). These changes in impulsive choice may be a result of improved delay tolerance or improved temporal perception caused by the fluctuations in sex hormones, specifically the increase in estradiol that occurs during proestrus.

Thus, impulsivity may be influenced by the hormonal changes occurring across estrous stages. However, it is unclear what mechanism(s) of impulsive choice is affected by female sex hormones. Impulsive choices may be a result of errors in temporal perception, delay aversion,

and/or magnitude insensitivity. A few studies have examined sex differences in accuracy and precision in timing intervals. Accuracy is the ability to time the interval as close as possible to the target duration while precision refers to the degree to which the interval is timed consistently. The few studies conducted in humans examining sex as a biological variable suggest that men can discriminate short intervals lasting milliseconds to seconds better than females (Rammsayer & Lustnauer, 1989; Wittmann & Szelag, 2003). In addition, females tended to overestimate time compared to males (Wittmann & Szelag, 2003). These results suggest that sex hormones may influence temporal perception.

Three studies have examined this possibility with rats using supra-second durations. Ross and Santi (2000) administered estradiol systemically to a group of ovariectomized females for 14 days and then tested them on a temporal discrimination task two weeks after injections. Rats that received chronic estradiol treatment had decreased precision on a temporal discrimination task. This effect was no longer present three weeks after treatment stopped. Likewise, Sandstrom (2007) found that ovariectomized female rats underestimated trained signal durations after a single administration of estradiol given before the testing session. Pleil, Cordes, Meck, and Williams (2011) found similar results after a single injection of estradiol given to ovariectomized females, but the injection did not alter the performance of gonadectomized (castrated) males. Taken together, these results suggest that estradiol alters temporal perception in females, which likely contributes to sex differences observed in humans and animals.

To date, published studies assessing the influence of sex hormones on temporal perception have been conducted in ovariectomized rats. Panfil et al. (in preparation) assessed timing in naturally cycling females to determine whether the estrous cycle was related to timing behavior. Female rats' ability to time the different delays was assessed with peak trials given

during the impulsive choice task. Rats in proestrus were more accurate when timing a 5-s delay, t = -3.09, b = -0.30, p < .01 (see Table 1.1 for peak times and spreads). Furthermore, when rats were in proestrus, they were more precise in timing the 5-s delay compared to when rats were in other estrous stages, t = -3.89, b = -0.38, p < .001 (Table 1.1; Figure 1.4A). At a 10-s delay, rats did not differ in peak time or peak spread based on estrous stage (Table 1.1; Figure 1.4B). At the 20-s delay (Table 1.1; Figure 1.4C), rats in proestrus underestimated the delay, t = -6.30, b = -0.96, p < .001, and rats in estrus overestimated the delay, t = 7.25, b = 0.82, p < .001. When rats were in proestrus, they were more precise in timing the 20-s delay compared to when rats were in other estrous stages, t = -3.29, b = -0.50, p < .01, and when rats were in estrus, they were less precise in timing the 20-s delay compared to when rats were in other estrous stages, t = 4.62, b = -0.52, p < .001 (Table 1.1; Figure 1.4C). Rats also differed in timing a 30-s delay based on estrous stage. Rats in proestrus underestimated the delay while rats in estrus and met/diestrus overestimated the delay, t = -2.33, b = -0.36, p < .05 (Table 1.1; Figure 1.4D). There were no differences in peak spread across estrous stages on the 30-s delay. Overall, the rats timed the 30-s delay precisely, but rats in proestrus underestimated the delay. Taken together, these results suggest that the effects of estrous cycle on temporal accuracy and precision appear to be complex and require further investigation to understand how sex hormones affect timing. In addition, these measures of timing were obtained during the impulsive choice task, which shows how females were timing the delays within the task. The effects of the estrous cycle on impulsive choice could interact with timing measurements or vice versa, creating challenges for interpreting the complex results.

Overall, impulsive choices and timing ability differ when comparing male and female humans. Many of these differences are found in rodents as well, although the correspondence

across species is not well understood. Behaviors such as self-administration of drugs, impulsive choices, and temporal perception may change across the estrous cycle or as a result of administration of female sex hormones. However, there is no established mechanism for these changes.

Current Study

The estrous cycle likely affects impulsive choice behaviors, and the mechanism through which these changes are mediated remains ambiguous. In addition, little research has examined the effects of estrous cycle on temporal processing—a critical component of impulsive choice. The current study aims to elucidate the mechanistic relationship between the estrous cycle, specifically estradiol, and impulsive choices and clarify the link between timing and the estrous cycle.

Female rats will complete an impulsive choice task embedded with peak trials with and without an estrogen receptor antagonist (antiestrogen). Based on preliminary data collected in Panfil et al. (in preparation), when rats were in proestrus, they made more LL choices at the longest delay (20 s). The impulsive choice task will test a wider range of delays to fully understand how the length of delay may interact with estrous cycle. Peak trials will be delivered on the full range of delays as well. The timing differences between previous literature and the preliminary data from Panfil et al. (in preparation) may be a result of naturally occurring versus induced hormone levels or task dependency. Previous research that used bisection tasks to assess timing ability also ovariectomized the rats and administered sex hormones (Pleil et al., 2011). Panfil et al. (in preparation) used embedded peak trials and naturally cycling female rats. Resulting data from the impulsive choice task with peak trials should replicate and extend the findings from Panfil et al. (in preparation).

I propose that the increase in estradiol that occurs naturally in proestrus will increase self-control and increase temporal accuracy and precision. When estradiol is lowest during estrus, self-control and temporal accuracy and precision will worsen. The estrogen receptor antagonist should inhibit activation of estrogen receptors. When estradiol is blocked from binding to ERs, females will make more impulsive choices and will have decreased temporal accuracy and precision similar to that observed during estrus.

Figure 1.1. Samples collected via vaginal lavage and stained with crystal violet. Panel A was scored as proestrus because of the presence of nucleated epithelial cells clustered together. Panel B was scored as estrus because of the cornified epithelial cells often clumped together (small clumps shown towards the bottom of the photo). Panel C was scored as met/diestrus because of the presence of leukocytes (small, dark, and granulated) and some nucleated epithelial cells.

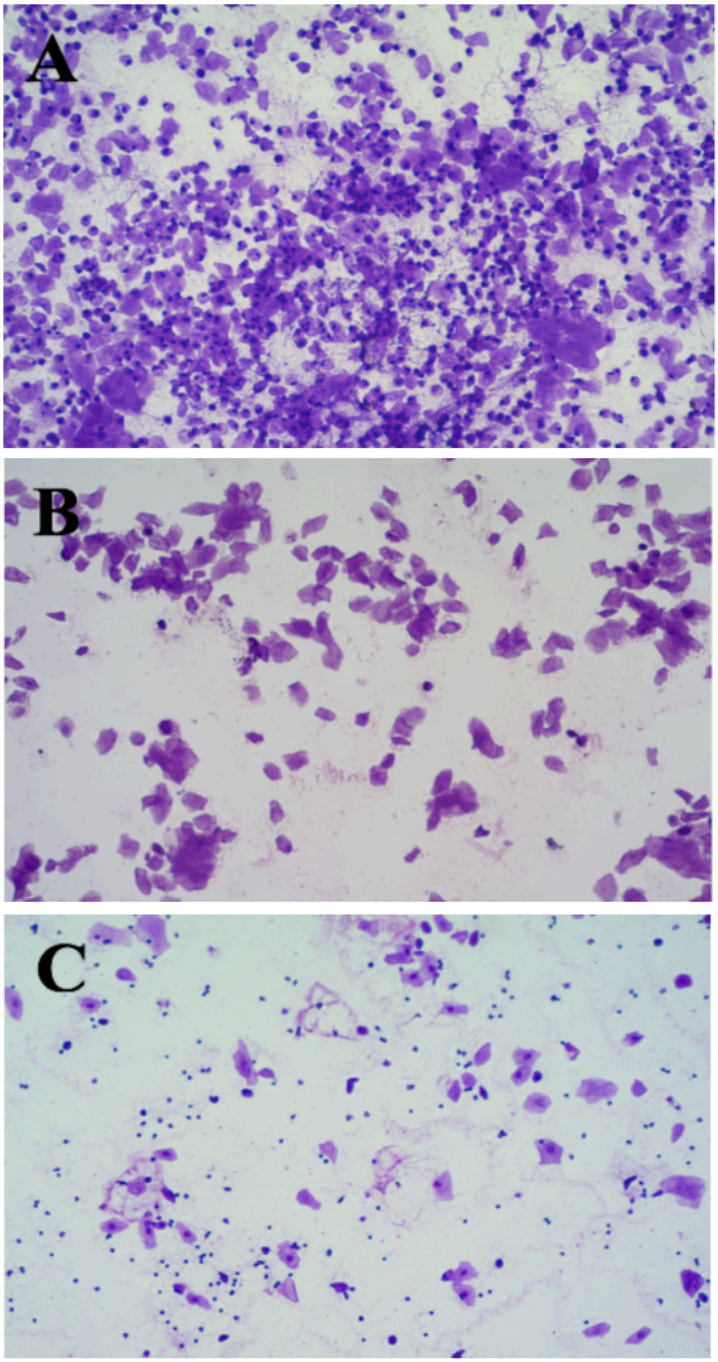


Figure 1.2. Approximate levels of estradiol and progesterone across the estrous cycle. Adapted from Yoest et al. (2018).

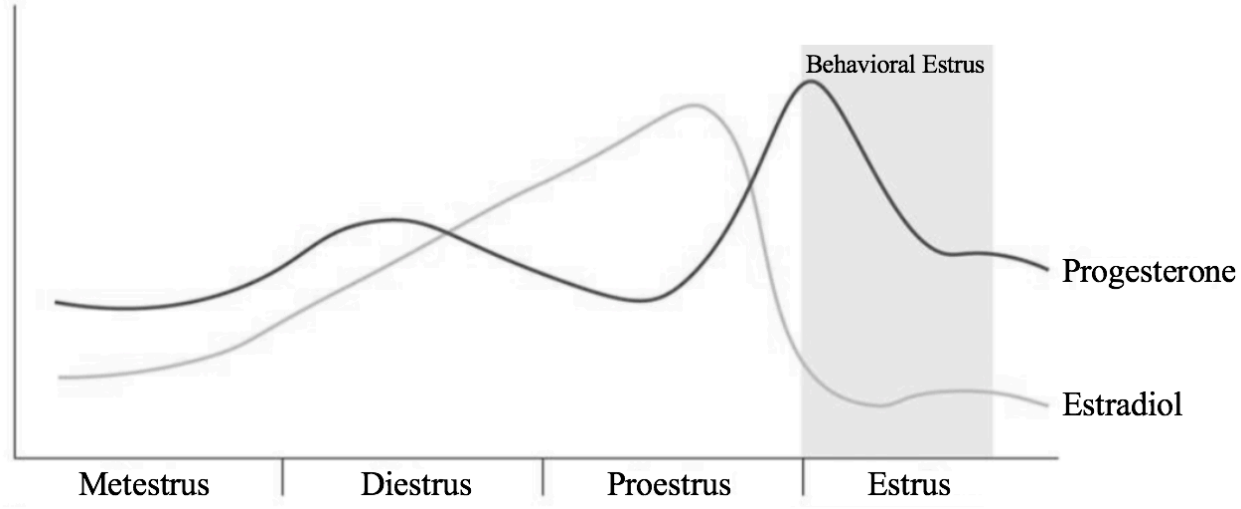


Figure 1.3. Mean proportion of LL choices across SS delays from preliminary study (Panfil et al., in preparation). Error bars (+/− SEM) were computed with respect to the estimated marginal means of the fitted repeated measures multi-level logistic regression.

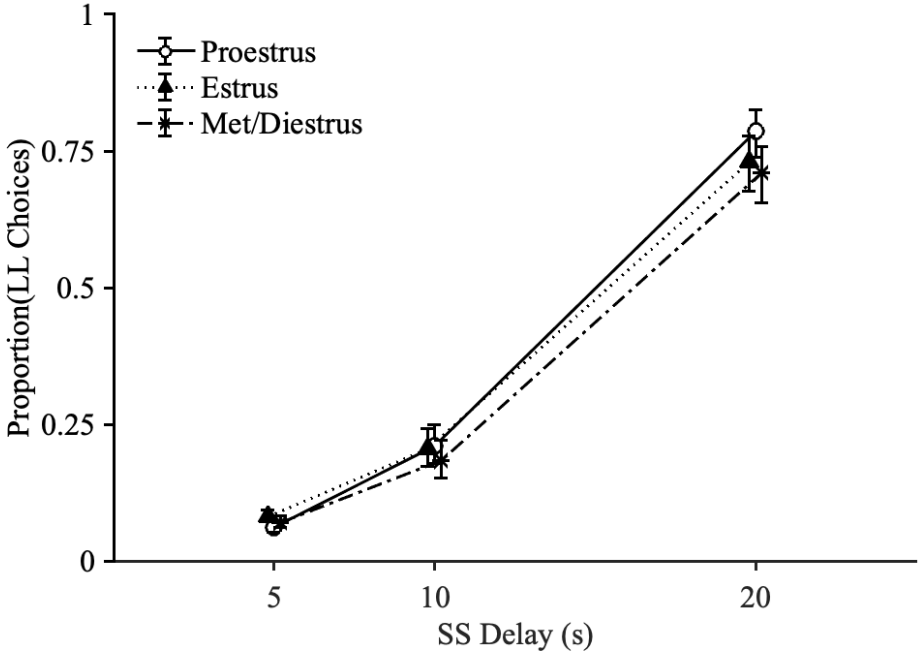


Figure 1.4. Mean responses per minute as a function of time into peak trials for a 5-s (A), 10-s (B), 20-s (C), and 30-s (D) delays from preliminary study (Panfil et al., in preparation). Markers represent mean responses and lines represent fitted repeated measures multi-level nonlinear regression values. Solid vertical lines represent the target interval.

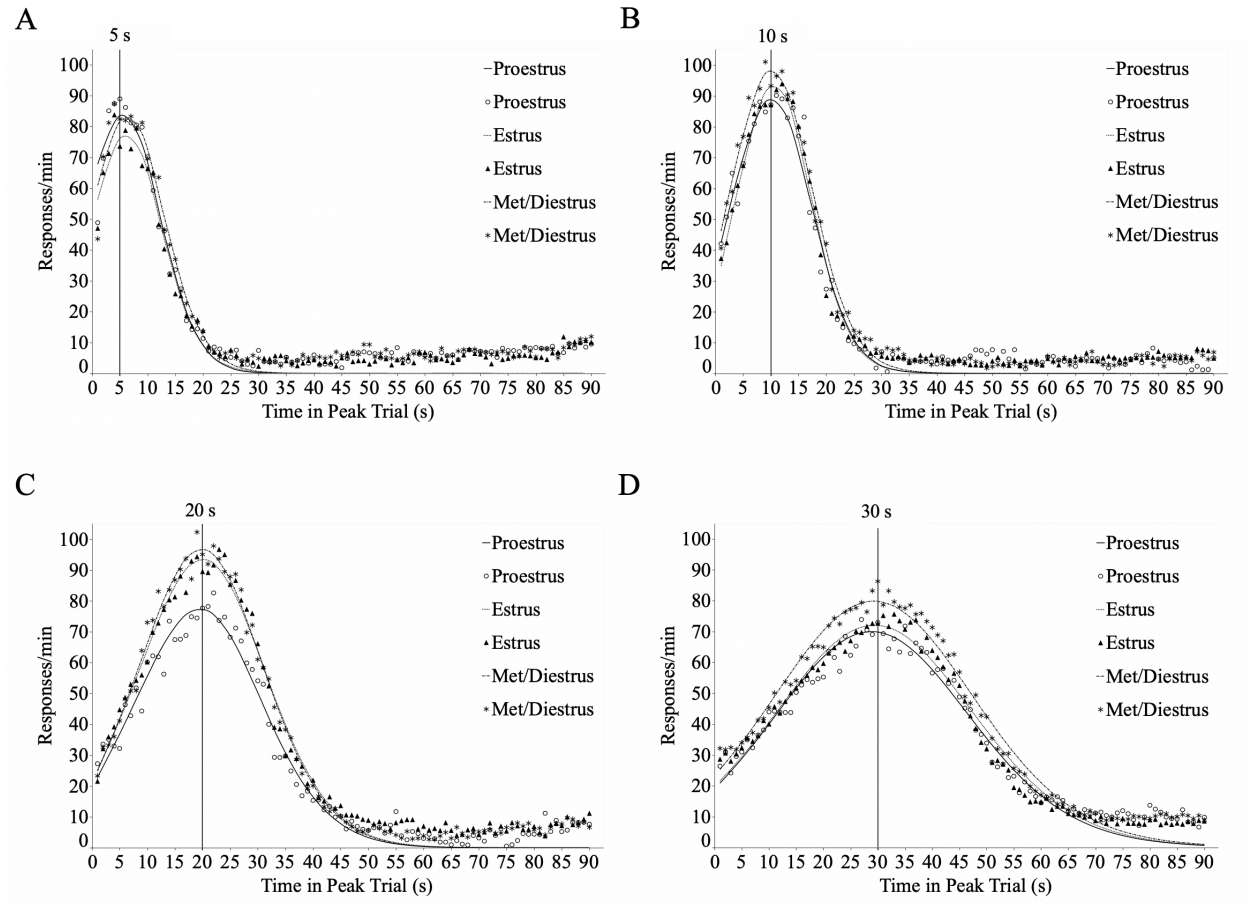


Table 1.1. Peak time, spread, and coefficient of variation for each delay and estrous cycle from preliminary study (Panfil et al., in preparation).

Delay	Phase	Peak Time (s)	Peak Spread	Coefficient of Variation
5 s	Proestrus	6.2	6.1	0.98
	Estrus	6.5	6.5	1.00
	Met/diestrus	6.9	6.8	0.96
10 s	Proestrus	10.1	7.3	0.72
	Estrus	10.3	7.3	0.71
	Met/diestrus	10.2	7.5	0.73
20 s	Proestrus	18.7	11.0	0.59
	Estrus	20.5	12.0	0.59
	Met/diestrus	19.8	11.5	0.58
30 s	Proestrus	29.9	18.8	0.63
	Estrus	30.4	18.7	0.61
	Met/diestrus	30.4	19.4	0.64

Note: Coefficient of variation is calculated by dividing peak spread by peak time.

