Sex differences in and neural activity of fixed-interval and fixed-time interventions to promote self-control

by

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B.S., Drake University, 2017
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AN ABSTRACT OF A DISSERTATION

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Abstract

Impulsive behavior is associated with many maladaptive behaviors and diseases, which manifest and affect females and males differently. Because neuroscience research is often conducted in male subjects only, the mechanisms for these differences are unclear. The current study examined the effects of two abbreviated time-based interventions in female and male rats to further understand how these interventions may alter impulsive behavior both at behavioral and neurobiological levels. In the current study, male and female rats were randomly assigned to one of four conditions: FI (fixed-interval) intervention with FI choice task (FI-Exp), FT (fixed-time) intervention with FT choice task (FT-Exp), no training control with FI choice task (FI-Con), and no training control with FT choice task (FT-Con). In the Exp (experimental) conditions, rats received training on 10- and 30-s delays over the course of six sessions, and rats in the Con (control) groups did not receive any training but experienced the same environmental stimuli as Exp groups. After the intervention phase, all rats completed an impulsive choice task with corresponding response-initiated FI or FT contingencies. The FI schedule delivered during the intervention and/or choice task required a lever press to make a choice and a second lever press after the delay elapsed to receive food. The FT schedule required a lever press to make a choice but no further responses were required to receive food. Following the impulsive choice phase, rats were euthanized and perfused, and brains were processed for c-Fos, a marker of neural activity, in two prefrontal cortical brain regions and three subregions of the striatum.

During the intervention and impulsive choice tasks, rats that received the FI schedules increased lever pressing in anticipation of food rewards. Rats that received the FT schedules did not enter the food cup in anticipation of food rewards but interacted with the levers often even though no response was required to receive food rewards. Rats in the Exp groups made more LL

choices than the Con groups when the delay to reward was 0 s, but there were no differences between schedule or sex. In addition, all conditions showed similar sensitivity to delay in the choice task. Analyses of c-Fos showed that females had higher levels of c-Fos than males in all brain regions. We also found that rats in the Exp groups had higher levels of c-Fos in the dorsomedial striatum, dorsocentral striatum, and prelimbic cortex, and rats that received the FI schedule showed higher levels of c-Fos in the dorsomedial striatum, dorsocentral striatum, dorsolateral striatum, and prelimbic cortex. Based on the group, schedule, and sex effects in neurobiology, it is possible that time-based interventions are effective in both sexes but through different cognitive mechanisms that rely on a complex network of multiple brain regions. Time-based interventions may primarily decrease impulsive action in males and improve interval timing ability in females, both of which result in enhanced self-control. However, while multiple brain regions showed differential activity based on conditions, these differences did not strongly relate with behavioral measures. The current study produced relatively weak intervention effects, which could be due to the limited number of training sessions, the female estrous cycle's effects on learning, and/or lever availability during the FT schedule. In the current study, the lever remained in the chamber until the delay elapsed and food was delivered whereas previous studies retracted the lever after the delay was initiated. Altogether, lever availability during the FT schedule may have affected delay sensitivity in a way that promoted temporal attention, but impaired interval timing ability compared to rats that received the FI schedule. The current study offers a variety of avenues for future research to further probe the cognitive and neurobiological mechanisms of time-based interventions for females and males.

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Approved by:

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Dedication

For my family. My degrees were certainly a group effort. Thank you all for your help in making this possible and pushing through with me until the end.

Chapter 1 - Introduction

Choices often involve a tradeoff between the size of reward and time of receipt associated with each option. Humans and animals face thousands of choices each day, which may gradually coalesce to a pattern of impulsive choices. An impulsive choice may be defined as opting for a small reward available immediately or after a short delay (smaller-sooner; SS) over a larger reward available after a longer delay (larger-later; LL). Impulsive choices are associated with multiple disorders and maladaptive behaviors such as Attention-Deficit/Hyperactivity Disorder (Antrop et al., 2006; Fox et al., 2008; Marco et al., 2009), major depressive disorder (Pulcu et al., 2014), schizophrenia (Ahn et al., 2011), gambling (Dixon et al., 2003), substance abuse (Perry & Carroll, 2008; Perry et al., 2008), and obesity (Rasmussen et al., 2010). On the other hand, selecting the larger-later reward instead of the smaller-sooner reward may be viewed as the self-controlled choice. Self-control is typically predictive of positive outcomes such as health, socioeconomic status, and safety (Moffitt et al., 2011).

Men and women differ in impulsive behaviors, evident across multiple tasks designed to measure facets of impulsivity. On delay discounting tasks, participants are asked to make choices between contrasting size rewards that also differ in delays to receipt. Women discount or experience a subjective decrease in reward value more rapidly than men when choosing between hypothetical rewards (Beck & Triplett, 2009; Reynolds et al., 2006; Smith & Hantula, 2008). Men discount more rapidly than women when money is used as the reward (Doi et al., 2015; Kirby & Marakovic, 1996; Weafer & de Wit, 2014). However, these effects were not found in some studies with healthy adults (Cross et al., 2011; de Wit et al., 2007; Lucas & Koff, 2010). Taken together, impulsive behaviors in men and women vary, which is similar to patterns observed in animals.

When examining sex differences in preclinical studies, male and female rodents vary in impulsive behavior across tasks. In an impulsive choice task involving choices between fixed rewards associated with fixed delays, male rats made more SS choices than females (Bayless et al., 2013). Similarly, females were more LL preferring than males across two cohorts of rats that completed multiple impulsive choice tasks after a behavioral intervention to improve self-control, but the effects may be task-dependent as the females' behavior was less reliable when comparing abbreviated and extended choice tasks (Panfil et al., 2020). On the other hand, when male and female mice were grouped 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). When tested on an adjusting delay choice task where the delay to reward changed based on previous choices, male and female rats did not differ in LL choices (Eubig et al., 2014; Perry et al., 2008). Altogether, there are indications of sex differences but there are also inconsistencies in impulsive behavior in humans and animals (see Orsini & Setlow, 2017; Weafer & de Wit, 2014 for reviews).