Chapter 2 - **Method**

Animals

Forty-eight female experimentally naïve Sprague-Dawley rats (Charles River, Stone Ridge, NY) were used in this experiment. The rats arrived in two squads of 24 rats each because behavioral testing was limited by 24 operant chambers. The second squad arrived as the first squad completed behavioral testing. Treatment of squads was identical, and all counterbalancing was completed across squads as well. Both squads arrived at the facility (Kansas State University, Manhattan, KS) between 51-75 g, which is approximately post-natal (PND) 28 (Figure 2.1). Experimentation began at PND 52. The rats were pair-housed and maintained on 12-hr light:dark schedule (lights on at approximately 7 am). The rats were tested during the dark phase of the cycle overnight. There was *ad libitum* access to water in the home cages and in the experimental chambers. Rats were food restricted and maintained at approximately 90% of their projected *ad libitum* weight, as derived from growth-curve charts obtained from the supplier. Rats received reward pellets (Product #F0021; Bio-Serv, Flemington, NJ) in the operant chambers as well as supplemental rat chow (Product #T.2016.15; Envigo, Huntingdon, United Kingdom) to maintain their weight. Both food sources had reduced levels of phytoestrogens (dietary estrogens) compared to standard pellets and chow to remove outside sources of estrogen. Daily feeding amounts typically ranged from 13-23 g per rat.

Apparatus

The experiment was conducted in 24 operant chambers (Med-Associates, St. Albans, VT), each housed within a sound-attenuating, ventilated box (74 x 38 x 60 cm). Each operant chamber (25 x 30 x 30 cm) was equipped with a stainless-steel grid floor, two stainless steel walls (front and back), and a transparent polycarbonate side wall, ceiling, and door. Two pellet

dispensers (ENV-203), mounted on the outside of the front wall of the operant chamber, delivered 45-mg food pellets (Product #F0021; Bio-Serv, Flemington, NJ) to a food cup (ENV-200R7) that was centered on the lower section of the front wall. Two retractable levers (ENV-112CM) were located on opposite sides of the food cup. The chamber was also equipped with a house light (ENV-227M) that was centered at the top of the chamber's front wall, as well as two nose-poke key lights (ENV-119M-1) that were each located above the left and right levers. Water was always available from a sipper tube that protruded through the back wall of the chamber. Experimental events were controlled and recorded with 1-ms resolution by the software program MED-PC V.

Procedure

Estrous Tracking

Starting on PND 76, estrous cycles were tracked via vaginal lavage at key time points throughout the experiment for a total of 20 collections (Figure 2.1). Rats typically reach sexual maturity at 6 or 7 weeks old (Adams & Boice, 1983; Long & Evans, 1920), so no collections occurred before that window. Physiological saline (.9%; ~0.2 mL) was drawn up with a tapered pipette tip, and the tip was inserted 5-10 mm into the vaginal opening. The solution was flushed in and out 2-3 times or until the solution was cloudy. The solution was then plated on a microscope slide. A new pipette was used for each sample collection. The collection procedure occurred at the same times every day: before (5PM) and after behavioral testing (8AM). Collection took place before and after testing because the rats spent 14 hr in the operant chambers. Preliminary data suggested that rats were more self-controlled during the proestrus stage, which lasts for 12-14 hr. Rats could have been in proestrus at the start but transitioned to

estrus by the end of a session. Collection after testing showed if the rats transitioned to a new stage during testing.

Pre-Training

Pre-training consisted of magazine training and lever press training. Magazine training involved the delivery of food pellets to the food cup on a random-time 60-s schedule. Following magazine training, the rats were trained to press both the left and right levers. First, food pellets were delivered on a fixed-ratio (FR) 1 schedule of reinforcement until 20 food pellets were delivered for responding on each lever. The FR 1 was followed by a random ratio (RR) 3 schedule of reinforcement where 3 responses were required on average per reinforcer, which lasted until 20 reinforcers were delivered for responding on each of the two levers. The RR 3 was followed by a RR 5, which lasted until the rats earned 20 food pellets for responding on each of the two levers. Magazine training lasted for one 14-hr session and lever press training lasted two 14-hr sessions (Figure 2.1).

Estrous Synchronization

In an attempt to synchronize rats' cycles to obtain behavioral data in all phases of the estrous cycle for each rat at the same time points, an injection of gonadotropin-releasing hormone receptor agonist, Leuprolide (Tocris Bioscience, Bristol, United Kingdom), was administered subcutaneously (2 ug in 200 uL of 0.1 N acetic acid) at 9 am and 2 pm two days before behavioral testing began (Rivier & Vale, 1990; A. J. Roberts et al., 1998). These injection times were planned to produce a cumulative rise in LH, which causes ovulation. On the second morning after injections, all rats should have been in metestrus and continued to be synchronized for at least one cycle. Vaginal lavage was performed two days after the injection and testing began that evening. Vaginal lavage occurred before they were loaded into the operant chambers

and after they completed the behavioral testing session. Cycles were induced before each testing phase (Figure 2.1).

Bisection Task

Rats received initial training with a 4-s short and a 12-s long signal for 6 days. Each trial began with the onset of the house light that lasted for either the short or long duration. Following the signal, both levers were inserted, and a choice response was collected. Correct responses were followed by a 1-pellet food delivery and a 15-s inter-trial interval (ITI). Incorrect responses were followed by a correction trial that was composed of a 5-s ITI and a repeat of the previous trial until the correct response was made and food was delivered. Rats earned up to 320 reinforcers in a session. Sessions lasted for a maximum of 14 hr with 4 blocks and corresponding 90-min inter-block intervals (IBIs). Each block contained 80 trials in 4 sub-blocks of 20 trials each. Each sub-block consisted of 10 long and 10 short trials presented in a random order. The correct response for the short 4-s duration was the same lever as the SS lever and the correct response for the 12-s duration was the same lever as the LL lever in the impulsive choice task. There were 6 training sessions with 4-s short and 12-s long signals, but rats did not reach the performance criterion of 80% correct on both levers at the group level for 2 consecutive days. Instead of administering more of the same training, the task was modified to make the distinction between signals easier. All other parameters remained the same except the short signal was changed to 3 s. Rats received another 7 sessions of training with the modified task but still did not reach performance criteria (Figure 2.2). In order to complete the other tasks before the rats reached anestrus, further training and testing was not pursued.

Impulsive Choice Task

Rats chose between a smaller-sooner (SS) reward and a larger-later (LL) reward. The SS delay was 5 s throughout all sessions. The LL delay ramped up or down across four blocks from 5 s to 60 s. One group of rats (n = 24) received the LL delays in ascending order while the other group (n = 24) received the LL delays in descending order. Previous literature suggested that the order of delays presented in choice tasks may produce differences in choice behavior (e.g., Robles & Vargas, 2007), so both orders were included.

Each session included a mixture of free-choice, forced-choice, and peak trials. On free-choice trials, both levers were available to the rat. After one lever was pressed, the other lever retracted, the cue light above the lever was illuminated, and the scheduled delay began. The first lever press following this delay resulted in food delivery, cue light offset, and onset of the 60-s ITI. Forced-choice trials were identical to free-choice trials, but only one lever was inserted. Peak trials were similar to forced-choice trials with only one lever inserted, except that the lever remained for 3 times the duration of the FI, and no reinforcement was provided. For example, the 5-s SS peak trial lasted for 15 s. All responses made on the available lever were recorded. Each session contained 272 trials delivered in 4 blocks. Each block had 32 free-choice, 12 SS forced-choice, 12 LL forced-choice, 6 SS peak, and 6 LL peak trials. Between blocks, there was a 90-minute IBI. Each session lasted for approximately 14 h and delivered a maximum of 400 reinforcers. The SS choice always resulted in 1 food pellet reward, and the LL choice always resulted in 2 food pellets. There were 10 sessions of the impulsive choice task in total.

Estrogen Receptor Antagonist Administration

Rats received injections of estrogen receptor antagonist, antiestrogen ICI 182,780 (Tocris Bioscience, Bristol, United Kingdom), to block the effects of estradiol on the brain (Figure 2.1).

Antiestrogen ICI blocks both types of estrogen receptors (Alfinito et al., 2008). The peak effects of antiestrogen ICI occur 1 hr after administration but remain in effect for up to 24 hr. In addition, antiestrogen ICI can cross the blood brain barrier, making it an effective blocker for the receptors located in the brain (Alfinito et al., 2008). While this antagonist has not been used systemically before, it successfully blocked estrogenic effects in the brain when delivered intracranially during in vivo microdialysis experiments (Song et al., 2019; Xiao & Becker, 1997). Injections were delivered subcutaneously at a dose of 0 mg/kg for 5 days of testing and at a dose of 1 mg/kg for 5 days of testing. The 0 mg/kg dose was included to test for any effects of the vehicle or injection on behavior. The 1 mg/kg dose resulted in biologically relevant levels of antiestrogen ICI in the hypothalamus for up to 24 hr (Alfinito et al., 2008), suggesting this should be an effective dose for targeting the brain. The order of doses was counterbalanced across groups. The injections occurred before animals were placed in the operant chambers.

Estrous Sample Processing and Histology

Following collection by vaginal lavage (see *Estrous Synchronization*), estrous samples were allowed to air-dry. Slides were immersed in crystal violet (0.1%) solution for one minute. Then, the slides were transferred to distilled and de-ionized water for two rinses of 1 min each. Slides were air-dried again before cover slipping with Permount. Slides were assessed for estrous stage and imaged with the 10x objective. Two experienced individuals blindly evaluated a subset of the samples. An intra-class correlation was used to assess interrater reliability. After a level of at least 0.80 interrater reliability was achieved, to ensure consistency in scoring, the rest of the samples were scored by both individuals. A third experienced scorer evaluated samples without matching scores. If the third individual could not determine the stage, samples were not entered in the analyses and treated as missing values. These instances occurred most

commonly when the sample had very few cells, which can happen if the tip of the pipette is not inserted far enough into the vaginal opening. This increases the difficulty in scoring the sample and results in poor reliability.

Scorers used the three main cell types – nucleated epithelial cells, cornified cells, and leukocytes – to determine the stage. Proestrus is characterized by mostly round nucleated epithelial cells and few leukocytes (Figure 1.1A). Estrus is characterized by large cornified cells clumped together and few leukocytes and nucleated epithelial cells (Figure 1.1B). Due to the difficulty capturing metestrus before the transition to diestrus begins, metestrus and diestrus were collapsed into one stage. Met/diestrus is characterized by the presence of some nucleated epithelial cells and a large number of leukocytes (Figure 1.1C). The previous day's sample was consulted in cases where the scorer did not observe distinct differences in cell type ratios. If the scorer still had difficulty deciding between two stages, the sample was marked as the upcoming stage. For example, if the sample was between proestrus and estrus, the sample was labeled as estrus. Behavioral testing began approximately 1 hr after sample collection, which should put them closer in time to the next phase during behavioral testing.

Data Analysis

Data was imported and compiled with MATLAB 2020a (MathWorks). Repeated measures multi-level analyses were conducted using the *lme4* package in R for the impulsive choice data and *nlme* package in R for the peak trial data (Bates, Maechler, Bolker, & Walker, 2015; Pinheiro, Bates, DebRoy, Sarkar, & Team, 2020). Fixed effects were determined using a theory-based approach, so that the hypotheses were tested as fixed effects. Random effects were determined based on Akaike Information Criterion (AIC; Akaike, 1974) and any significant correlations between the intercept and other random effects as a metric of overparameterization,

which suggests that the terms are capturing the same variance (Baayen, Davidson, & Bates, 2008; Bates, Kliegl, Vasishth, & Baayen, 2015). Random effects that significantly correlated with intercept were removed from the random effects structure.

All analyses were conducted on the last three sessions of each phase of impulsive choice testing. No analyses were conducted on the bisection training data. Figure 2.2 depicting their performance across training sessions was included to show that the rats did not reach the criteria on either training duration. Categorical variables—estrous stage, group (ascending vs. descending), and dose—were effects coded. Dose was treated as categorical because only two doses were administered (0 mg/kg and 1 mg/kg).

Impulsive Choice Analyses

To assess impulsive choice behavior, an initial omnibus analysis via repeated measures multi-level logistic regression with logit link function was conducted to determine whether the order of delays significantly affected decision making. Group (ascending vs. descending) significantly affected choice behavior (see Table 2.1), so the two groups were analyzed separately for the rest of the analyses. Although the analyses for each order of delays were analyzed separately, the same fixed effects and random effects were included in the models. Delay, estrous stage, dose, and their interactions were entered as fixed effects. Rat (intercept) and delay were tested as random effects. Delay was scaled to assess the 30-s intercept. The models were conducted on 11,795 observations for the ascending and 11,082 observations for the descending groups. Across both models, if interactions were significant, main effect statistics were not included in the narrative but were included in the output tables.

Peak Timing Analyses

To assess peak trial data obtained during the choice task, nonlinear multi-level regressions were conducted on the 5-s SS FI, 15-s LL FI, and 30-s LL FI. Analyses were not conducted on the 5-s LL FI or 60-s LL FI because a significant portion of the rats did not complete all trials in the impulsive choice task. This resulted in a lack of peak data for the last block of trials, which was the 5-s LL or 60-s LL delays depending on what order the delays were presented. Order of delays significantly affected choice behavior, so the two groups were analyzed separately for the peak data as well.

The nonlinear component was specified with a three-parameter modified Gaussian distribution to fit responses per minute and took the following form:

$$me^{-(t-a)^2/2p^2} \quad (1)$$

where *t* represented the time into the peak trial, *a* was the time of the peak (accuracy), and *p* was the standard deviation of the peak (precision; see A. E. Fox, Visser, & Nicholson, 2019 for a similar analysis). The function included *m* to adjust for height of the peak (maximum rate). The analyses included focused on two meaningful dependent measures to assess timing ability: peak time (*a;* accuracy), and peak spread (*p;* precision).

The analyses for each order of delays were separate, but the same fixed effects and random effects were included in the models. The three parameters, *a, p*, and *m*, were allowed to vary across estrous stage, dose, and their interaction ($a + p + m \sim$ estrous stage × dose). Rat (intercept) was included as a random effect. For replication purposes, the seed was set to 2020 in all peak data analyses. Starting values for the model were chosen based on the average peak functions across all rats (within each separate group). For the ascending order of delays, starting values for the 5-s SS FI were 6.50 for *a*, 5.70 for *p*, and 64.48 for *m,* and the model was

conducted on 1905 observations. For the descending order of delays, starting values for the 5-s SS FI were 6.66 for *a*, 6.10 for *p*, and 65.17 for *m,* and the model was conducted on 1935 observations. For the ascending order of delays, starting values for the 15-s LL FI were 20.74 for *a*, 18.34 for *p*, and 55.12 for *m*, and the model was conducted on 5670 observations. For the descending order of delays, starting values for the 15-s LL FI were 20.70 for *a*, 23.38 for *p*, and 47.77 for *m*, and the model was conducted on 3825 observations. However, the model failed to converge, so no analysis for the descending group on the 15-s LL FI were included. Two rats did not press at all on any 15-s LL peak trials and multiple other rats did not press at all on 15-s LL peak trials during specific sessions. For the ascending order of delays, starting values for the 30-s LL FI were 30.07 for *a*, 33.03 for *p*, and 42.45 for *m*, and the model was conducted on 9540 observations. Again, the model failed to converge, so no analysis for the ascending group on the 30-s FI were included. Two of the twenty-four rats did not press at all on any 30-s LL peak trials, which resulted in less data for the model compared to the data for the 30-s LL peak trials for rats that received the delays in descending order. For the descending order of delays, starting values for the 30-s LL FI were 38.08 for *a*, 34.99 for *p*, and 39.5 for *m*, and the model was conducted on 10980 observations.

Figure 2.1. Timeline of the experiment based on age of the rats in post-natal day (PND). Rats typically reach sexual maturity between PND 42-49, so the estrous collections did not begin until after this period of time.

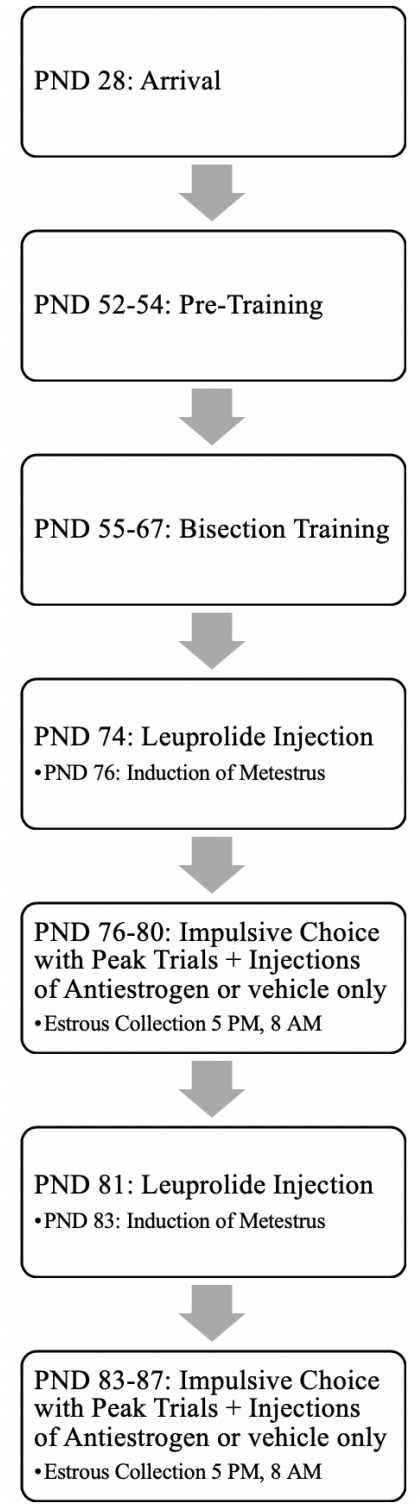


Figure 2.2. Proportion correct on the short and long durations during bisection training. Error bars (+/− SEM) were computed with respect to the marginal means of the raw data. Rats did not reach the criteria for either duration even with extended training.

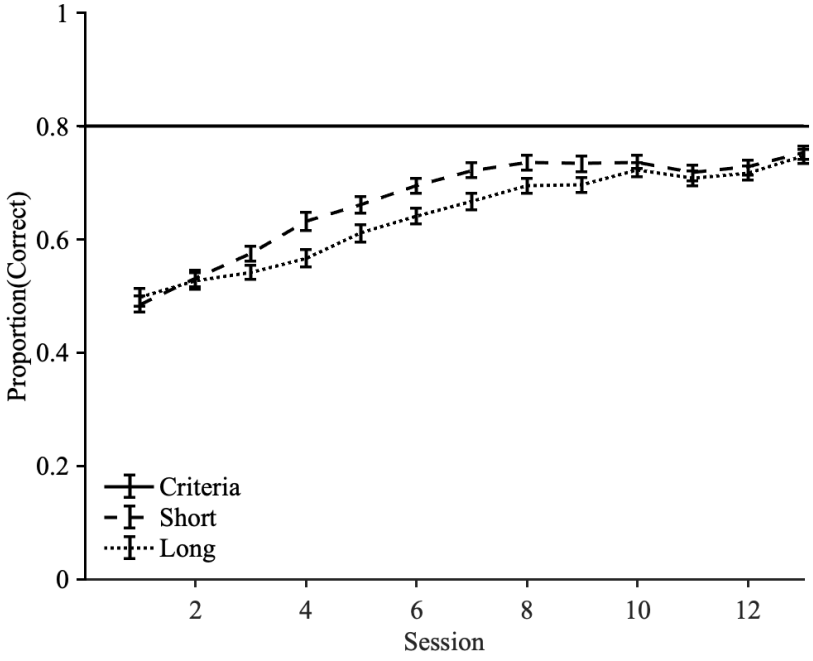


Table 2.1. Summary of fixed effects including group, which signifies order of delays, from the impulsive choice omnibus test model. See Appendix A for full model output.

	b	SE	*z*	
Group × LL Delay	-1.53	0.09	-16.96	***
Group × Proestrus	-0.08	0.04	-1.98	*
Group × Dose × LL Delay × Estrous	0.22	0.11	1.96	*

Note: For Group, the ascending condition was coded as 1 and the descending condition was coded as -1. The 0 mg/kg dose was coded as 1 and the 1 mg/kg dose was coded as -1. Proestrus was coded as 1, 0; estrus was coded as 0, 1; met/diestrus was coded as -1, -1.

* $p < .05$
** $p < .01$
*** $p < .001$

Chapter 3 - **Results**

Estrous Cycle Synchronization

Injections of leuprolide were timed to produce a cumulative rise in LH resulting in ovulation. Cycles were induced before each testing phase, so all rats should have been in met/diestrus for the first night of testing (Figure 2.1). However, when assessing the estrous samples, it was clear that the cycles were not synchronized. Ten of the 48 rats were in met/diestrus on the first night of testing phase 1. Only 3 of 48 rats were in met/diestrus on the first night of testing phase 2. Instead, 23 of 48 rats were in estrus on the first night of testing phase 1, and 34 of 48 were in estrus of the first night of testing phase 2.

Impulsive Choice

Ascending.

There was a significant Dose × Estrus interaction (see Table 3.1; Figure 3.1). However, further coefficient testing on the Dose × Estrus interaction showed rats in estrus that received 0 mg/kg of the estradiol antagonist did not differ from rats in estrus that received 1 mg/kg of the estradiol antagonist (see Figure A.1 for an alternative view of the Dose × Estrus interaction). Instead, there were significant differences in estrous phase in the 0 mg/kg condition. Specifically, rats in estrus on 0 mg/kg (b = -1.14) made more LL choices than rats in met/diestrus on 0 mg/kg (b = -1.45; t = 4.60, $p < .05$). This finding was unexpected because rats in proestrus were most self-controlled in the preliminary study. In addition, it was hypothesized that the estrogen receptor antagonist would result in more impulsive choices, but there were no differences between the 0 mg/kg and 1 mg/kg doses.

There was a significant effect of LL Delay such that as LL Delay increased, rats made more impulsive choices (see Table 3.1; Figure 3.1). There were no other significant effects involving LL Delay.

Descending.

There was a significant Dose × Estrus interaction (see Table 3.2; Figure 3.2). However, further coefficient testing on the Dose × Estrus interaction showed rats in estrus that received 0 mg/kg of the estradiol antagonist did not differ from rats in estrus that received 1 mg/kg of the estradiol antagonist (see Figure A.2 for an alternative view of the Dose × Estrus interaction). Instead, there were significant differences in estrous phase in the 0 mg/kg condition. Specifically, rats in estrus on 0 mg/kg (b = -1.80) made more LL choices than rats in met/diestrus on 0 mg/kg (b = -2.21; t = 9.71, p < .01). In addition, there were significant differences in estrous phase in the 1 mg/kg condition. Specifically, rats in estrus on 1 mg/kg (b = -1.85) made fewer LL choices than rats in proestrus on 1 mg/kg (b = -1.54; t = 10.29, p = .001). These findings did not concur with the hypotheses, which stated that rats in proestrus will be most self-controlled and that the estrogen receptor antagonist would result in more impulsive choices. Instead, rats in estrus were most self-controlled in the 0 mg/kg condition, and there were no differences between the 0 mg/kg and 1 mg/kg doses.

There was a significant LL Delay × Dose × Proestrus interaction (see Table 3.2), meaning that the slope of the functions differed between conditions (Figure 3.2). The slope of the choice function for rats in proestrus on the 0 mg/kg dose (b = -1.29) was significantly steeper compared to the choice function for rats in estrus on the 0 mg/kg dose (b = -1.86; t = 7.45, p < .01; Figure 3.2) and for rats in proestrus on the 1 mg/kg dose (b = -1.98; t = 6.12, p < .05; see Figure A.2 for another view). Also, the choice function for rats in proestrus on the 1 mg/kg dose (b = -1.98)

was significantly shallower compared to the choice function for rats in estrus on the 1 mg/kg dose (b = -1.42; t = 7.45, p < .01; Figure 3.2).

Peak Timing

Ascending.

On 5-s SS peak trials, there was a significant Dose × Estrus interaction on peak time (see Table 3.3; Figure 3.3). Pairwise comparisons were used to probe the interaction. Rats in proestrus on 0 mg/kg (b = 7.03) had later peak times on the 5-s FI compared to rats in met/diestrus on 0 mg/kg (b = 5.94; t = 2.50, p < .05) but were not significantly different from rats in estrus (b = 7.06; t = -0.07, p > .05; Figure 3.3). Rats in estrus on 0 mg/kg (b = 7.06) also had later peak times compared to rats in met/diestrus on 0 mg/kg (b = 5.94; t = 3.05, p < .01). These findings do not support the original hypothesis which stated that the increase in estradiol that occurs naturally in proestrus will increase temporal accuracy. In addition, when rats in estrus received 0 mg/kg (b = 7.06), they had later peak times (farther from the 5-s target) than when they received 1 mg/kg (b = 6.63; t = 2.97, p < .01; see Figure A.3 for an alternative view of this effect). However, when rats in met/diestrus received 0 mg/kg (b = 5.94), they had earlier peak times than when they received 1 mg/kg (b = 6.80; t = -2.34, p < .05; see Figure A.3 for another view). There were no differences in accuracy between the two doses when rats were in proestrus. Based on the preliminary study, it was hypothesized that blocking estradiol with the estrogen receptor antagonist would result in decreased temporal accuracy, but that was not observed in the current study.

On 5-s SS peak trials, there was also a significant Dose × Estrus interaction on peak spread (see Table 3.3; Figure 3.3). Rats in proestrus on 0 mg/kg (b = 7.00) had significantly smaller peak spreads (were more precise in timing) on the 5-s FI compared to rats in estrus on 0

mg/kg (b = 9.11; t = -4.71, p < .001) but had larger peak spreads compared to rats in met/diestrus (b = 4.30; t = 4.87, p < .001; Figure 3.3). Rats in estrus on 0 mg/kg (b = 9.11) had larger peak spreads than rats in met/diestrus on 0 mg/kg (b = 4.30; t = 10.55, p < .01). These results do not completely support the original hypothesis and preliminary study results where rats in proestrus had better temporal precision than all other stages of the estrous cycle. When rats in proestrus received 0 mg/kg (b = 7.00), they had larger peak spreads than when they received 1 mg/kg (b = 5.24; t = 4.30, p < .001; see Figure A.3 for an alternative view of this effect). In addition, when rats in estrus received 0 mg/kg (b = 9.11), they had larger peak spreads than when they received 1 mg/kg (b = 4.85; t = 24.65, p < .001; see Figure A.3 for another view). There were no differences in peak spread between the two doses when rats were in met/diestrus. Again, these results do not support the hypothesis that the estrogen receptor antagonist would result in worsened temporal precision.

Measures of accuracy and precision can be integrated into a single value, coefficient of variation (CV), to simplify comparisons of timing behavior between groups and across delays. CV values were calculated by dividing peak spread by peak time (see Table 3.4 for CV values). Lower CV values indicate reduced timing errors due to either overestimation of delay and/or increased variance in timing. Overall, on the 5-s SS, administration of the estradiol antagonist decreased CV values. This suggests that timing errors reduced with administration of the estradiol antagonist contrary to the original hypothesis.

On 15-s LL peak trials, there were significant Dose × Proestrus and Dose × Estrus interactions on peak time (see Table 3.5; Figure 3.4). Rats in proestrus on 0 mg/kg (b = 23.90) had later peak times on the 15-s FI compared to rats in estrus on 0 mg/kg (b = 20.10; t = 4.97, p < .001) and rats in met/diestrus (b = 20.90; t = 2.93, p < .01; Figure 3.4). These results were

inconsistent with the preliminary study where rats in proestrus underestimated longer delays. Rats in proestrus on 1 mg/kg (b = 18.00) had earlier peak times on the 15-s FI compared to rats in estrus on 1 mg/kg (b = 21.00; t = -2.49, $p < .05$). In addition, when rats in proestrus received 0 mg/kg (b = 23.90), they had later peak times on the 15-s FI compared to when they received 1 mg/kg (b = 18.00; t = 5.19, $p < .001$; see Figure A.4 for another view of this effect). This was opposite of the pattern anticipated where the estrogen receptor antagonist worsens temporal accuracy. There were no differences in accuracy between the two doses when rats were in estrus or met/diestrus.

On 15-s LL peak trials, there was a significant Dose × Proestrus effect on peak spread (see Table 3.5; Figure 3.4). For the 0 mg/kg dose, rats in proestrus (b = 14.30) and rats in estrus (b = 15.00) had smaller peak spreads on the 15-s FI compared to rats in met/diestrus (b = 17.60; t = -2.65 and -2.72, ps < .01). This result partially replicated the preliminary study where rats in proestrus were most precise than rats in estrus and met/diestrus. For the 1 mg/kg dose, when rats were in proestrus (b = 25.60), they had larger peak spreads than rats in estrus (b = 21.30; t = 2.43, $p < .05$) and met/diestrus (b = 20.70; t = 2.42, $p < .05$). Peak spreads got larger with administration of the estradiol antagonist across all estrous cycles (see Figure A.4 for an alternative view of this effect). On the 0 mg/kg dose, rats in proestrus (b = 14.30), estrus (b = 15.00), and met/diestrus (b = 17.60) had smaller peak spreads compared to when they received 1 mg/kg (Proestrus: b = 25.60; t = -7.01, $p < .001$; Estrus: b = 21.30, t = -5.76, $p < .001$; Met/diestrus: b = 20.70; t = -2.01, $p < .05$). This result was consistent with the original hypothesis which stated that the estrogen receptor antagonist would decrease temporal precision.

Overall, on the 15-s LL, administration of the estradiol antagonist resulted in higher CV values, suggesting that timing errors increased with administration of the estradiol antagonist

(see Table 3.4). There were no formal analyses on the 30-s LL FI for rats that received the delays in ascending order because the model failed to converge. In sum, timing errors decreased with administration of the estradiol antagonist on the 5-s SS, but increased with administration of the estradiol antagonist on the 15-s LL. The pattern of results on the 15-s FI supported the hypothesis that the estrogen receptor antagonist would impair timing, but this was not the case for the shorter 5-s FI.

Descending.

On 5-s SS peak trials, there was a significant main effect of Dose on peak time (see Table 3.6; Figure 3.5). When rats received 0 mg/kg, they had earlier peak times (closer to the 5-s target) than when they received 1 mg/kg (see Figure A.5 for an alternative view). This was consistent with hypotheses that predicted worsened temporal accuracy as a result of estrogen receptor antagonist administration. Unlike the preliminary study where rats in proestrus were most accurate on timing shorter delays, there were no other significant differences in peak time.

On 5-s SS peak trials, there were significant Dose × Proestrus and Dose × Estrus interactions on peak spread (see Table 3.6; Figure 3.5). For the 0 mg/kg dose, rats in proestrus (b = 7.25) had larger peak spreads on the 5-s FI compared to rats in estrus (b = 5.10; t = 5.70, p < .001) and met/diestrus (b = 6.21; t = 2.29, p < .05). This was unexpected because rats in proestrus were most precise in timing shorter delays in the preliminary study. Also, rats in estrus on 0 mg/kg (b = 5.10) had smaller peak spreads than rats in met/diestrus on 0 mg/kg (b = 6.21; t = -4.01, p < .001). When rats in proestrus received 0 mg/kg (b = 7.25), they had larger peak spreads than when they received 1 mg/kg (b = 6.04; t = 2.85, p < .01; see Figure A.5 for an alternative view). However, when rats in estrus received 0 mg/kg (b = 5.10), they had smaller peak spreads than when they received 1 mg/kg (b = 6.44; t = -7.50, p < .001; see Figure A.5).

There were no differences in precision between the two doses when rats were in met/diestrus. Again, the estrogen receptor antagonist should have decreased temporal precision by blocking naturally occurring estradiol, but this pattern was not found when rats were in proestrus.

Overall, on the 5-s SS, administration of the estradiol antagonist resulted in lower CV values when rats were in proestrus contrary to the original hypothesis. CV values were relatively stable across doses of the estradiol antagonist when rats were in estrus or met/diestrus (see Table 3.7 for CV values). This suggests that timing errors, particularly variance in timing, were reduced for rats in proestrus when they received the antagonist.

There were no formal analyses on the 15-s LL FI for rats that received the delays in descending order because the model failed to converge. On 30-s LL peak trials, there was a significant Dose × Proestrus effect on peak time (see Table 3.8; Figure 3.6). For the 0 mg/kg dose, rats in proestrus (b = 33.90) had earlier peak times (closer to 30-s target) compared to rats in estrus (b = 39.30; t = -4.54, $p < .001$) and met/diestrus (b = 38.80; t = -4.17, $p < .001$). Similar results occurred during the preliminary study where rats in proestrus underestimated longer delays compared to rats in estrus and met/diestrus. For the 1 mg/kg dose, rats in proestrus (b = 42.30) had earlier peak times compared to rats in estrus (b = 44.70; t = -2.20, $p < .05$) but did not significantly differ from rats in met/diestrus (t = 0.17, $p > .05$). In addition, on the 0 mg/kg dose, rats in proestrus (b = 33.90) and estrus (b = 39.30) had earlier peak times than when they received 1 mg/kg (Proestrus: b = 42.30; t = -7.80, $p < .001$; Estrus: b = 44.70; t = -5.23, $p < .001$; see Figure A.6 for alternative view of these effects). These results were consistent with the hypothesis that the estrogen receptor antagonist would worsen temporal accuracy. However, there were no differences in accuracy between the two doses when rats were in met/diestrus.

On 30-s LL peak trials, there was a significant Dose × Proestrus effect on peak spread (see Table 3.8; Figure 3.6). For the 0 mg/kg dose, rats in proestrus (b = 38.30) had larger peak spreads on the 30-s FI compared to rats in estrus (b = 27.90; t = 8.12, p < .001). Also, rats in estrus on 0 mg/kg (b = 27.90) had smaller peak spreads than rats in met/diestrus on 0 mg/kg (b = 36.00; t = -7.81, p < .001). However, in the preliminary study, there were no differences in precision on a 30-s delay. For the 1 mg/kg dose, rats in proestrus (b = 33.50) had smaller peak spreads than rats in estrus (b = 47.50; t = -10.09, p < .001) and met/diestrus (b = 67.20; t = -5.54, p < .001). Also, rats in estrus (b = 47.50) had smaller peak spreads than rats in met/diestrus on the 1 mg/kg dose (b = 67.20; t = -3.23, p < .01). Overall, precision decreased with administration of the estradiol antagonist when rats were in estrus and met/diestrus but not in proestrus (see Figure A.6 for another view). Compared to the 0 mg/kg dose, rats in proestrus (b = 38.30) had larger peak spreads than when they received 1 mg/kg (b = 33.50; t = 4.30, p < .001). Compared to the 0 mg/kg dose, rats in estrus (b = 27.90) and met/diestrus (b = 36.00) had smaller peak spreads than when they received 1 mg/kg (Estrus: b = 47.50, t = -15.07, p < .001; Met/diestrus: b = 67.20; t = -5.12, p < .001). These results were consistent with the hypothesis that the estrogen receptor antagonist would worsen temporal precision.

When comparing CV values across the 5-s SS and 30-s LL FIs, typically the estradiol antagonist resulted in lower CV values when rats were in proestrus but higher CV values when rats were in estrus or met/diestrus (see Table 3.7). These results partially support the original hypothesis that the estrogen receptor antagonist would worsen accuracy and precision. Particularly on the 30-s LL, administration of the estradiol antagonist resulted in higher CV values when rats were in estrus and met/diestrus, suggesting that timing errors increased with administration of the estradiol antagonist. In short, timing errors were reduced with

administration of the estradiol antagonist when rats were in proestrus, but administration of the estradiol antagonist increased timing errors when rats were in estrus and met/diestrus.