Sex differences in impulsive choice are increasingly complex when considered in conjunction with diseases, disorders, and problematic behaviors. Men and women differ in prevalence, progression to disease, and treatment outcomes in many disorders and behaviors associated with impulsive choice (Becker & Hu, 2008; Fattore & Melis, 2016; Hing et al., 2016; Iacono & Beiser, 1992; Kimokoti et al., 2013; Ramtekkar et al., 2010; Randall et al., 1999; Weafer et al., 2015; Williams et al., 2015). For example, females with obesity were less successful at losing weight across weight loss intervention strategies compared to males with obesity, which is associated with impulsive choice (Williams et al., 2015). Similar patterns are observed in preclinical studies examining disease-like states (Anker & Carroll, 2011; Becker &

Hu, 2008; Carroll & Anker, 2010; Hu & Becker, 2003; Lynch et al., 2002). Collectively, male and female humans and rodents display clearer sex differences when examining clinical levels of impulsivity-related disorders but inconsistent patterns in healthier samples.

An impulsive choice may be the result of one or more cognitive mechanisms such as delay aversion, delay discounting, temporal processes, and magnitude discrimination. Delay aversion is the subjective dislike of waiting, leading to avoidance of delays that limit experience with longer time intervals (Kirkpatrick et al., 2015; Marshall et al., 2014; Sonuga-Barke et al., 1992; Winstanley et al., 2006). Delay discounting is the gradual reduction of reward value as a function of time. Individuals who steeply discount reward value as the delay increases may opt for SS rewards over LL rewards (Baumann & Odum, 2012; Mazur, 2000). Temporal processing, or interval timing, refers to the ability to time a delay both accurately and precisely. An individual may perceive a delay to reward as longer than it is as a result of inaccurate estimation. Imprecision impairs temporal discrimination, so an individual may not be able to differentiate between short and long delays. These temporal processing errors may influence delay discounting processes and selection of the self-controlled choice (Kirkpatrick et al., 2015; Litrownik et al., 1977; Marshall et al., 2014; Takahashi, 2005; Wittmann & Paulus, 2008). Impulsive choices may be a product of impaired magnitude discrimination, or the ability to differentiate reward sizes such as one food reward versus three food rewards. Differing magnitude of rewards may influence accuracy and/or precision in timing delays associated with the rewards (Galtress & Kirkpatrick, 2009). Altogether, multiple cognitive mechanisms may underlie impulsive choice behavior, offering a variety of treatment avenues to promote self-control.

Time-Based Interventions

To treat disorders related to impulsive choices, time-based interventions have been developed in pre-clinical rodent models (Marshall et al., 2014). Time-based interventions typically involve multiple sessions of forced exposure to delays and may be delivered according to different reinforcement schedules. Time-based interventions successfully increased LL choices in male and female rats across multiple experiments (Bailey et al., 2018; Fox et al., 2019; Marshall et al., 2014; Mazur & Logue, 1978; Panfil et al., 2020; Peck et al., 2020; Peterson & Kirkpatrick, 2016; Renda & Madden, 2016; Renda et al., 2018; Rung et al., 2018; Smith et al., 2015; Stuebing et al., 2018). Similar results were found with humans (Dixon et al., 1998; Eisenberger & Adornetto, 1986; Eisenberger et al., 1985; Vessells et al., 2018). Time-based interventions appear to be durable and generalizable (Bailey et al., 2018; Renda & Madden, 2016), and produce effects after only a few sessions of training (Panfil et al., in preparation). Recently, we examined the effect of the number of training sessions of a response-initiated fixed interval (FI) time-based intervention on LL choices in male rats. Results suggest that interventions with as few as 6 sessions (or 200 trials) promoted selection of the LL choice and decreased sensitivity to delay (Figure 1.1; Panfil et al., in preparation). The effects of the abbreviated FI intervention were further replicated in another set of male rats where groups received the intervention with or without a pre-intervention choice test and with or without a break between the test and the intervention all compared to a no-delay (ND) control condition. Regardless of receiving a pre-intervention choice test or a break between testing and training, the abbreviated FI intervention promoted self-control (Figure 1.2; Panfil et al., in preparation). The current study used this abbreviated FI intervention. While time-based intervention effects are

robust in multiple dimensions, it is unclear what cognitive mechanisms underlie the interventions.

The FI intervention has been proposed to promote LL choices by improving temporal perception (Marshall et al., 2014). Some studies show positive correlations between timing parameters and self-control (Peterson & Kirkpatrick, 2016; Smith et al., 2015; Stuebing et al., 2018), but others show no relationship (Fox et al., 2019; Rung et al., 2018). These differences may be due to procedural differences in how the time-based intervention was delivered. One possibility is that the delay and response contingencies of schedules during the intervention or choice task may affect the relationship between impulsive choice and timing. Specifically, some studies used the FI schedule where the delay to reward must be initiated with a response, such as pressing a lever or poking a cue light (Fox et al., 2019; Smith et al., 2015; Stuebing et al., 2018). After the delay has elapsed, a second response must be made to receive reinforcement (Figure 1.3). Other experiments have exposed rats to response-initiated fixed-time (FT) schedules where a response is required to initiate the delay, but no response is necessary to collect the reward (Rung et al., 2018). Reinforcement is delivered after the delay elapses (Figure 1.3).

In a recent study, FI and FT interventions were compared in a mixed sex sample to determine whether the delay and response contingencies influenced the relationship between impulsive choice and timing (Smith et al., under review). The contingencies were also tested in the context of the impulsive choice tasks pre- and post-intervention. Assessment of interval timing was in a separate test following the choice task where rats received peak interval trials. Peak interval trials are typically used within animal research to assess accuracy and precision in timing a fixed-interval duration. Peak interval trials are at least three times the duration of the FI, and responses are collected throughout the entire trial. Overall, the FI and FT interventions

increased self-controlled choices, suggesting that both interventions promote delay tolerance, or the ability to wait through the delays instead of avoiding them. Also, the FI choice contingency increased delay sensitivity (slope) while the FT choice contingency did not, suggesting that the FI choice task may demand a greater level of temporal attention than the FT choice task. In addition, rats that received the FI contingency in choice and/or intervention phases had comparable timing accuracy and precision during peak interval trials, but rats that received FT contingencies only (FT choice tasks and FT intervention) showed the poorest temporal precision (Smith et al., under review). Smith and colleagues hypothesize that the FI intervention and choice test promoted active waiting which may have invoked greater temporal attention during the delays while the FT intervention and choice test allow for passive waiting.

Insights into the cognitive mechanisms of the interventions may be elucidated by testing abbreviated interventions. As noted above, previous research in male rats showed that an abbreviated FI 200 (200 trials, or 6 sessions total) intervention promoted LL choices (Panfil et al., in preparation). However, it is unclear whether an abbreviated FT intervention would be equally effective at increasing LL choices compared to the abbreviated FI intervention. The FT intervention does not require a response to receive a reward after the delay elapses, so any temporal information learned through this passive waiting may require more sessions to learn compared to the FI intervention. The FT intervention was tested in males for 125 sessions (Rung et al., 2018) and a mixed-sex sample for 45 sessions (Smith et al., under review). Altogether, rats may require more than 6 sessions of the FT intervention to produce improvements in self-control because the FT intervention likely invokes temporal attention to a lesser degree compared to the FI intervention.

Measuring interval timing ability during the impulsive choice task may clarify the importance of timing ability, attention to time, and delay tolerance. FI and FT interventions may result in different accuracy and precision values but similar improvements in self-control, suggesting that both interventions may increase self-control through delay tolerance. In this case, interval timing or more active attention to delays may not drive improvements in self-control. Waiting, in any form, may increase the ability to tolerate the LL delay. Alternatively, interval timing ability may be essential to improvements in self-control. However, because interval timing ability may be improved via attention to delays, the FI intervention may improve accuracy and precision compared to the FT intervention and result in increases in self-control. It is also possible that FI and FT interventions may result in similar accuracy and precision in timing but differences in impulsive choice, suggesting temporal attention (but not interval timing) in the FI schedule drives differences in impulsive choice. Finally, the FT intervention may promote self-control to a higher degree than the FI intervention by biasing rats toward specific stimuli or goals during the intervention, which may be assessed by measuring responding during the interventions.

Responding during the interventions may indicate possible cognitive processes at work. When examining rats' behavior during the interventions, rats in the FI condition interact often with the lever while rats in the FT condition spend more time interacting with the food cup where rewards are delivered (Smith et al., under review). This suggests that the FI intervention may promote sign-tracking (i.e., lever pressing) and the FT intervention may promote goal-tracking (i.e., food cup entries). Sign-tracking is typically defined as approaching and interacting with a conditioned stimulus that is associated with food even though no response is required for delivery of the reward (Hearst & Jenkins, 1974). While the FI intervention contingency requires

a response for food, rats are only required to press the lever one time after the delay elapses. However, rats typically respond on the lever throughout the delay, ramping up responding as the target delay approaches. Goal-tracking is defined as spending time in and around the location where rewards are delivered instead of interacting with the conditioned stimulus (Boakes et al., 1978). The FT intervention does not require a response for reward delivery, so rats often spend time around the food cup. In sum, the FI intervention may bias rats towards sign-tracking while the FT intervention may promote goal-tracking. This has not been confirmed in previous studies administering these interventions because the lever was retracted after the delay was initiated in the FT condition (Rung et al., 2018; Smith et al., under review). This prevented a formal comparison of lever presses and head entries between the conditions. In the current study, the lever remained extended in the operant chamber in both experimental conditions, so lever presses and head entries to the food cup may be compared as measures of sign- and goal-tracking.

Sign-tracking and goal-tracking behavior may be linked to impulsivity. These behaviors have been evaluated in relation to impulsive action, impulsive choice, and risky choice, all facets of the broader construct of impulsivity (Bari & Robbins, 2013; Evenden, 1999). Impulsive action typically refers to behavioral inhibition, or the ability to suppress or withhold an initial response while impulsive choice refers to selection of smaller-sooner rewards over larger-later rewards (Bari & Robbins, 2013; Evenden, 1999). Risky choice is often defined as selecting a larger but uncertain reward over a smaller but certain reward (Rachlin et al., 1991). Rats and humans that display sign-tracking behavior are also more impulsive than rats and humans that display goal-tracking behavior when assessing impulsive action (Flagel et al., 2010; King et al., 2016; Lovic et al., 2011). However, results are mixed when comparing sign- and goal-trackers on impulsive choice with some studies showing sign-trackers as more SS preferring (Garofalo & di Pellegrino,

2015; Olshavsky et al., 2014; Tomie et al., 1998) and others not (Flagel et al., 2010; Lovic et al., 2011). These differences may be attributed to how impulsive choice was measured and how individuals were classified as sign- or goal-trackers. Some studies were conducted by assessing self-reported hypothetical choices in humans (Garofalo & di Pellegrino, 2015). Other experiments examined impulsive choice behavior in rats with delays to reward that increased within a session but classified rats as sign- or goal-trackers (Flagel et al., 2010; Lovic et al., 2011; Tomie et al., 1998) or orienter and non-orienters (Olshavsky et al., 2014) based on different criteria from the appetitive Pavlovian conditioning procedure. In terms of risky choice, sign-trackers are riskier than goal-trackers (Olshavsky et al., 2014; Swintosky et al., 2021). Altogether, these results suggest that sign-trackers are often more impulsive than goal-trackers across dimensions of impulsivity.

Sign- and goal-tracking behaviors may interact with the FI and FT interventions to affect impulsive choices. No previous studies (to my knowledge) have evaluated sign- and goal-tracking within a time-based intervention or within an impulsive choice task as a function of response contingency. Based on these differing response contingencies, the FI intervention may bias rats towards sign-tracking while the FT intervention may influence rats towards goal-tracking. Given the literature indicating that sign-trackers are often more impulsive, this suggests that the FI intervention may increase impulsive choices compared to an FT intervention. However, FI and FT interventions typically result in similar effects on reducing SS choices when delivered for extended periods of time (Smith et al., under review; see Figure 1.4). Instead, sign- and goal-tracking behavior may interact with the interventions at the individual subject level. The relationship between sign- and goal-tracking and intervention efficacy may be antagonistic such that sign-tracking counteracts the intervention effects at the individual level. Sign-trackers

may show smaller intervention effects compared to goal-trackers based on previous literature suggesting sign-trackers are more impulsive.

Sign-tracking and goal-tracking studies suggest there may be sex differences in these behaviors, which may extend to the FI and FT interventions. Across experiments, female rats sign-track more than male rats (Hilz et al., 2021; Hughson et al., 2019; King et al., 2016; Pitchers et al., 2015; Stringfield et al., 2019), suggesting that females may lever press more during the interventions while males may enter the food cup more during the interventions, regardless of response contingency. In addition, the intervention response contingency may exaggerate these behaviors so that FI females may sign-track more than FI males and FT males may goal-track more than FT females. If sign-tracking behavior counteracts the intervention effect, this would suggest that FI females should be more impulsive and FT males should be more self-controlled. However, recent data suggests the opposite, such that sign-tracking is synergistic with temporal attention.