Exploratory Analysis of Food Earnings

As previously noted, a large portion of the rats did not complete all trials on multiple sessions in the impulsive choice task. This resulted in a less data for the last block of trials, which was the 5-s LL or 60-s LL delays depending on what order the delays were presented. It was unclear whether the rats did not complete the sessions during specific phases of the estrous cycle. Previous studies have shown that estradiol decreased food intake (Yoest, 2018). Estradiol is highest during proestrus, so exploratory analyses were conducted to assess any differences in food earned based on the estrous cycle. Repeated measures multi-level Poisson regressions assessed number of food pellets earned per session of the impulsive choice task for each order of delays delivered. In both models, dose, estrous cycle, and their interactions were used as fixed effects, and rat (intercept) was included as a random effect. In these analyses, the estrous cycle was coded as the cycle that the rats were in when loaded into the operant chambers. Estrous cycle and dose were effects coded. Each model was conducted on 230 observations. The dispersion factors for both models were 1, indicating that a quasi-Poisson distribution was not necessary for either model.

For the rats that received the LL delays in ascending order (5 s → 60 s), when rats began the impulsive choice task in proestrus, they earned slightly more food than the average earnings across all estrous cycles, $b = 0.02$, $z = 2.35$, $p < .05$ (see Table 3.9; Figure 3.7). Administration of the estradiol antagonist did not significantly affect food earnings.

For the rats the received the delays in descending order (60 s → 5 s), there was a significant Dose × Proestrus interaction, $b = -0.02$, $z = -2.57$, $p < .05$ (see Table 3.10; Figure 3.8).

Further pairwise comparisons showed that rats in proestrus that received 1 mg/kg of the estradiol antagonist (b = 5.32) earned more food than when they received 0 mg/kg of the antagonist, (b = 5.25; t = 11.02, $p < .01$). Also, rats in estrus that received 1 mg/kg of the antagonist (b = 5.31) earned more food than when they received 0 mg/kg (b = 5.28, t = 4.20, $p < .05$). There were significant differences in estrous phase in the 0 mg/kg condition as well. Rats in proestrus that received 0 mg/kg of the antagonist (b = 5.25) earned less food than rats in met/diestrus that received 0 mg/kg (b = 5.34, t = 16.09, $p < .001$). Rats in estrus that received 0 mg/kg of the antagonist (b = 5.28) earned less food than rats in met/diestrus that received 0 mg/kg (b = 5.34, t = 16.70, $p < .001$). There were no significant differences in estrous phase in the 1 mg/kg condition.

Table 3.1. Summary of fixed effects from repeated measures multi-level logistic regression assessing impulsive choice for the rats that received the delays in ascending order only.

	b	SE	*z*	
(Intercept)	-1.29	0.23	-5.63	***
LL Delay	-4.70	0.15	-32.20	***
Dose	-0.03	0.04	-0.69	
Proestrus	-0.03	0.07	-0.46	
Estrus	0.07	0.06	1.20	
LL Delay × Dose	-0.12	0.14	-0.88	
LL Delay × Proestrus	0.29	0.21	1.40	
LL Delay × Estrus	0.01	0.19	0.07	
Dose × Proestrus	-0.03	0.06	-0.39	
Dose × Estrus	0.12	0.06	2.06	*
LL Delay × Dose × Proestrus	0.10	0.21	0.48	
LL Delay × Dose × Estrus	0.22	0.18	1.21	

Note: The 0 mg/kg dose was coded as 1 and the 1 mg/kg dose was coded as -1. Proestrus was coded as 1, 0; estrus was coded as 0, 1; met/diestrus was coded as -1, -1.

* $p < .05$
** $p < .01$
*** $p < .001$

Table 3.2. Summary of fixed effects from repeated measures multi-level logistic regression assessing impulsive choice for the rats that received the delays in descending order only.

	b	SE	*z*	
(Intercept)	-1.82	0.22	-8.45	***
LL Delay	-1.64	0.11	-15.43	***
Dose	-0.13	0.03	-4.04	***
Proestrus	0.14	0.05	2.68	**
Estrus	-0.01	0.04	-0.17	
LL Delay × Dose	0.02	0.11	0.17	
LL Delay × Proestrus	0.42	0.14	3.03	**
LL Delay × Estrus	0.09	0.13	0.70	
Dose × Proestrus	-0.02	0.05	-0.34	
Dose × Estrus	0.15	0.04	3.43	***
LL Delay × Dose × Proestrus	0.34	0.14	2.48	*
LL Delay × Dose × Estrus	-0.22	0.13	-1.67	

Note: The 0 mg/kg dose was coded as 1 and the 1 mg/kg dose was coded as -1. Proestrus was coded as 1, 0; estrus was coded as 0, 1; met/diestrus was coded as -1, -1.

* $p < .05$
** $p < .01$
*** $p < .001$

Table 3.3. Summary of fixed effects from a repeated measures multi-level nonlinear regression assessing 5-s SS peak timing for the rats that received the delays in ascending order only.

	Peak Time				Peak Spread			
	b	SE	*z*		*b*	SE	*z*	
(Intercept)	6.69	0.22	31.20	***	5.86	0.30	19.43	***
Dose	-0.01	0.08	-0.09		0.94	0.10	9.07	***
Proestrus	0.16	0.15	1.02		0.26	0.19	1.37	
Estrus	0.16	0.13	1.20		1.12	0.17	6.64	***
Dose × Proestrus	0.20	0.13	1.60		-0.06	0.16	-0.39	
Dose × Estrus	0.22	0.09	2.37	*	1.19	0.12	10.30	***

Note: The 0 mg/kg dose was coded as 1 and the 1 mg/kg dose was coded as -1. Proestrus was coded as 1, 0; estrus was coded as 0, 1; met/diestrus was coded as -1, -1.

* $p < .05$
** $p < .01$
*** $p < .001$

Table 3.4. Peak times and spreads for each delay, dose, and estrous cycle for rats that received the delays in ascending order.

Delay	Dose	Phase	Peak Time (s)	Peak Spread	Coefficient of Variation
5-s SS	0 mg/kg	Proestrus	7.03	7.00	0.99
		Estrus	7.06	9.11	1.29
		Met/diestrus	5.94	4.30	0.72
	1 mg/kg	Proestrus	6.65	5.24	0.79
		Estrus	6.63	4.85	0.73
		Met/diestrus	6.80	4.67	0.69
15-s LL	0 mg/kg	Proestrus	23.90	14.30	0.60
		Estrus	20.10	15.00	0.75
		Met/diestrus	20.90	17.60	0.84
	1 mg/kg	Proestrus	18.00	25.60	1.42
		Estrus	21.00	21.30	1.01
		Met/diestrus	20.50	20.70	1.01

Note: Coefficient of variation is calculated by dividing peak spread by peak time.

Table 3.5. Summary of fixed effects from a repeated measures multi-level nonlinear regression assessing 15-s LL peak timing for the rats that received the delays in ascending order only.

	Peak Time				Peak Spread			
	b	SE	*z*		*b*	SE	*z*	
(Intercept)	20.73	0.71	29.34	***	19.09	0.84	22.85	***
Dose	0.91	0.28	3.26	**	-3.46	0.41	-8.55	***
Proestrus	0.19	0.50	0.37		0.86	0.69	1.24	
Estrus	-0.16	0.37	-0.44		-0.95	0.52	-1.83	
Dose × Proestrus	2.04	0.45	4.51	***	-2.23	0.65	-3.44	***
Dose × Estrus	-1.36	0.36	-3.79	***	0.33	0.52	0.62	

Note: The 0 mg/kg dose was coded as 1 and the 1 mg/kg dose was coded as -1. Proestrus was coded as 1, 0; estrus was coded as 0, 1; met/diestrus was coded as -1, -1.

* $p < .05$
** $p < .01$
*** $p < .001$

Table 3.6. Summary of fixed effects from a repeated measures multi-level nonlinear regression assessing 5-s SS peak timing for the rats that received the delays in descending order only.

	Peak Time				Peak Spread			
	b	SE	*z*		*b*	SE	*z*	
(Intercept)	7.22	0.28	25.98	***	6.28	0.27	23.72	***
Dose	-0.33	0.09	-3.80	***	-0.10	0.10	-0.92	
Proestrus	0.06	0.14	0.45		0.36	0.16	2.30	*
Estrus	0.12	0.10	1.19		-0.51	0.12	-4.38	***
Dose × Proestrus	0.21	0.15	1.43		0.70	0.17	4.20	***
Dose × Estrus	-0.19	0.11	-1.84		-0.58	0.12	-4.81	***

Note: The 0 mg/kg dose was coded as 1 and the 1 mg/kg dose was coded as -1. Proestrus was coded as 1, 0; estrus was coded as 0, 1; met/diestrus was coded as -1, -1.

* $p < .05$
** $p < .01$
*** $p < .001$

Table 3.7. Peak times and spreads for each delay, dose, and estrous cycle for rats that received the delays in descending order.

Delay	Dose	Phase	Peak Time (s)	Peak Spread	Coefficient of Variation
5-s SS	0 mg/kg	Proestrus	7.15	7.25	1.01
		Estrus	6.81	5.10	0.75
		Met/diestrus	6.68	6.21	0.93
	1 mg/kg	Proestrus	7.40	6.04	0.82
		Estrus	7.86	6.44	0.82
		Met/diestrus	7.38	6.64	0.90
30-s LL	0 mg/kg	Proestrus	33.90	38.30	1.13
		Estrus	39.30	27.90	0.71
		Met/diestrus	38.80	36.00	0.93
	1 mg/kg	Proestrus	42.30	33.50	0.79
		Estrus	44.70	47.50	1.06
		Met/diestrus	41.80	67.20	1.61

Note: Coefficient of variation is calculated by dividing peak spread by peak time.

Table 3.8. Summary of fixed effects from a repeated measures multi-level nonlinear regression assessing 30-s LL peak timing for the rats that received the delays in descending order only.

	Peak Time				Peak Spread			
	b	SE	*z*		*b*	SE	*z*	
(Intercept)	40.15	1.20	33.54	***	41.72	2.09	19.97	***
Dose	-2.80	0.56	-5.03	***	-7.65	1.06	-7.25	***
Proestrus	-2.03	0.72	-2.82	**	-5.85	1.15	-5.07	***
Estrus	1.89	0.65	2.92	**	-4.02	1.12	-3.58	***
Dose × Proestrus	-1.39	0.64	-2.17	*	10.05	1.11	9.08	***
Dose × Estrus	0.09	0.65	0.14		-2.13	1.12	-1.91	

Note: The 0 mg/kg dose was coded as 1 and the 1 mg/kg dose was coded as -1. Proestrus was coded as 1, 0; estrus was coded as 0, 1; met/diestrus was coded as -1, -1.

* $p < .05$
** $p < .01$
*** $p < .001$

Table 3.9. Summary of fixed effects from a repeated measures multi-level Poisson regression testing food earned in the impulsive choice task for the ascending order only.

	b	SE	*z*	
(Intercept)	5.45	0.03	164.35	***
Dose	0.00	0.00	-0.22	
Proestrus	0.02	0.01	2.35	*
Estrus	0.00	0.01	0.71	
Dose × Proestrus	-0.01	0.01	-1.57	
Dose × Estrus	0.01	0.01	1.00	

Note: The 0 mg/kg dose was coded as 1 and the 1 mg/kg dose was coded as -1. Proestrus was coded as 1, 0; estrus was coded as 0, 1; met/diestrus was coded as -1, -1.

* $p < .05$
** $p < .01$
*** $p < .001$

Table 3.10. Summary of fixed effects from a repeated measures multi-level Poisson regression testing food earned in the impulsive choice task for the descending order only.

	b	SE	*z*	
(Intercept)	5.31	0.05	104.68	***
Dose	-0.01	0.01	-2.45	*
Proestrus	-0.02	0.01	-2.46	*
Estrus	-0.01	0.01	-1.70	
Dose × Proestrus	-0.02	0.01	-2.57	*
Dose × Estrus	0.00	0.01	-0.14	

Note: The 0 mg/kg dose was coded as 1 and the 1 mg/kg dose was coded as -1. Proestrus was coded as 1, 0; estrus was coded as 0, 1; met/diestrus was coded as -1, -1.

* $p < .05$
** $p < .01$
*** $p < .001$

Figure 3.1. Mean proportion of LL choices across LL delays for the rats that received the delays in ascending order only. Error bars (+/− SEM) were computed with respect to the estimated marginal means of the 30-s intercept fitted repeated measures multi-level logistic regression and jittered for readability.

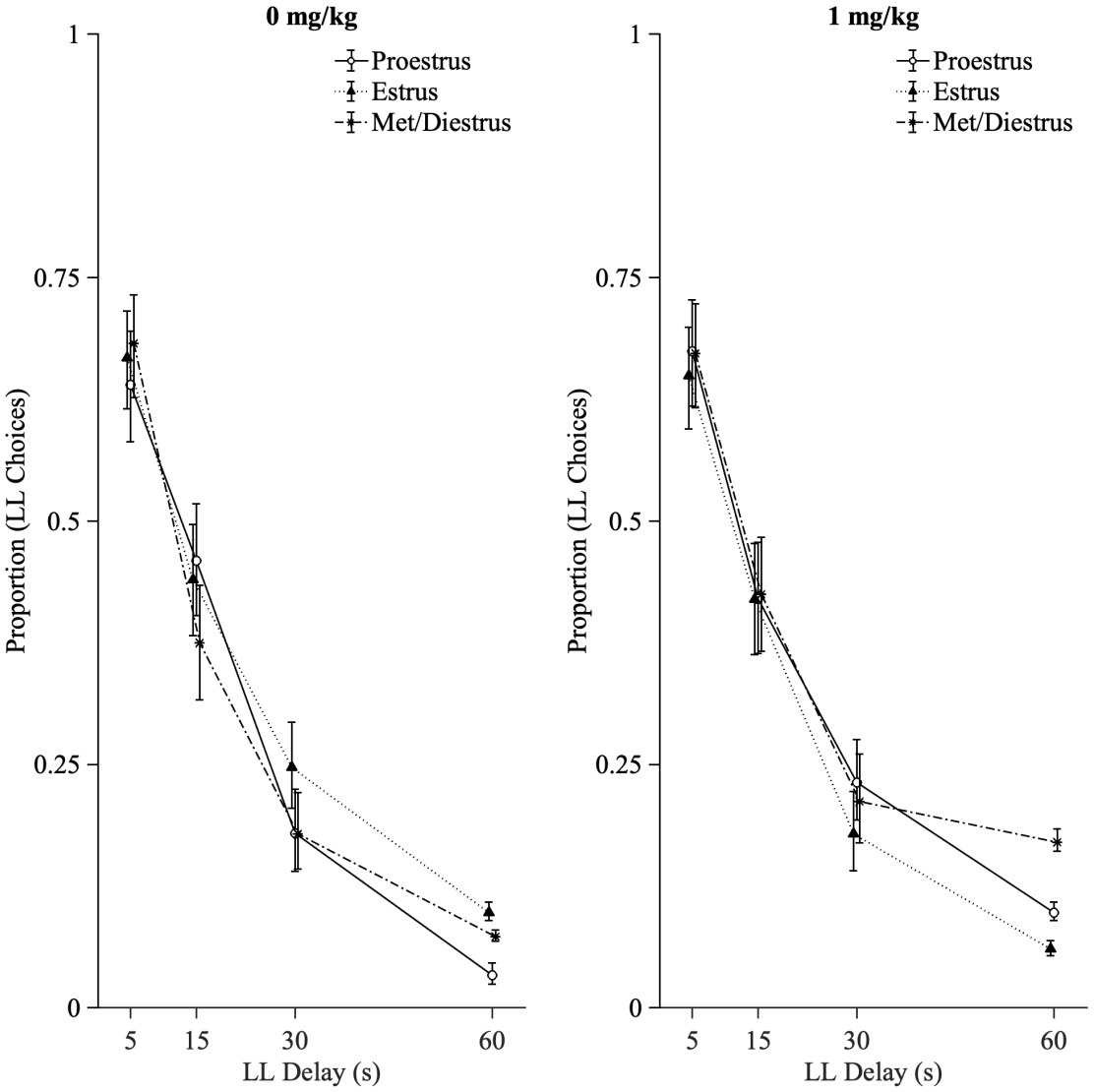


Figure 3.2. Mean proportion of LL choices across LL delays for the rats that received the delays in descending order only. Error bars (+/− SEM) were computed with respect to the estimated marginal means of the 30-s intercept fitted repeated measures multi-level logistic regression and jittered for readability.