While Smith et al. (under review) was not designed to examine sex differences, therefore underpowered to analyze sex, the data suggests that males and females may respond differently to the FT intervention. Particularly, male rats that received the FT intervention and FT choice task were minimally affected by the intervention when comparing pre- and post-intervention choices (Figure 1.4). Based on sign- and goal-tracking sex differences, it is possible that the male FT intervention and FT choice task rats were biased towards goal-tracking, which may have further reduced temporal attention to the delays in the intervention and choice task. This suggests that sign-tracking may have a synergistic relationship with temporal attention in the FI intervention condition. Sign-tracking behavior may promote attention to delays because rats are interacting with the conditioned stimulus often, which may invoke greater attention to delays.

Taken together with sex differences in sign- and goal-tracking, FI females may sign-track most and be more receptive to the intervention while FT males may goal-track most and show a weaker intervention effect, as was the case in Smith et al. (under review). While this hypothesis is inconsistent with previous literature demonstrating a relationship between sign-tracking and higher impulsive choices, previous research did not examine sign- and goal-tracking during interventions or choice tasks where sign-tracking behavior may align with conditions to promote self-control. Altogether, the current study aimed to examine sex differences in self-control after abbreviated FI and FT interventions.

Neurobiological Mechanisms of Impulsive Choice and Time-Based Interventions

Examining the neurobiology of FI and FT interventions may inform the understanding of cognitive mechanisms. Multiple brain regions may be active during these interventions (Bailey et al., 2016; Balleine et al., 2007; da Costa Araujo et al., 2010; Kim et al., 2009; Meck, 2006; Sackett et al., 2019), but research in this area has been largely limited to male rodents (But see da Costa Araujo et al., 2010). Examining the biological mechanisms of time-based interventions in conjunction with the more established biological mechanisms of impulsive choice may clarify the cognitive mechanisms of FI and FT interventions. Several brain regions and circuits contribute to impulsive choices, suggesting the possibility that multiple neural targets could be affected by time-based interventions. Furthermore, it is possible that males and females show similar behavioral effects after a time-based intervention but through different neural mechanisms. Based on previous research, the current study seeks to evaluate the effects of abbreviated FI and FT interventions on a marker of neural activity in the dorsal striatum, the prelimbic cortex, and the infralimbic cortex.

The dorsal striatum (DS) may be subdivided into anatomically distinct regions, and subregions of DS may play different roles in choice, timing, and attention (Kim & Im, 2019). This functionally heterogeneous area is comprised of the dorsomedial (DMS), dorsocentral (DCS), and dorsolateral (DLS) striatal regions. The dorsal striatum is recruited during interval timing, which may contribute to impulsive choice (Kirkpatrick et al., 2015; Litrownik et al., 1977; Marshall et al., 2014; Meck, 2006; Smith et al., 2015; Takahashi, 2005; Wittmann & Paulus, 2008). Dopaminergic lesions of DS (mostly DCS only lesions but DMS and DLS were affected in some subjects as well) result in poor accuracy and precision in timing (Meck, 2006). In addition, dopaminergic lesions to DLS increased SS choices in male rats when compared to sham lesions (Tedford et al., 2015). However, in a separate experiment, excitotoxic lesions made to DLS and DCS after training was completed on a delay discounting task resulted in more LL choices for two weeks after the lesion before returning to baseline (Dunnett et al., 2012). Taken together, these experiments suggest the DS is linked to both timing and impulsive choice behaviors, but the subregions may play functionally distinct roles.

Given the links between DS, timing, and impulsive choice, subregions of DS may be recruited based on the type of intervention received in the current study. FI interventions and FI choice tasks may promote attention to delay because of the response requirement after the delay elapses for food delivery. Temporal attention may recruit core timing processes via DS. FT interventions and choice tasks do not require a response to collect the reward, so temporal attentional processes may not be as strongly recruited. This suggests the FI intervention and choice task may activate DS more so than the FT intervention and choice task. However, few studies have examined the unique contributions of each DS subregion to timing and choice, so it

remains unclear which portion of DS may be recruited during the FI intervention and choice tasks. Projections to DS subregions may illuminate these distinctions.

Likely in conjunction with the striatum, the prefrontal cortex (PFC) may contribute to the neurobiological mechanism(s) of impulsive choice and time-based interventions based on the cognitive processes underlying them. Prefrontal cortical activity often reflects control and allocation of attentional processes (Bailey et al., 2016). The prelimbic (PL) cortex, a medial subregion of the PFC, may be involved in interval timing (Dietrich et al., 1997; Kim et al., 2009). In particular, PL may be responsible for tracking multiple delays during impulsive choice tasks, and this region projects to DMS creating a possible core timing circuit (Coull et al., 2011; Finnerty et al., 2015; Matell & Meck, 2004; Tallot & Doyère, 2020). More specifically, pyramidal neurons in PL respond differentially to SS and LL choices during free-choice trials (Sackett et al., 2019), and PL neurons display activation patterns that may reflect scalar variance (Kim et al., 2018; Tiganj et al., 2017), or less precision/certainty surrounding longer time intervals than short ones (Gibbon, 1977). Pyramidal neurons are located in layer V of PL and project to the nucleus accumbens with collateral projections to DMS as well, further demonstrating a connection to impulsive choice (Emmons et al., 2017; Emmons et al., 2019; Gorelova & Yang, 1996; Groenewegen et al., 1991; McGeorge & Faull, 1989; Vertes, 2004). Overall, the FI intervention and choice task may increase self-control by increasing neural activity in a network of brain regions possibly including the circuit between PL and DMS, which attends to timing information associated with multiple delays and choices.

If DMS and PL activity are increased, this may promote stronger functional connectivity between these brain regions, strengthening core timing processes. Improved interval timing ability may allow individuals to make more informed, and therefore, more self-controlled

choices because the delays to reward are known. The FI intervention and choice task may be reliant on interval timing processes, which likely promotes neural activity in this functional circuit between PL and DMS. The FT intervention and choice task may not heavily rely on interval timing, suggesting the functional circuit between PL and DMS may not be involved in FT conditions. Across the FI and FT schedules, it is still possible that DCS and DLS are recruited in the current study based on their role in choice behavior as demonstrated in previous research (Dunnett et al., 2012; Tedford et al., 2015). Activation in these areas may suggest that other pathways such as the circuitry connecting the medial agranular cortex and the DCS may be involved (Cheatwood et al., 2003; Reep et al., 2003). Along the same lines, both time-based interventions may increase self-control through other facets than timing and rely on other cortical regions.

The infralimbic (IL) cortex may be affected by both time-based interventions through a mechanism distinct from timing processes. The infralimbic cortex is another medial subregion of PFC, situated ventrally to PL. Previous research suggests IL plays an important role in reward seeking behavior such that populations of neurons in this region encode cue-reward associations (Pfarr et al., 2018). In addition, lesions to IL increase impulsive action, or the ability to inhibit an initial response (Chudasama et al., 2003), and neuronal activity in IL also correlates with impulsive action (Tsutsui-Kimura et al., 2016). Overall, the infralimbic cortex may be recruited by both time-based interventions when forming associations between levers, rewards, and delays and crucial to suppression of impulsive action, resulting in increased self-control. It is also possible that the infralimbic cortex may be involved in the FT intervention and choice task more so than the FI intervention and choice task if the FI schedule relies on interval timing and/or temporal attention while the FT schedule may not.

The neurobiological effects of FI and FT interventions may be measured with immunohistochemical techniques to localize markers of neural activity. Neural activity can be indirectly measured with c-Fos, which is an immediate early gene marker (Krukoff, 1999). Previous research measuring c-Fos after an adjusting delay impulsive choice task in female rats suggest that c-Fos is a sensitive measure for choice tasks with a delay contingency (da Costa Araujo et al., 2010). Along the same lines, female rats showed increased c-Fos expression in the orbitofrontal cortex, but not other prefrontal or striatal regions, after an FI 30-s schedule compared to a VI 75-s schedule (Valencia-Torres et al., 2012). This suggests that the orbitofrontal cortex was active during an FI schedule where rats were only exposed to one time interval. The prelimbic cortex appears to be particularly important when multiple delays are involved (Coull et al., 2011; Finnerty et al., 2015; Matell & Meck, 2004; Tallot & Doyère, 2020), suggesting time-based interventions offering training on multiple delays may recruit PL instead of or in addition to the orbitofrontal cortex. This may be specific to the FI intervention, which may heavily rely on interval timing whereas the FT intervention may not.

With regard to sign- versus goal-tracking with food rewards, male rats showed differential c-Fos mRNA expression in the orbitofrontal cortex, thalamus, nucleus accumbens, and DS (Flagel, Cameron, et al., 2011; Flagel & Robinson, 2017). In particular, rats displaying sign-tracking behavior showed increased c-Fos mRNA expression in the DMS and DLS compared to goal-trackers, suggesting sign-tracking behavior in the current study may be associated with higher levels of c-Fos in DMS and DLS. Further correlational analysis of c-Fos mRNA expression across brain regions also suggest that sign-trackers show activity in the paraventricular nucleus of the thalamus (PVT) and the nucleus accumbens while goal-trackers show activity in the orbitofrontal cortex and PVT (Flagel, Cameron, et al., 2011). Taken

together, sign-tracking behavior may result from activity in subcortical circuits and goal-tracking behavior may result from cortico-striatal and cortico-cortical circuitry (Flagel, Cameron, et al., 2011; Flagel & Robinson, 2017). If interval timing does not appear to underlie FI and FT interventions, sign- and goal-tracking behaviors may serve as an alternative explanation. PL and DMS regions are likely involved in timing, but PL is not necessarily involved in sign- and goal-tracking. Instead, DS and other brain regions such as PVT, the nucleus accumbens, and the orbitofrontal cortex may show differential c-Fos expression in sign- and goal-trackers. Overall, c-Fos appears to be an appropriate marker for measuring neural activation after the proposed interventions in male and female rats. Altogether, no studies (to my knowledge) have examined neural activity in male and female rats after time-based interventions in regions associated with interval timing.

Current Study

Given the growing literature highlighting sex differences in impulsive decision-making in both humans and animals coupled with a lack of consistency in patterns across behavioral tasks, more research is needed to understand how males and females differ in both cognitive and neurobiological processes underlying impulsive choices. In recent experiments comparing males and females, or using mixed sex samples, time-based interventions consisting of FI and FT schedules effectively promoted self-control (Panfil et al., 2020; Smith et al., under review). In these studies, rats received the FI and FT interventions for 45 sessions, but the current study used 6 sessions of intervention training. This abbreviated FI intervention was effective at promoting self-control in male rats (Panfil et al., in preparation; Figures 1.1 and 1.2), but it is unclear if the abbreviated FI intervention will promote LL choices in females and if an abbreviated FT intervention will increase LL choices in males and females. Therefore, the current experiment

investigated efficacy of abbreviated FI and FT interventions to promote self-control and examined sex differences in abbreviated time-based interventions. In the current experiment, we also measured sign- and goal-tracking like behaviors to examine how these responses may interact with the intervention and choice task contingencies in males and females, which has not been assessed in previous literature. Finally, we examined c-Fos following time-based interventions in male and female rats to provide insights into neurobiological mechanisms of FI and FT schedules in male and female rats.

Male and female rats completed lever training and were randomly assigned to one of four conditions: FI intervention with FI choice task (FI-Exp), FT intervention with FT choice task (FT-Exp), no training control with FI choice task (FI-Con), and no training control with FT choice task (FT-Con). In the FI and FT intervention conditions, rats received training on two delays, 10 and 30 s, delivered in separate sessions. Rats completed two sessions of training on the 10-s delay and four sessions of training on the 30-s delay, equating to 200 total trials on each delay. Rats in the no training control conditions (FI-Con and FT-Con) received the same amount of operant chamber time and food rewards but did not receive any experimental stimuli as described in Peterson and Kirkpatrick (2016). Most of the previous research investigating time-based interventions used a no-delay control condition where control animals did not experience any delays to reward but were given similar response requirements and food earning rates (Bailey et al., 2018; Panfil et al., 2020; Smith et al., 2015). The current experiment aimed to examine neurobiology as a result of the FI and FT interventions, so the no training control groups captured neurobiology as a result of the choice tasks only. A no-delay condition plus a choice task would further complicate neurobiology results, particularly where recent research showed a no-delay control condition may increase impulsive choices (Fox, 2022). After the

intervention phase, all rats completed an impulsive choice task with response-initiated FI or FT contingencies. The FI choice task required a lever press to make a choice and a lever press after the delay elapsed to receive food rewards. The FT choice task required a lever press to make a choice but no response to receive food rewards, which were automatically delivered after the delay elapsed. Following the impulsive choice task, all rats were euthanized and perfused, and brains were processed for c-Fos. This neurobiological marker was quantified in the dorsomedial striatum, dorsocentral striatum, dorsolateral striatum, prelimbic cortex, and infralimbic cortex.