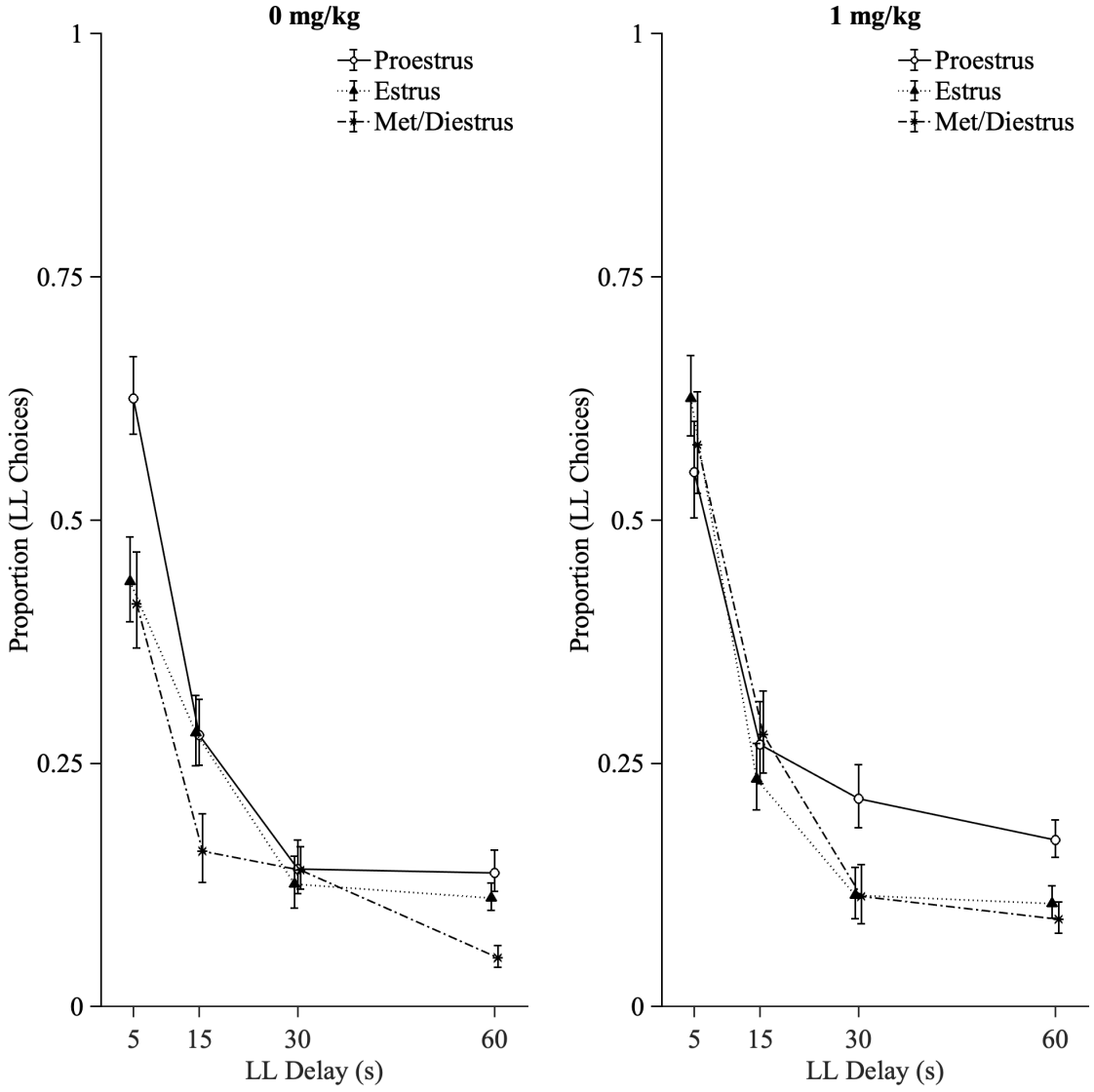


Figure 3.3. The top panel represents mean responses per minute as a function of time into 5-s SS peak trials for the ascending order only. Markers represent mean responses and lines represent fitted repeated measures multi-level nonlinear regression values. Solid vertical and horizontal lines represent the target interval. The bottom two panels represent mean peak time and spread per estrous cycle with error bars (+/− SEM).

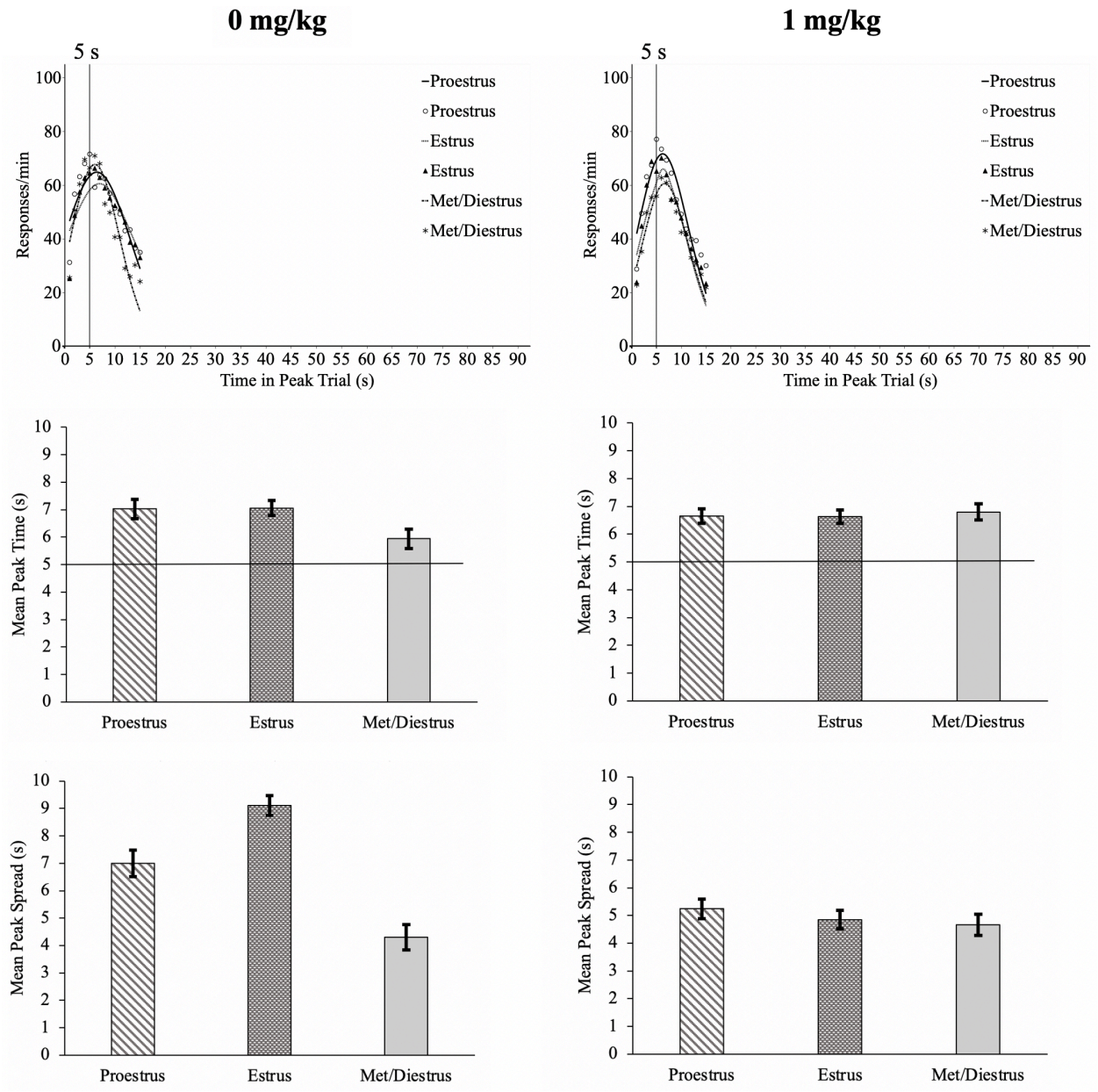


Figure 3.4. The top panel represents mean responses per minute as a function of time into 15-s LL peak trials for the ascending order only. Markers represent mean responses and lines represent fitted repeated measures multi-level nonlinear regression values. Solid vertical and horizontal lines represent the target interval. The bottom two panels represent mean peak time and spread per estrous cycle with error bars (+/− SEM). Note the truncated axes in the bottom two panels.

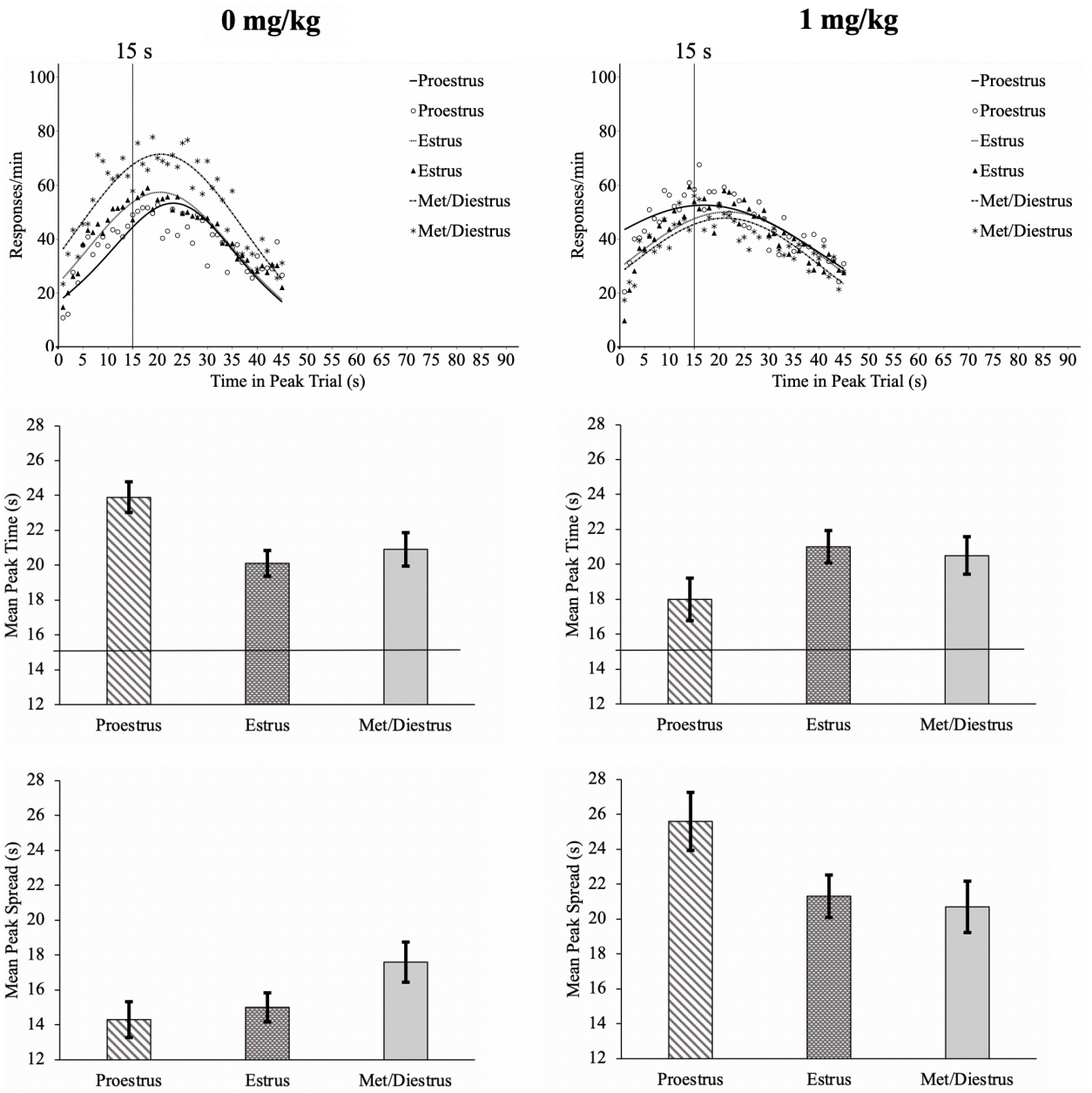


Figure 3.5. The top panel represents mean responses per minute as a function of time into 5-s SS peak trials for the descending order only. Markers represent mean responses and lines represent fitted repeated measures multi-level nonlinear regression values. Solid vertical and horizontal lines represent the target interval. The bottom two panels represent mean peak time and spread per estrous cycle with error bars (+/− SEM).

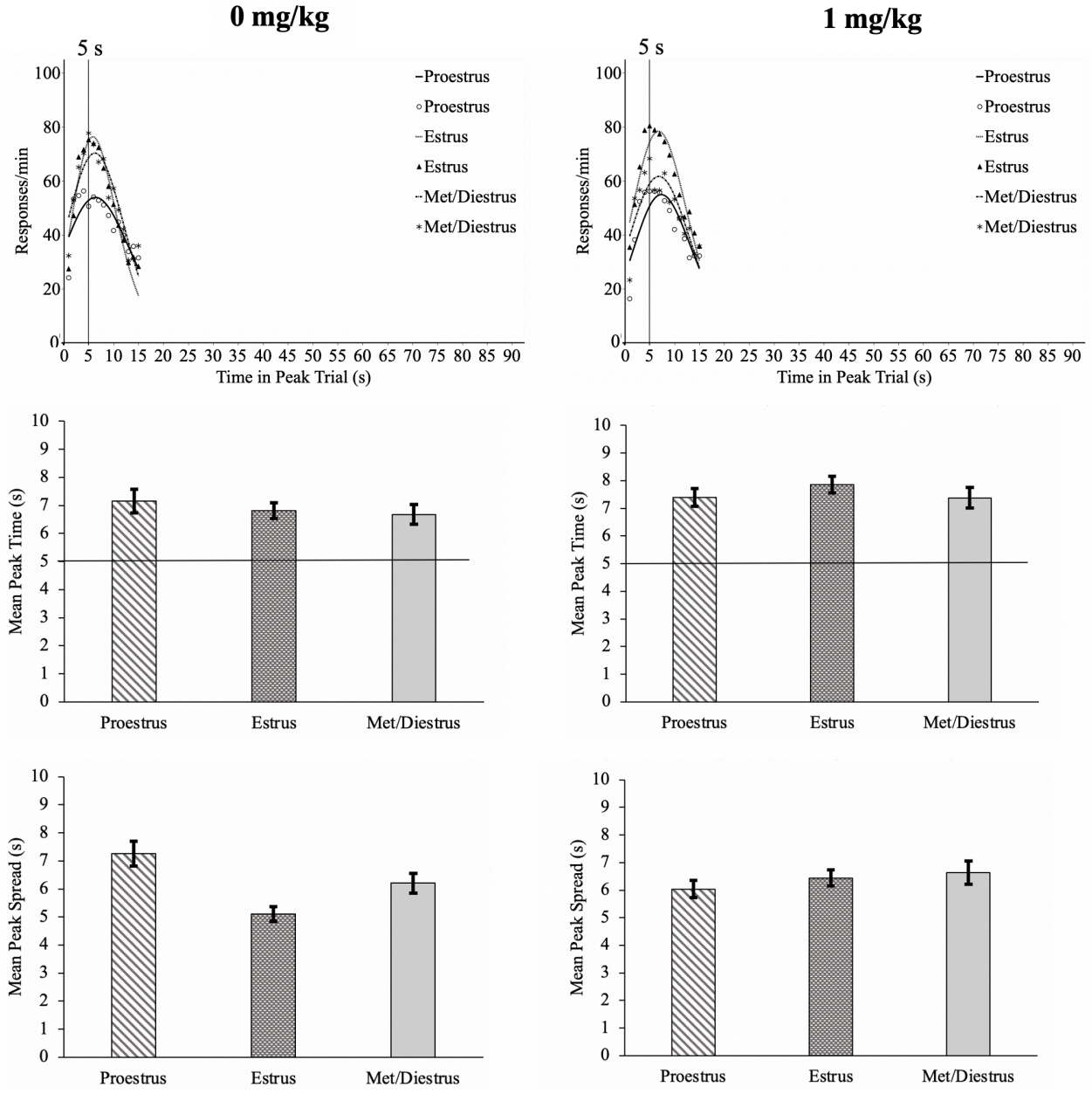


Figure 3.6. The top panel represents mean responses per minute as a function of time into 30-s LL peak trials for the descending order only. Markers represent mean responses and lines represent fitted repeated measures multi-level nonlinear regression values. Solid vertical and horizontal lines represent the target interval. The bottom two panels represent mean peak time and spread per estrous cycle with error bars (+/− SEM). Note the truncated axes in the bottom two panels.

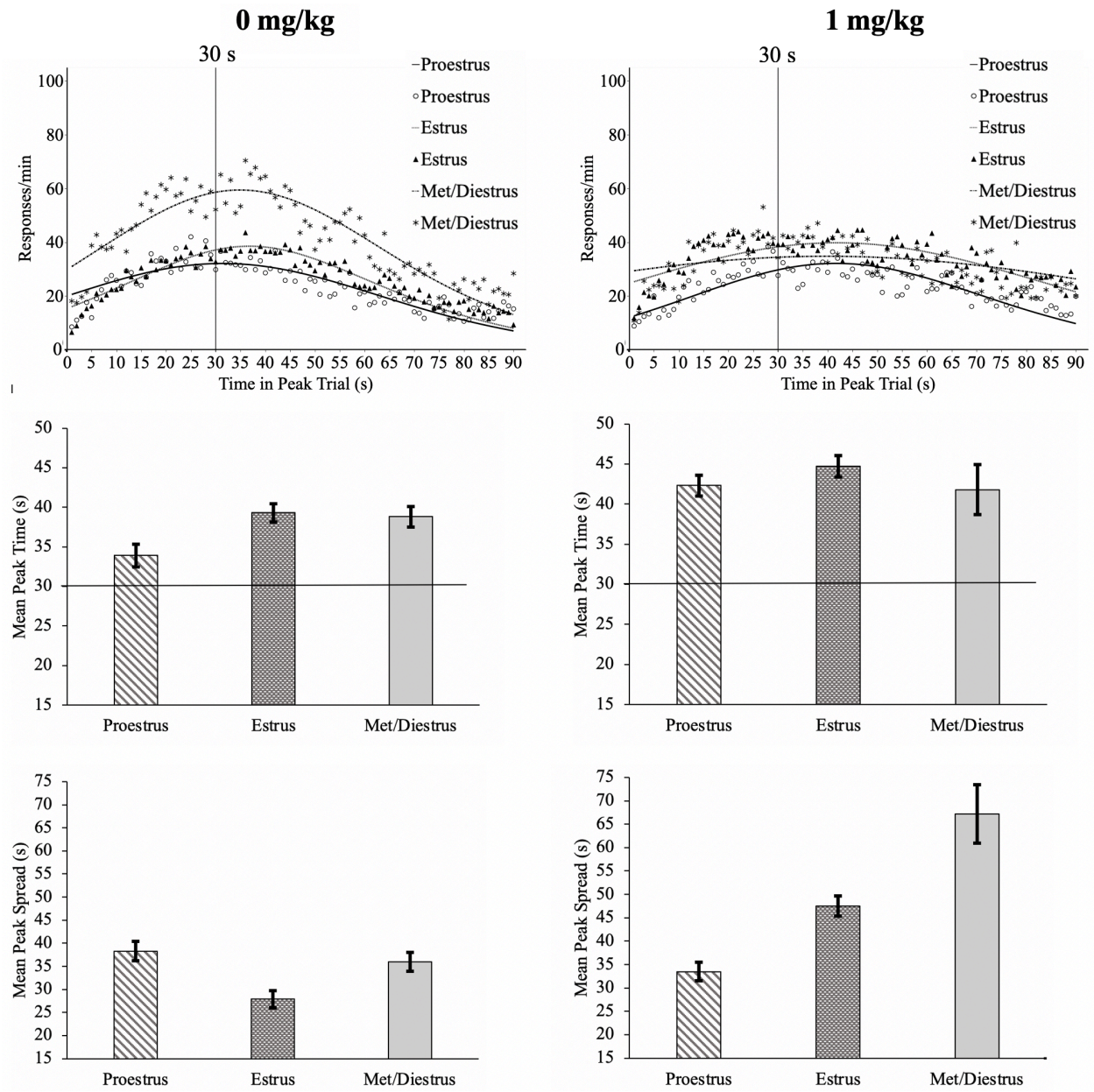


Figure 3.7. Mean number of food pellets earned for the rats that received the delays in ascending order only. Error bars (+/− SEM) were computed with respect to the estimated marginal means of the fitted repeated measures multi-level Poisson regression. Converted b-values are displayed above each bar.

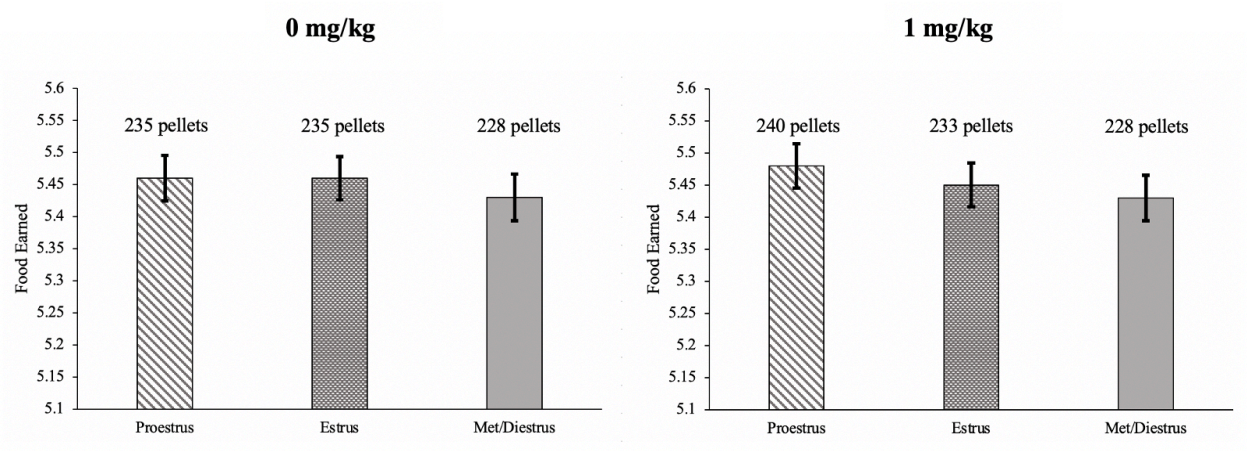
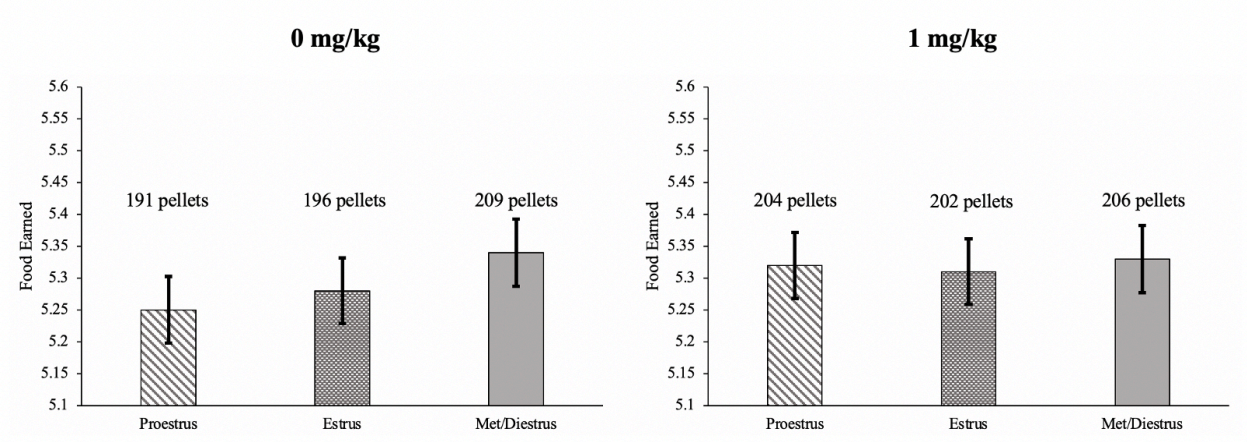


Figure 3.8. Mean number of food pellets earned for the rats that received the delays in descending order only. Error bars (+/− SEM) were computed with respect to the estimated marginal means of the fitted repeated measures multi-level Poisson regression. Converted b-values are displayed above each bar.



Chapter 4 - **Discussion**

Based on preliminary research, the natural increase in estradiol that occurs in proestrus may increase self-control while low levels of estradiol that occur in estrus may decrease self-control. However, in the current study, rats in proestrus made the fewest LL choices at both 0 mg/kg and 1 mg/kg of the estradiol antagonist in the ascending condition. In the descending condition, on 0 mg/kg, rats in met/diestrus made the fewest LL choices while rats in estrus made the most LL choices. On 1 mg/kg, rats in proestrus made the most LL choices and rats in estrus made the least LL choices. This pattern of results across both order of delays, specifically with 0 mg/kg of the estradiol antagonist, does not replicate the preliminary study, which showed rats made the most LL choices in proestrus.

Furthermore, preliminary research suggests that the increase in estradiol that occurs naturally in proestrus may increase temporal accuracy and precision and the decrease in estradiol that occurs during estrus may decrease accuracy and precision. In the current study, there was little consistency in the effects of naturally occurring or manipulated hormones on accuracy and precision across the estrous cycle. Rats in proestrus were not more accurate or precise on all the delays. In fact, most of the time, rats in proestrus were the least accurate or the least precise in the ascending and descending conditions. Rats in estrus did not differ dramatically from the other estrous stages in terms of accuracy but were most precise in general across all conditions. In the ascending condition, administration of 1 mg/kg of the estradiol antagonist worsened timing on a short delay but improved timing on a longer delay, but in the descending condition, the estradiol antagonist improved timing when rats were in proestrus but worsened timing when rats were in estrus or met/diestrus.

Overall, the current study results did not replicate the preliminary study, but hormonal manipulations, differences in satiety and body weight, and added complexity in design may explain these discrepancies. Rats in the current study experienced a choice task with a short SS delay, which may have influenced their self-control and timing behavior. Reward value associated with the delays may have resulted in regression to the mean effects and reward-timing interactions.

Delay Length and Order

The differences in choice task parameters, particularly delay lengths and order, may account for some of the discrepancies in results between the preliminary study and current study. The preliminary study used an impulsive choice task where the SS delay increased across three phases and the phases were several sessions each. The SS delay was 5 s in Phase 1, 10 s in Phase 2, and 20 s in Phase 3. The LL delay remained at 30 s in all phases. Each phase lasted for 10 consecutive sessions, and sessions were only 2 hr in length. The impulsive choice task in the current study varied the LL delay across a wider range of delays but always with a relatively short SS option. Recent research suggests that short SS delays negatively affect self-control. Specifically, male rats received a choice task where the SS delay was 5 or 10 s while the LL delay increased across sessions. Rats that experienced the longer SS delay made more LL choices (Smith, Panfil, & Kirkpatrick, in preparation). While this effect has not been investigated in female rats, the prolonged exposure to the short SS delay in the current study may have been detrimental to self-control across all conditions. Along the same lines, previous research comparing within-session and between-session changes in delay showed decreased delay sensitivity, particularly on short delays, in male rats that received the within-session changes (Peterson, Hill, & Kirkpatrick, 2015). Taken together, the within-session task with a

short SS delay in the current study may have resulted in increased impulsivity and decreased sensitivity to changes in delay.

Consistent exposure to the short 5-s SS delay in the current study may also have affected rats' timing behavior on longer delays. In the preliminary study, where rats had prolonged exposure to a 30-s LL delay, rats across all phases of the estrous cycle timed the longest delays better than the shortest delays, as evident in lower CV values. Lower CV values suggest fewer timing errors due to either overestimation of delay and/or increased variance in timing. Along these lines, one could expect rats in the current study to time the shortest delay better than the longest delays because of prolonged exposure to a 5-s SS delay. Instead, CV values mostly decreased from 5 s to 15 s in the ascending condition but stayed the same on the 5-s and 30-s FIs in the descending condition when looking at the 0 mg/kg dose of the estrogen receptor antagonist. Overall, prolonged exposure to the 5-s SS delay does not appear to have negatively affected rats' timing behavior on longer delays.

It is possible that the lower CV values observed on longer delays in the preliminary study may have occurred because of a regression to the mean effect. When considering timing behavior in a Bayesian context, rats have no information about the delays used in the impulsive choice task at first resulting in wide, flat distributions representing each time interval. The certainty in timing each interval should increase with experience so that the distributions representing each interval become sharper at the target interval with a narrower standard deviation around the interval. Prolonged exposure to the 30-s LL delay in the preliminary study should have decreased uncertainty in timing the interval, resulting in a sharper and more narrow distribution representing this interval. Briefer exposure to the shorter delays should have resulted in more uncertainty in the interval distributions compared to the 30-s distribution.

Based on the increased uncertainty of the shorter intervals, rats should have overestimated the shorter delays towards the direction of the 30-s LL, resulting in larger CV values. In other words, prolonged exposure results in a more informative prior distribution of information which influences timing of intervals that were experienced less often. Previous research has shown this effect where timing on the uncertain interval is either under- or overestimated towards the more certain interval (Jazayeri & Shadlen, 2010; Petzschner & Glasauer, 2011; Zimmermann & Cicchini, 2020). This suggests that rats in the current study may underestimate the LL delays because of less certainty in the LL intervals and more certainty in the 5-s SS delay. However, timing of the 30-s and 60-s LL delays were not analyzed because of a lack of data in the ascending condition. In the descending condition, rats overestimated the 30-s delay across estrous cycles and doses of the estradiol receptor antagonist. There was a larger degree of uncertainty in timing the 30-s interval in the current study compared to the preliminary study evident in the peak spreads. It is possible that the perceived distribution of the 30-s interval was still too wide and flat (indicating uncertainty in timing) for a regression to the mean effect to occur.

It is important to note that the current study provided significantly less exposure to all delays compared to the preliminary study. Exposure to delays was reduced in the current study's design to test rats on multiple doses and tasks before they reached anestrus. While the current study had 14-hr sessions, there were multiple inter-block intervals per session and fewer sessions than the preliminary study, which delivered the task in 2-hr sessions for 30 days. While the number of peak trials per delay were the same within a session (6 SS and 6 LL peak trials per delay per session), there were fewer total forced choice and free choice trials in the current study. Less training could have resulted in more uncertainty in the intervals across all delays. Shorter,

spaced sessions likely enhanced acquisition and performance on the embedded peak trials in the preliminary study. Longer, massed sessions in the current study likely impaired timing behavior across all conditions to some degree.

Regardless of increased timing errors in the current study, there were key differences between estrous cycles when comparing accuracy and precision measures between the current and preliminary studies. In the preliminary study, rats in proestrus were more accurate and precise in timing the 5-s SS delay. In the current study's ascending condition, rats in proestrus and estrus were less accurate and precise than rats in met/diestrus timing the 5-s SS delay. In the current study's descending condition, there were no differences in accuracy but rats in proestrus and estrus were more precise than rats in met/diestrus timing the 5-s SS delay. In the preliminary study, rats in proestrus were more accurate in timing the 30-s LL delay compared to when rats were in estrus and met/diestrus, but there were no differences in peak spread across estrous stages on the 30-s delay. In the current study's descending condition, rats in proestrus were more accurate than rats in estrus and rats in met/diestrus, and rats in estrus were more precise than rats in proestrus and rats in met/diestrus timing the 30-s LL delay. These differences in accuracy and precision on the same delays suggest that ascending versus descending presentation of delays affected rats' timing behavior.

The reward values associated with the delays during initial experience with the task may also have influenced timing behavior. Previous research has shown that performance on embedded peak trials (like those used in the current study) were affected by the value associated with each of the changing delays (Galtress, Garcia, & Kirkpatrick, 2012; Galtress & Kirkpatrick, 2009, 2010a; Galtress, Marshall, & Kirkpatrick, 2012). More specifically, larger magnitude rewards associated with a delay increase precision in responding and changing magnitudes can

shift peak functions left and right (Galtress & Kirkpatrick, 2009). Increasing the magnitude of reward associated with a delay shifts the peak function left while decreasing the magnitude of reward shifts the peak function right (Galtress & Kirkpatrick, 2009, 2010a; Ludvig, Balci, & Spetch, 2011; Ludvig, Conover, & Shizgal, 2007). Across studies, these effects were found based on the initial experience in the task. Taken together with the current study design, the contrasting reward magnitude value established in the initial experience with the first block of delays may have resulted in additional timing variance on the fluctuating LL delays. The value imbued to each delay based on the order they were presented may explain the complexities in the timing results.

In sum, differences in task parameters such as SS delay length, order of delays, and experience with the delays likely influenced choice and timing behavior in the current study, which may explain some of the discrepancies when compared to the preliminary study. In particular, extended experience with the 5-s SS delay likely negatively affected choice behavior and reward value associated with each delay during initial tasking acquisition likely interacted with timing behavior and estrous cycle. Unfortunately, no previous research has examined the effects of sex hormones on reward magnitude effects on timing behavior. Future research is needed to evaluate the role of sex hormones in reward-timing interactions.

Satiety and Body Weight

Due to the extended length of the overnight sessions, rats may have reached satiation before the end of each session. Rats in the current study typically did not complete all the trials. Up to 400 reinforcers could be earned in a session, but on average, rats earned 220 reinforcers.

In the ascending condition, when rats began the impulsive choice task in proestrus, they earned more food than the average earnings across all estrous cycles, and administration of the

estradiol antagonist did not significantly affect food earnings. This is unexpected based on the impulsive choice assessment, which showed that rats in proestrus made the fewest LL choices at the 30-s intercept. However, the food earnings analyses examined earnings across entire sessions and did not assess earnings per delay. Also, food intake usually decreases during periods of fertility because females are spending time looking for a mate, not eating (Yoest, 2018). Estradiol is at its highest levels during proestrus when females are preparing for ovulation and mating. Estradiol can decrease food intake, which may direct more attention to mating as well (Yoest, 2018). However, studies assessing food intake did not use choices between differing food reward amounts like the current study. While increased estradiol may decrease food intake in studies offering choices between food and access to sexually mature males, females in proestrus did not earn less food as a result of impulsive choices for food rewards.

The pattern of results in the descending condition is more consistent with previous food intake literature. In the descending condition, rats in proestrus and estrus that received 1 mg/kg of the estradiol antagonist earned more food than when they received 0 mg/kg of the antagonist, and rats in proestrus and estrus that received 0 mg/kg of the antagonist earned less food than rats in met/diestrus that received 0 mg/kg. When estradiol was blocked by the ER antagonist, rats in proestrus earned more food. Without the ER antagonist, rats in proestrus also earned less food than rats in met/diestrus. When delays were presented in descending order, naturally occurring increases in estradiol may have decreased food intake while blocking these effects increased food intake.

When considering the ascending and descending condition results together, it is possible that estrous cycle and satiety interacted with delay length to produce these differences. Choosing

the LL can reduce satiety because there are longer periods between trials due to the longer delay to reward. In addition, receiving the LL delays in ascending order may have helped rats avoid satiety later in the session. As the LL delay increases in the session, rats prefer the SS. As the session progresses, they earn less food because they chose the SS more, which may reduce satiety. This pattern occurred in rats in proestrus in the ascending condition. They made the most SS choices at the 30-s LL delay but still earned more food on average compared to the other estrous cycles. Overall, resistance to satiety may have allowed rats to work longer.

Across both conditions, the average food earnings amounted to roughly 10 g, which was about half of a rat's daily food ration. Rats received the rest of their food ration after the session. While it is possible that rats were satiated by the end of the session, it is also likely that they experienced fatigue and/or anticipated their upcoming food delivery towards the end of the session. It is also important to note that the rats were food restricted and maintained at approximately 90% of their projected *ad libitum* weight, which was a relatively mild level of deprivation compared to most of the rodent literature assessing impulsive choice and timing. Typically, rats are food restricted to 85% or below. However, food restriction at or below 85% of free feeding weight may interact with dopaminergic functioning (Cabib & Bonaventura, 1997; Costall, Fortune, Naylor, & Nohria, 1980). Impulsive choice and temporal perception are strongly associated with dopamine (DA) in the striatum (Cardinal, 2006; Meck, 2006). Where dopaminergic functioning underlies impulsive choice and timing, rats were maintained at 90% to minimize this potential confound.

In sum, rats consistently did not complete all trials and may have experienced satiety and/or fatigue as a result of the extended session length. Consistent with previous literature showing estradiol decreases food intake, rats in proestrus and estrus earned more food when

estradiol was blocked with the antagonist in the descending condition. They earned less food than rats in met/diestrus without the antagonist. Resisting satiety may have been easier in the ascending condition because preference for the SS increased as LL delay increased, resulting in fewer food rewards towards the end of the session. Specifically, rats in proestrus in the ascending condition may have resisted satiety by choosing the SS most on later delays, allowing them to work for longer.