This experiment extends on the time-based intervention literature to examine sex differences in abbreviated FI and FT interventions and the associated neural mechanisms. The FI or FT intervention may be more or less effective in males or females based on sign- and goal-tracking behaviors, interval timing, temporal attention, or neurobiology. It is also possible that time-based interventions alter delay tolerance, so exposure to waiting in any fashion may promote self-control in males and females. Across measures, a lack of sex differences is still an interesting contribution to the field. Understanding the cognitive and neural processes associated with time-based interventions in males and females may further their development as treatments for disorders related to impulsive choices.

Figure 1.1. Proportion of LL choices with error bars (+/− SEM) pre- and post-intervention where male rats received varying lengths (200-1500 total trials across both delays, or 6-45 sessions) of FI intervention on 10- and 30-s schedules. Rats that received the FI intervention for 200 trials per delay showed increased LL choices post-intervention compared to pre-intervention that was comparable to or better than the other training conditions.

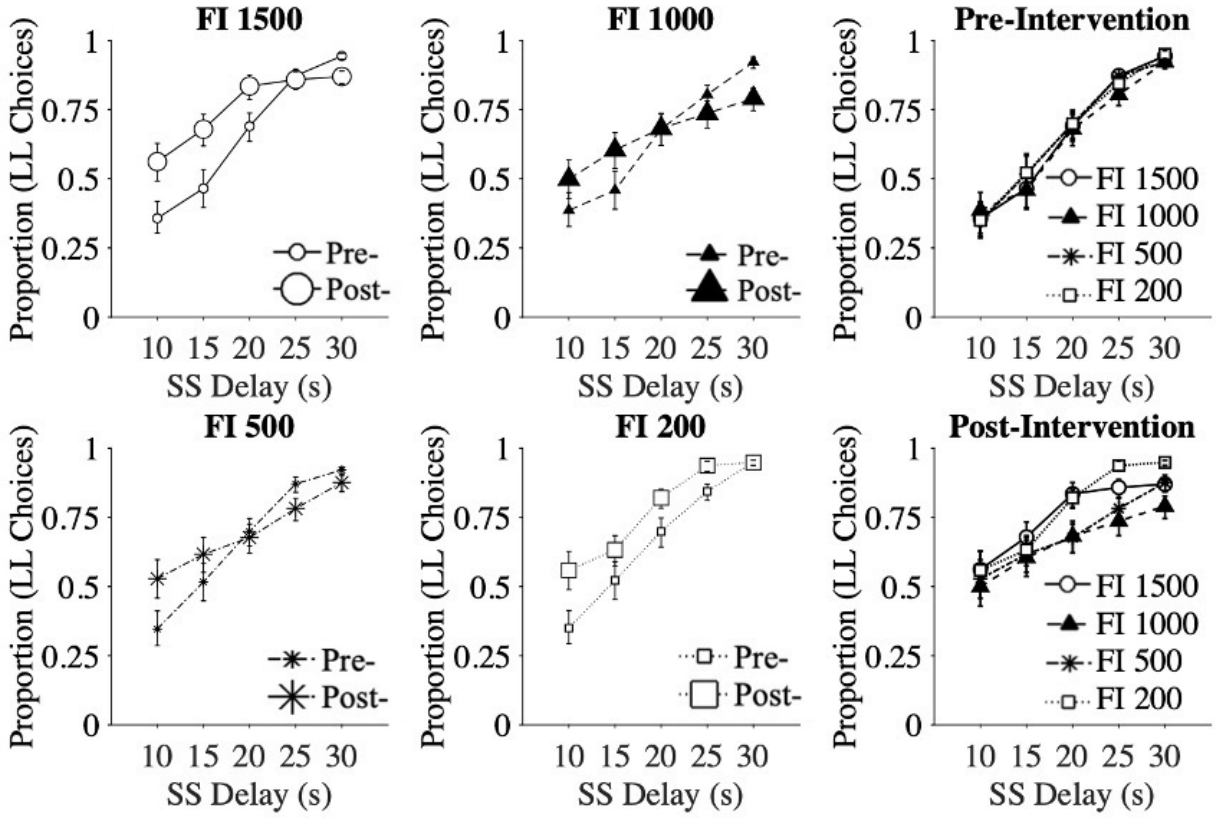


Figure 1.2. Proportion of LL choices with error bars (+/− SEM) post-intervention where male rats received the FI 200 intervention or a no-delay (ND) control task. Two groups of FI 200 intervention rats completed a pre-intervention impulsive choice task while the third group did not receive a pre-intervention choice test. Half the rats that received a pre-test got a 30-day break (a typical length of time needed for recovery from invasive neuroscientific manipulations) before training while the other half did not. The FI 200 intervention was successful at increasing LL choices in all three groups (compared to the control group), particularly at short SS delays. Error bars

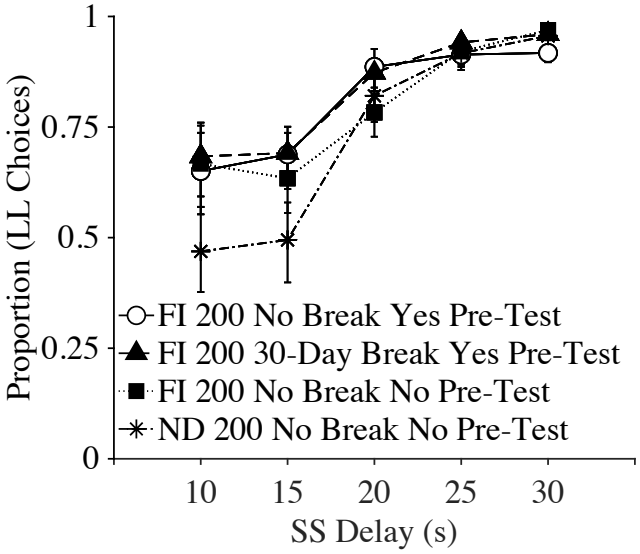


Figure 1.3. A comparison of the response requirements in fixed-interval and fixed-time schedules. Across schedules, rats must press a lever to initiate the delay. In fixed-interval schedules, rats must press the lever again after the delay has elapsed to receive the food reward. In fixed-time schedules, food is delivered immediately after the delay has elapsed.