Hormonal Manipulations

Throughout the study, drugs were administered to manipulate naturally occurring hormones. Injections of leuprolide should have induced all rats' cycle to met/diestrus for the first night of both testing phases. A small portion of the rats were in met/diestrus on the first night of both testing phases while a little over half of the rats were in estrus on the first night of both testing phases. Increases in luteinizing hormone (LH) occur as a result of natural increases in estradiol and increases in LH further increase levels of estradiol. The dose or timing of leuprolide injections may not have caused a sufficient rise to trigger this cascade of hormone fluctuations. Along the same lines, the pH of the leuprolide solution was adjusted to comply with IACUC (Institutional Animal Care and Use Committee) guidelines during mixing, which may have decreased its efficacy. Previous studies using the estrous synchronization technique reported 80% or more of the rats' cycles were induced to met/diestrus two days after (Rivier & Vale, 1990; A. J. Roberts et al., 1998). Taken together, this suggests that leuprolide did not induce estrous cycles in the current study.

It is unclear whether leuprolide affected the rats' behavior at all in the current study. Any acute effects on LH caused by leuprolide were no longer present on the first day of testing based on the half-life of leuprolide. Furthermore, these injections occurred two days before each

testing phase, suggesting that any stress caused by the injections of leuprolide likely dissipated before testing. As previously noted, only 10 of 48 rats were in met/diestrus on the first night of testing phase 1 and 3 of 48 rats on the first night of testing phase 2, but 23 of 48 rats were in estrus on the first night of testing phase 1 and 34 of 48 rats on the first night of testing phase 2. The number of rats in each stage of the estrous cycle was not randomly distributed. However, the preliminary study did not have a randomly distributed number of rats in each stage either. Unfortunately, it remains unclear whether the leuprolide had any effect at all because of the non-random patterns in both studies.

While the leuprolide injections did not induce estrous cycles to the degree of success that previous studies have achieved, it is possible that there was a long-term effect of the leuprolide injections. The results obtained in the current study when examining the 0 mg/kg condition of the estrogen receptor antagonist were inconsistent with the preliminary study where estrous cycles occurred naturally. A longer-term hormonal cascade may have altered choice and timing behavior, which could at least partially explain the discrepancies in results. No other studies have examined the effects of a gonadotropin-releasing hormone receptor agonist on impulsive choice or timing behavior in male or female rodents, so additional study is needed to determine whether a longer-term hormonal cascade occurred in the current study.

Sex hormones such as estradiol may affect impulsive choice and timing behavior but possibly to varying degrees depending on whether estrous cycles are naturally occurring or manipulated. Currently, no other studies besides the preliminary study have measured estrous cycles and timing behavior in naturally cycling female rats, but multiple studies have examined females' timing behavior in response to administration of estradiol. Chronic administration of estradiol decreased temporal discrimination in ovariectomized rats, and single injections of

estradiol resulted in underestimation of delays (Pleil et al., 2011; Ross & Santi, 2000; Sandstrom, 2007). In the preliminary study where hormones were not manipulated at all, when estradiol was highest rats were more precise in timing short delays and underestimated longer delays. In the current study, the timing results were unsystematic across conditions and delays. In addition, no studies have examined impulsive choice behavior in female rats while assessing estrous cycles besides the preliminary and current studies, which were inconsistent. Taken together, these results suggest that timing and impulsive choice behaviors may be sensitive to natural versus manipulated hormones. These patterns may be confirmed with future studies that directly compare natural and manipulated hormonal effects on impulsive choice and timing.

Taking into account that the leuprolide may have affected choice and timing in a long-term manner, the majority of rats were not in same estrous stage throughout testing, which has multiple implications for assessing how the rats learned the impulsive choice task. Because rats were not in the same stage of the estrous cycle on the first night of testing, learning of the impulsive choice task may have differed based on initial estrous cycle. The incomplete synchronization resulted in rather uneven numbers of rats in each of the estrous cycles during the first session of the task. The rats did not sync up naturally either, so the inconsistency in numbers of rats in a specific estrous cycle on any particular session continued throughout the experiment. In addition, cycle lengths varied, so as few as three rats were in a specific estrous cycle during a specific session. Unfortunately, the lack of control over the rats' estrous cycles complicates the statistical analysis of impulsive choice task learning.

The same issue arises when assessing bisection task learning. Estrous cycles were not collected during the bisection training task but would have been synchronized and collected during bisection testing if rats acquired the training task. The rats likely had widely varying

estrous cycles similar to the choice task on the first few days of the bisection training task. Without any estrous cycle collections, it remains unclear whether estrous cycle affects bisection training task acquisition or performance.

Previous research suggests female sex hormones may impact task acquisition. Typically, females in proestrus or who received injections of estradiol acquire tasks faster than females in other stages of the estrous cycle or ovariectomized females (see Dalla & Shors, 2009 for a review). For example, in eye-blink conditioning, females learn to time the interval between the conditioned stimulus and unconditioned stimulus faster when training begins while rats are in proestrus (Dalla & Shors, 2009). However, there are some paradigms where the opposite effect is observed. For example, high levels of estrogen during the conditioning phase of a latent inhibition paradigm caused females to perform worse during testing, possibly because they were unable to ignore irrelevant stimuli (Quinlan, Duncan, Loiselle, Graffe, & Brake, 2010). Further research is needed to determine whether female rats in a specific estrous cycle would acquire the bisection task faster when compared to the other estrous cycles. At least in the impulsive choice task, many rats were in estrus on the first session, so low levels of estradiol may affect impulsive choice task acquisition.

In addition to injections of leuprolide, rats received injections of an estrogen receptor antagonist. The estrogen receptor antagonist should inhibit activation of estrogen receptors. Based on the preliminary study, it was hypothesized that blocking estradiol from binding to ERs would make females more impulsive and decrease their temporal accuracy and precision. However, there were no differences in impulsive choices between the 0 mg/kg and 1 mg/kg doses for each estrous phase across both orders. It is possible that the estradiol antagonist dose was too low to produce effects. Rats received 1 mg/kg injections of estrogen receptor antagonist,

antiestrogen ICI 182,780 to block the effects of estradiol on the brain, which has been shown to result in biologically relevant levels of antiestrogen ICI in the hypothalamus for up to 24 hr (Alfinito et al., 2008). This dose may be effective for specific functions associated with the hypothalamus. For example, antiestrogen ICI did not block the effects of estradiol on progesterone receptor expression in the hypothalamus or prolactin surges before birth (Steyn, Anderson, & Grattan, 2007). This suggests that brain regions may be sensitive to specific doses of antiestrogen ICI in order to affect functioning (Steyn et al., 2007). Dose-specific effects may explain the weak effects on impulsive choice behavior when considering the stronger effects of antiestrogen ICI on peak timing.

In the ascending condition, on the 5-s SS FI, the estradiol antagonist decreased CV values, suggesting reduced timing errors, but on the 15-s LL FI, administration of the estradiol antagonist increased CV values, suggesting increased timing errors. In the descending condition, the estradiol antagonist decreased CV values when rats were in proestrus but increased CV values when rats were in estrus or met/diestrus. While complex, these results suggest that 1 mg/kg of antiestrogen ICI was an effective dose for influencing timing behavior. Differences in brain regions underlying impulsive choice and temporal perception may at least partially explain the response to antiestrogen ICI.

Brain Regions and Neurotransmitters Underlying Impulsive Choice and Timing

As previously noted, impulsive choice and temporal perception are strongly associated with DA in the striatum (Cardinal, 2006; Meck, 2006). The nucleus accumbens core (NAc), located in the ventral striatum, plays an important role in impulsive choices (see Cardinal, 2006 for a review). The NAc receives dopaminergic projections from the ventral tegmental area also known as the mesolimbic pathway (Salgado & Kaplitt, 2015; Ungerstedt, 1971). Lesions or

inactivations to NAc increase impulsive choices in rats (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001; da Costa Araujo et al., 2009; Feja, Hayn, & Koch, 2014; Galtress & Kirkpatrick, 2010b; Steele, Peterson, Marshall, Stuebing, & Kirkpatrick, 2018; Valencia-Torres et al., 2012). Caudate and putamen nuclei (CPu), located in the dorsal striatum, are involved in temporal perception (Meck, 2006). Dopaminergic cell bodies in the substantia nigra synapse in the CPu region, which is part of the nigrostriatal pathway (Ungerstedt, 1971). Dopaminergic lesions to CPu result in poor accuracy and precision when timing durations (Meck, 2006). Overall, the striatum and dopamine are directly involved in impulsive choice and timing behaviors.

Previous research has reported extensive sex differences in dopaminergic functioning in the striatum (Becker & Ramirez, 1981; Levesque, Gagnon, & Di Paolo, 1989; Morissette & Di Paolo, 1993; Xiao & Becker, 1994). These studies were originally examining sex differences in DA release in the hypothalamus with the striatum serving as a control (e.g., Becker & Ramirez, 1980). Researchers found that females had higher levels of DA release in the striatum than males did after administration of amphetamine (Becker & Ramirez, 1981). However, these sex differences were specific to when females were in estrus. When females were in proestrus, DA release was similar to that in males after amphetamine administration (Becker & Ramirez, 1981). Along the same lines, extracellular dopamine in the striatum peaks during estrus (Xiao & Becker, 1994), and the number of striatal dopamine uptake sites is highest during proestrus (Morissette & Di Paolo, 1993). DA receptor density fluctuates across the estrous cycle as well (Di Paolo, Falardeau, & Morissette, 1988; Levesque et al., 1989). Thus, striatal dopaminergic functioning may be directly affected by fluctuating estradiol levels across the estrous cycle.

Estradiol, an agonist of estrogen receptors, likely mediates these sex differences observed in the striatum. Previous research has shown that estradiol affects the striatum to change dopamine in multiple ways. For example, direct injection of estradiol into the NA (nucleus accumbens core and shell) in ovariectomized rats before potassium (K+) stimulation produced a small increase in the concentration of DA released. This effect subsided 15 min later. Continued measurement of DA via cyclic voltammetry showed that the stimulated DA release was highest 60 min after administration which lasted for another 60 min (Thompson & Moss, 1994). These results suggest that estradiol administration caused less DA release at first, but DA was available in the extracellular space for an extended duration. Also, estradiol administration slows down DA clearance significantly in the NA of ovariectomized rats, which means that estradiol affects DA reuptake (Thompson, 1999). Specifically, in the CPu, females in proestrus and estrus had higher extracellular dopamine than during diestrus, but there were no differences in DA concentration for intact versus castrated males (Xiao & Becker, 1994). This suggests that ovarian hormones, but not testicular hormones, modulate DA activity.

Estradiol increases DA release via GABAergic medium spiny neurons (MSNs) in the dorsal striatum (Mermelstein, Becker, & Surmeier, 1996). Estradiol blocks Ca^{2+} (calcium) needed to activate the ERs on GABAergic MSNs, which decreases the inhibitory influence of GABA on DA release. The same mechanism of action has been proposed for the ventral striatum, although that remains unconfirmed. Alternatively or in addition to this mechanism, estradiol may increase cell firing in the ventral tegmental area which projects to the ventral striatum, or estradiol may exert its effects on the hypothalamic projections to the ventral striatum (Yoest et al., 2018). Altogether, estradiol acts directly in the dorsal striatum and likely in the ventral striatum and other regions to modulate dopamine release.

In sum, previous research suggests the estradiol acts directly in the dorsal striatum, the structure associated with temporal perception, and blocking estradiol affected accuracy and precision depending on the order delays were delivered and the length of the interval. It is important to note that the timing measures from the current study were collected during the impulsive choice task, which shows how females were timing the delays within the task. Future research focused on the role of estradiol in timing may benefit from measuring timing behavior outside of tasks that could interact with timing measurements. While there is an established mechanism of action of estradiol on DA in the dorsal striatum, there is no confirmation that estradiol affects the ventral striatum in the same way. Future research testing the mechanism of estradiol in the ventral striatum would significantly enhance current understanding of how estradiol affects reward-based decision making.

Another brain region associated with impulsivity may be responsible for the previous research reporting sex differences and hormonal influences on decision making. The prefrontal cortex (PFC) plays a key role in impulsivity and interacts with the ventral striatum to regulate impulsive choices (Bailey, Simpson, & Balsam, 2016). The PFC actively tracks delays during impulsive choice tasks and projects to the ventral striatum to create a feedback loop with other structures such as the basal ganglia and the amygdala (Sackett, Moschak, & Carelli, 2019). In addition, there are ERs present in PFC (Kuiper et al., 1997; Shughrue, Lane, & Merchenthaler, 1997). Taken together, it is possible that the PFC is directly affected by estradiol to produce the sex differences in decision-making. However, the dose of antiestrogen ICI may not have been effective for the PFC because the systemic injections in this study did not robustly affect choice behavior.

Results from the current experiment may be better explained by another sex hormone, progesterone, instead of estradiol. While estradiol was the major focus of this study, it is possible that any differences in decision making may be due progesterone. Progesterone is highest at the beginning of estrus and relatively higher during diestrus than the metestrus and proestrus stages (Figure 1.2). In both the ascending and descending conditions without the estradiol antagonist, rats made more self-controlled choices during estrus. Previous research suggests that progesterone increases self-control in rodents. Progesterone administration to ovariectomized female rats decreased impulsive burying on a marble burying task (Llaneza & Frye, 2009; Schneider & Popik, 2007) and decreased impulsive action in female rats on a go/no-go task for sucrose pellets (Swalve, Smethells, & Carroll, 2016) and for cocaine infusions (Swalve et al., 2018). In short, natural increases in progesterone caused by the estrous cycle may result in increased self-control.

Conclusions

Humans make daily choices based on reward and timing information associated with different options. While the effects of hormonal fluctuations on choice and timing may be small, these influences quickly accumulate when considering the frequency at which they may occur. Female rodents cycle through proestrus, estrus, and met/diestrus every 4 to 5 days on average, and female humans cycle through similar hormonal fluctuations every 28 days on average. Understanding how female sex hormones affect choice and timing behavior can contribute significantly to current knowledge of the neurobiology of decision making and treatment outcomes associated with impulse control disorders in females.

Overall, there were multiple inconsistencies between the preliminary study and the current study, signifying that further research is needed to determine the influence of sex

hormones on impulsive choice and temporal perception. Rats in estrus made the most self-controlled choices without the estradiol receptor antagonist, suggesting that natural increases in progesterone may result in increased self-control. The estradiol receptor antagonist did not alter impulsive choices for each estrous phase, further indicating that estradiol may not influence impulsive choices. However, the estradiol receptor antagonist did affect temporal perception. Particularly the order of delays may mediate the mechanism of estradiol on temporal perception. These effects may be due in part to timing measurements being obtained during the impulsive choice task, and therefore, influenced by the reward values associated with each delay.

Future research should investigate the role of progesterone on impulsive choice in addition to how estradiol and progesterone interact to affect impulsive choice. In addition, future research should parse out the effects of estradiol on temporal perception with respect to the length of the timing intervals with a task that does not have differing magnitudes of reward associated with the intervals. These studies will further current knowledge of the effects of female sex hormones on the neurobiological processes involved in decision-making and timing and highlight the importance of sex as a biological variable in these fields.

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Appendix A - Supplemental Data

The following supplemental materials contain the full model summary of the omnibus analysis to assess whether the order of delays affected impulsive choice behavior and alternative views of higher order interactions for impulsive choice and peak timing results. Figure A.1 depicts the impulsive choice behavior of the rats that received the delays in ascending order, and Figure A.2 shows the impulsive choice behavior of the rats that received the delays in descending order. Figures A.3 and A.4 show peak timing of the 5-s SS and 15-s LL peak intervals for the ascending order (5 s → 60 s). Figures A.5 and A.6 depict peak timing of the 5-s SS and 30-s LL peak intervals for the descending order (60 s → 5 s).

Omnibus Test

The omnibus analysis was a repeated measures multi-level logistic regression with a logit link function. The model included group, which signifies the order of delays in the impulsive choice task, dose, estrous cycle, and LL delay. Rat (intercept) was included as a random effect. Group, dose, and estrous cycle were effects coded, and LL delay was scaled to the assess the 30-s delay. There were multiple group effects including the interaction between group and LL delay, the interaction between group and proestrus, and the four-way interaction between group, LL delay, dose, and estrus (see Table A.1). These significant effects confirm that the order of delays presented in the choice task affected decision making. The two orders were analyzed separately for the analyses included in the Results section.

Table A.1. Full summary of fixed effects from the impulsive choice omnibus test model where delay was scaled to the 30-s delay.

	b	SE	*z*	
(Intercept)	-1.56	0.16	-9.91	***
LL Delay	-3.17	0.09	-35.13	***
Group	0.26	0.16	1.68	
Dose	-0.08	0.03	-2.92	**
Proestrus	0.05	0.04	1.25	
Estrus	0.03	0.04	0.89	
LL Delay × Group	-1.53	0.09	-16.96	***
LL Delay × Dose	-0.05	0.09	-0.60	
Group × Dose	0.05	0.03	1.79	
LL Delay × Proestrus	0.35	0.12	2.85	**
LL Delay × Estrus	0.05	0.11	0.46	
Group × Proestrus	-0.08	0.04	-1.98	*
Group × Estrus	0.04	0.04	1.08	
Dose × Proestrus	-0.02	0.04	-0.51	
Dose × Estrus	0.13	0.04	3.72	***
LL Delay × Group × Dose	-0.07	0.09	-0.81	
LL Delay × Group × Proestrus	-0.06	0.12	-0.51	
LL Delay × Group × Estrus	-0.04	0.11	-0.35	
LL Delay × Dose × Proestrus	0.22	0.12	1.79	
LL Delay × Dose × Estrus	0.00	0.11	-0.01	
Group × Dose × Proestrus	0.00	0.04	-0.12	
Group × Dose × Estrus	-0.02	0.04	-0.46	
LL Delay × Group × Dose × Proestrus	-0.12	0.12	-0.99	
LL Delay × Group × Dose × Estrus	0.22	0.11	1.96	*

Note: For Group, the ascending condition was coded as 1 and the descending condition was coded as -1. The 0 mg/kg dose was coded as 1 and the 1 mg/kg dose was coded as -1. Proestrus was coded as 1, 0; estrus was coded as 0, 1; met/diestrus was coded as -1, -1.