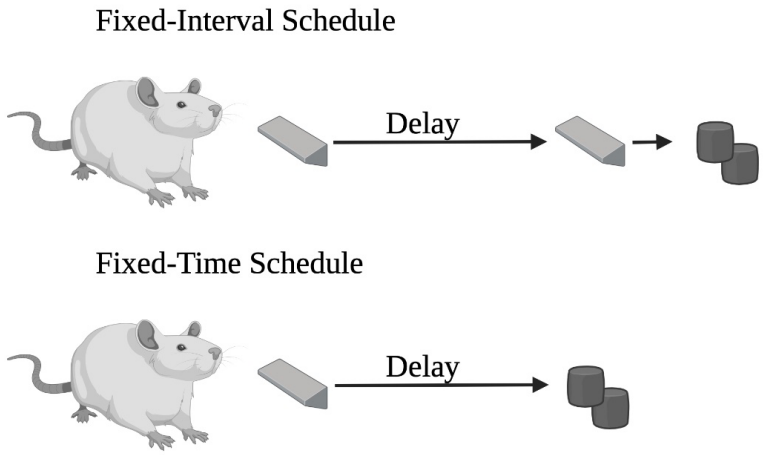
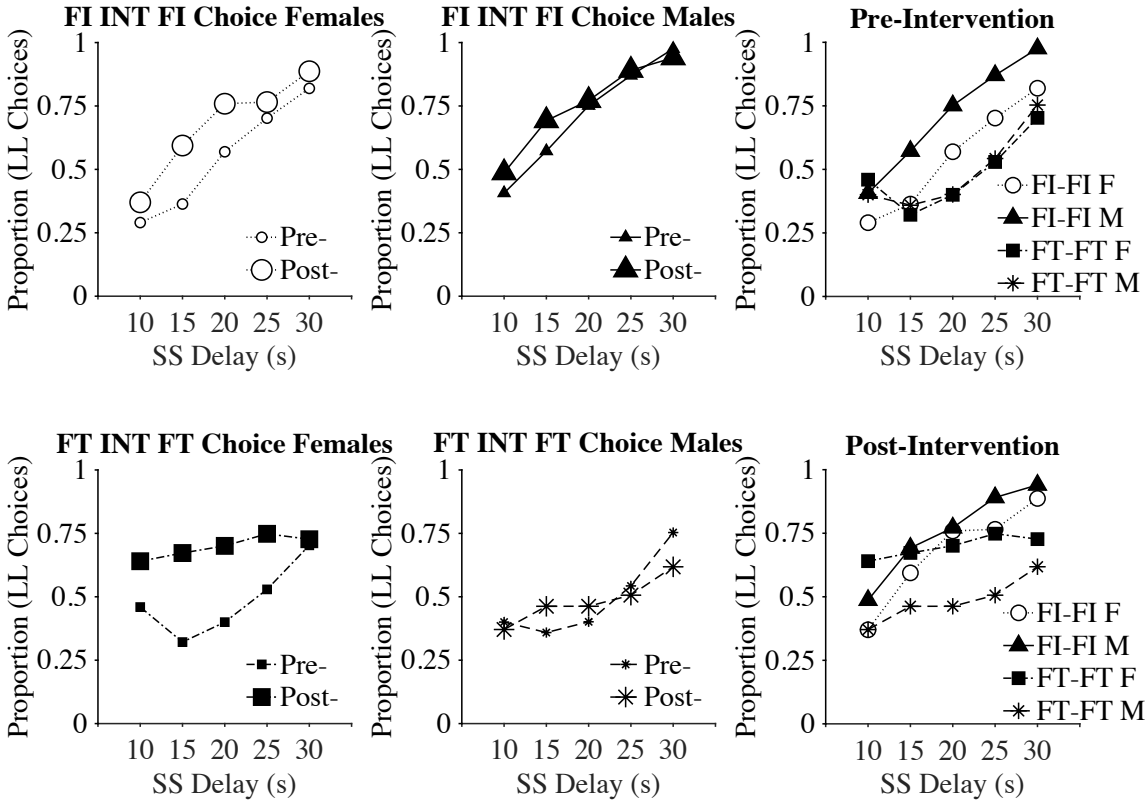


Figure 1.4. Proportion of LL choices pre- and post-intervention where a mixed sex sample received FI (fixed-interval) or FT (fixed-time) impulsive choice tasks (FI Choice or FT Choice) before and after an FI or FT intervention (FI INT or FT INT). While the study was not designed to examine sex differences, and therefore, underpowered to detect sex effects, the graphical representation of the data suggests that males and females may respond differently to the FT intervention. Males (M) appeared to be minimally affected by the FT intervention compared to females (F). Overall, FI INT FI Choice females and males were more sensitive to delay changes than FT INT FT Choice females and males.



Chapter 2 - Method

Animals

Forty-eight female and forty-eight male experimentally naïve Sprague-Dawley rats (Charles River, Stone Ridge, NY) were used in this experiment. Additional female and male rats were ordered as spare animals and used to replace eight rats that did not learn to press levers in the operant chambers in a timely manner. The rats arrived in squads of 48 rats (plus spare rats of each sex) because behavioral testing was limited to testing in 24 operant chambers at once. Treatment of squads were identical, and all counterbalancing was completed across squads.

Rats arrived at the facility (Kansas State University, Manhattan, KS) weighing 51-75 g, which was approximately post-natal (PND) 30. Experimentation began at approximately PND 62 after rats were acclimated to the facility and were accustomed to food restriction. Rats arrived at the facility during the pre-adolescent stage of development and transitioned through adolescence and into adulthood over the course of the experiment. The rats were pair-housed and maintained on a reverse 12-hr light:dark schedule (lights off at approximately 7 am). The rats were tested during the dark phase of the cycle, and sessions lasted approximately 2 hr. There was *ad libitum* access to water in the home cages and in the experimental chambers. Rats were food restricted and maintained at approximately 87% of their projected *ad libitum* weight, as derived from growth-curve charts obtained from the supplier. Daily feeding amounts typically ranged from 13-25 g per rat.