* $p < .05$
** $p < .01$
*** $p < .001$

Figure A.1. Alternative view of mean proportion of LL choices across LL delays for the rats that received the delays in ascending order only. Error bars (+/− SEM) were computed with respect to the estimated marginal means of the 30-s intercept fitted repeated measures multi-level logistic regression and jittered for readability.

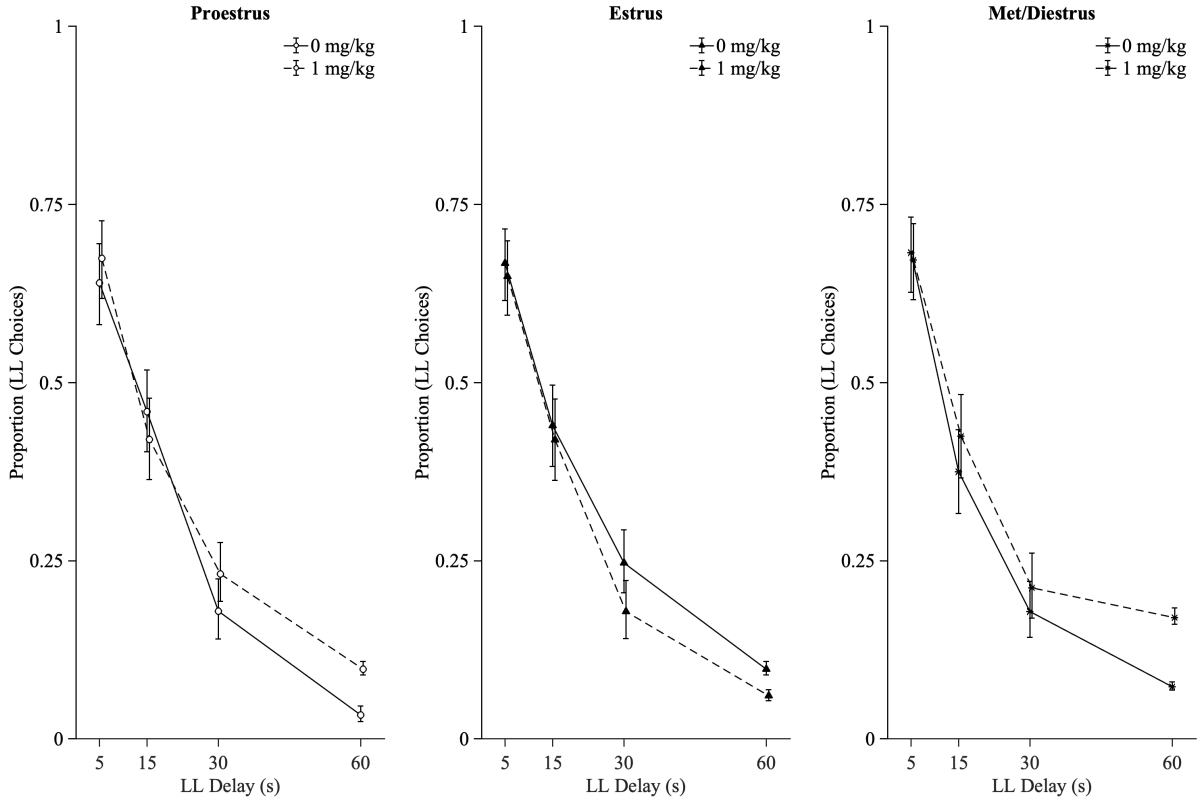

Figure A.2. Alternative view of mean proportion of LL choices across LL delays for the rats that received the delays in descending order only. Error bars (+/− SEM) were computed with respect to the estimated marginal means of the 30-s intercept fitted repeated measures multi-level logistic regression and jittered for readability.

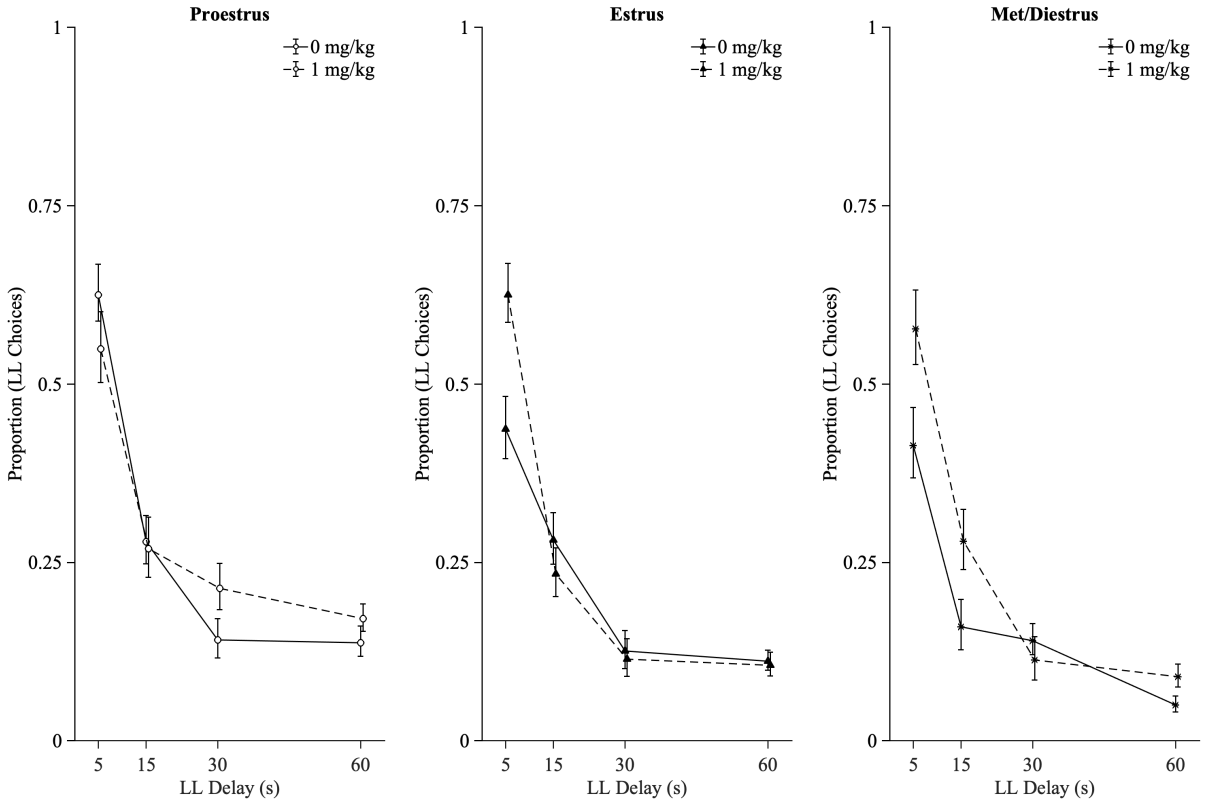

Figure A.3. Alternative view of mean responses per minute as a function of time into 5-s SS peak trials for the ascending order only. Markers represent mean responses and lines represent fitted repeated measures multi-level nonlinear regression values. Solid vertical lines represent the target interval.

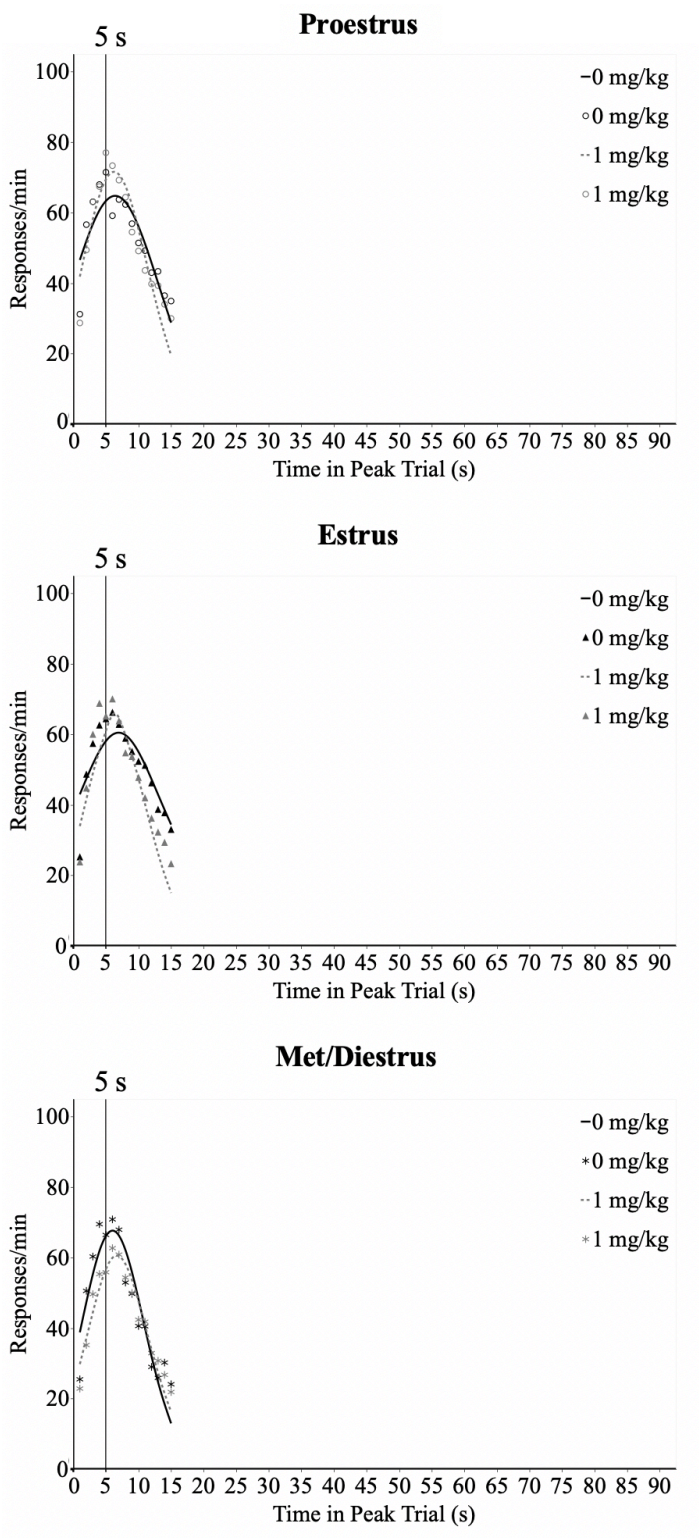

Figure A.4. Alternative view of mean responses per minute as a function of time into 15-s LL peak trials for the ascending order only. Markers represent mean responses and lines represent fitted repeated measures multi-level nonlinear regression values. Solid vertical lines represent the target interval.

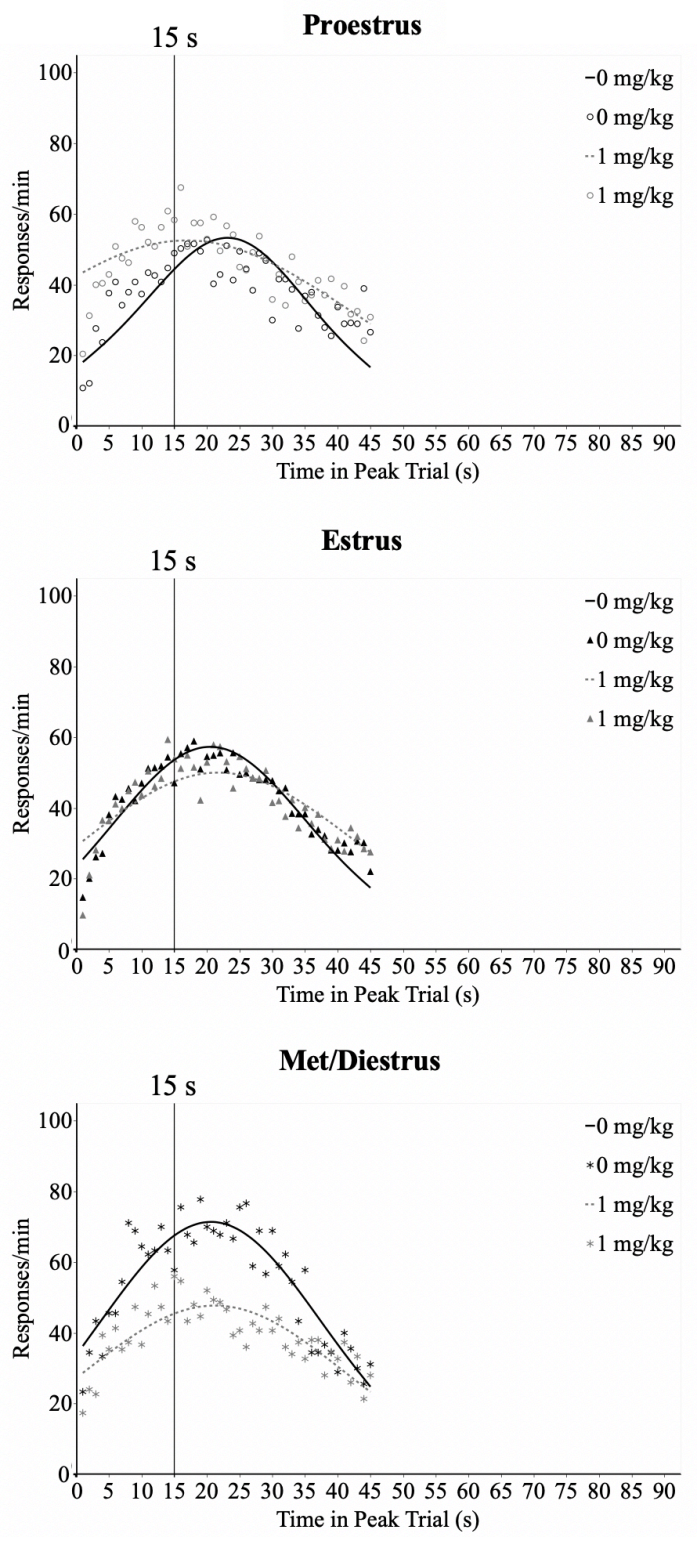

Figure A.5. Alternative view of mean responses per minute as a function of time into 5-s SS peak trials for the descending order only. Markers represent mean responses and lines represent fitted repeated measures multi-level nonlinear regression values. Solid vertical lines represent the target interval.

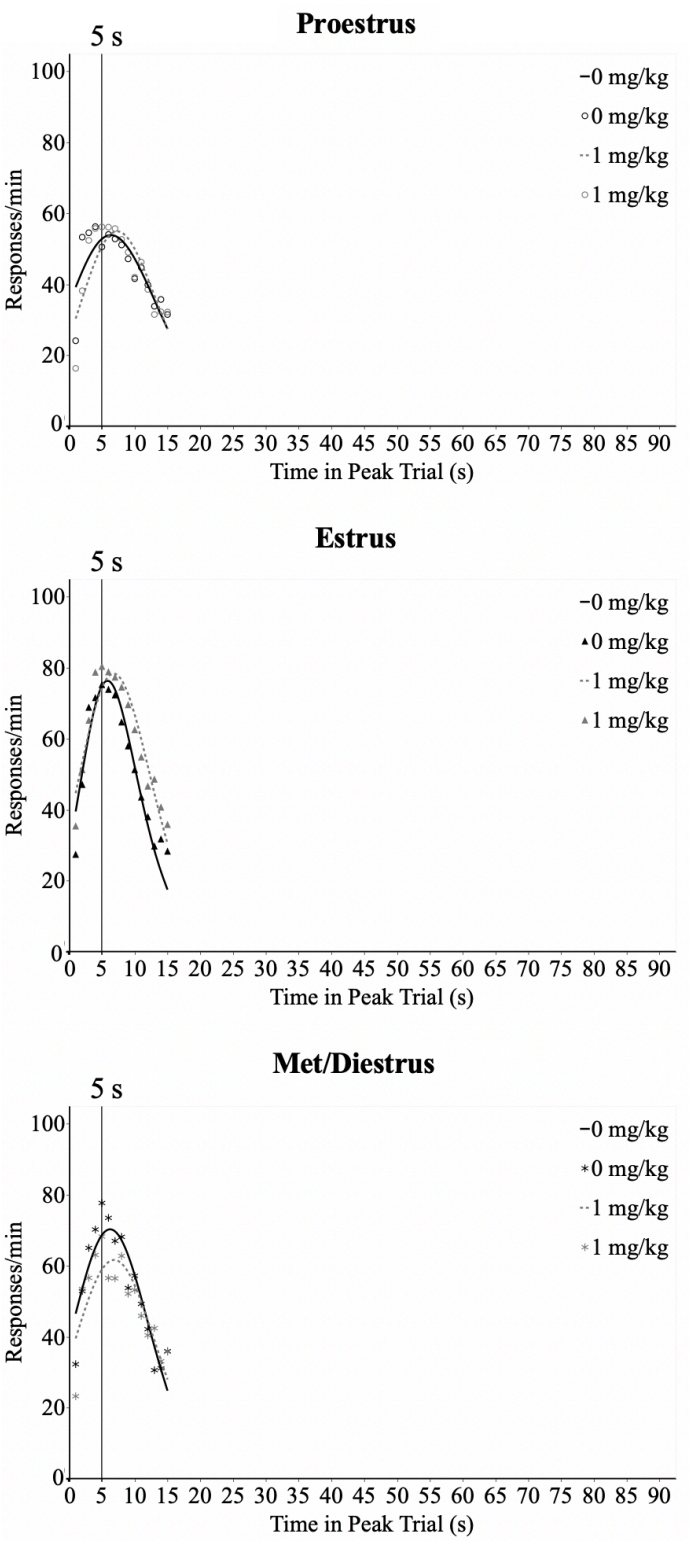

Figure A.6. Alternative view of mean responses per minute as a function of time into 30-s LL peak trials for the descending order only. Markers represent mean responses and lines represent fitted repeated measures multi-level nonlinear regression values. Solid vertical lines represent the target interval.