Apparatus

Experimentation occurred in 24 operant chambers (Med-Associates, St. Albans, VT), each housed within a sound-attenuating, ventilated box (74 × 38 × 60 cm). Each operant chamber (25 × 30 × 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

Rats were randomly assigned to one of four conditions: FI-Exp, FT-Exp, FI-Con, and FT-Con with the restriction of equal numbers of male and female rats in each group (n = 12 males, n = 12 females). Rats in the FI-Exp and FT-Exp conditions received intervention training and corresponding choice tests while rats in the FI-Con and FT-Con groups only received choice testing. During the intervention phase, rats in the FI-Con and FT-Con conditions experienced the same amount of time and food in the operant chambers as the FI-Exp and FT-Exp conditions, so neurobiological differences between the experimental and control groups were likely due to receipt of intervention training, not environmental factors such as age upon arrival to the facility, experimenter handling, pre-training, etc. Following behavioral training and testing, rats were euthanized and perfused to process brain tissue for c-Fos (see Figure 2.1 for experimental timeline).

Pre-Training

All rats in all conditions received pre-training, which 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 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 an RR 5, which lasted until the rats earned 20 food pellets for responding on each of the two levers. Magazine training lasted for one 2-hr session. Rats received lever press training for four 2-hr sessions. Eight rats that did not learn to lever press in a timely manner were replaced with spare animals, so the experiment continued according to the timeline.

Fixed-Interval (FI) Intervention Training

Twenty-four (12 female and 12 male) rats received an FI intervention designed to promote self-control (Smith et al., 2015). The FI intervention was effective in promoting self-control in males and females when delivered for 45 sessions in total (Panfil et al., 2020). More recently in males only, the FI intervention promoted self-control when delivered for 6 sessions in total (see Figure 1.1; Panfil et al., in preparation). The current experiment was an extended replication of Panfil et al. (in preparation) to evaluate the 6-session intervention in male and females rats.

The house light illuminated at the start of the trial. A single lever was inserted into the chamber and a lever press initiated the delay, turned off the house light, and turned on the cue

light. The first lever press after the target delay resulted in food delivery (1 pellet for FI 10 s or 2 pellets for FI 30 s). Any presses during the delay were recorded but these responses did not have any consequence. In addition, head entries to the food cup (measured by breaking a LED photocell beam) during the delay were recorded and did not result in any programmed consequences.

The FI schedules delivered fixed delays of 10 s or 30 s in separate sessions and the inter-trial interval (ITI) was 60 s. These sessions lasted until 100 total food pellets were delivered, approximately 2 hr. Rats completed 2 sessions of training on FI 10 s and 4 sessions of training on FI 30 s. The order of delays was counterbalanced across individuals.

Fixed-Time (FT) Intervention Training

Twenty-four (12 female and 12 male) rats received an FT intervention designed to promote self-control (Rung et al., 2018). This condition expanded upon information learned in Rung et al. (2018) and Smith et al. (under review) where rats (males only and a mixed-sex sample, respectively), received a fixed-time (FT) intervention and subsequent impulsive choice tasks. The FT intervention matched the FI intervention in all regards except no responses (lever press or head entry) were required to deliver the reward. In previous studies administering the FT intervention, the lever was retracted after the delay was initiated (Rung et al., 2018; Smith et al., under review). In the current study, the lever remained inserted in both experimental conditions, so lever presses and head entries to the food cup could be compared to examine sign- and goal-tracking. The FT intervention delivery matched the FI intervention delivery in the number of food pellets and sessions.

No-Training Control

Forty-eight (24 female and 24 male) rats received the same treatment as the no-training control in Peterson and Kirkpatrick (2016). Rats spent the same amount of time in the operant chambers but did not receive any stimuli. Control rats were trained to lever press (see above) but did not receive any lever press opportunity during the intervention phase (i.e., the levers remained retracted from the chamber). Control rats were weighed, handled, and placed into the operant chambers at the same time as the rats in other conditions, and received 4.5 g of food pellets in the food cup. This amount was equivalent to the maximum number of pellets available in training sessions for rats in other conditions. These sessions lasted for approximately 2 hr. Rats spent the same number of sessions in the operant chambers as rats in experimental conditions for a total of 6 sessions.

Impulsive Choice Task

In the impulsive choice task, rats chose between a smaller-sooner (SS) reward and a larger-later (LL) reward (Panfil et al., 2020). The response requirement matched the contingency delivered during the intervention phase. Thus, rats in the FI intervention group received an FI choice task, and rats in the FT intervention group received an FT choice task. Recent research in our laboratory suggests that congruency in response requirements during the intervention and choice task can affect impulsive choice behavior (Smith et al., under review). Thus, incongruency of response requirements between the intervention and choice task may complicate interpretation. No training control rats received FI or FT choice tasks, depending on group assignment.

The impulsive choice task consisted of 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 illuminated, and the scheduled delay began. For rats receiving the FI choice task, the first lever press following the delay resulted in food delivery, cue light offset, and onset of the 60-s intertrial interval (ITI). For rats receiving the FT choice task, food delivery occurred after the delay elapsed along with the lever retraction, cue light offset, and onset of the ITI. Forced-choice trials were identical to free-choice trials, but only one lever was inserted. The lever remained available throughout the delay. Peak trials were identical to forced-choice trials but lasted for 90 s and food was not delivered. Across all trial types for both FI and FT choice tasks, lever presses and head entries into the food cup were recorded.

Each session contained 84 trials delivered in four blocks. The beginning of each session was a block of 6 SS forced-choice trials for rats to orient to the SS delay, which changed frequently during the task. After the first training block, the remaining three blocks contained 14 free-choice, 4 SS forced-choice, 4 LL forced-choice, 2 SS peak, and 2 LL peak trials. Each session lasted for approximately 2 hr and delivered a maximum of 126 food pellets. The initial SS delay was 10 s for 4 consecutive sessions; once completed, the SS delay increased after two sessions (15→20→25 s). The choice task lasted 10 sessions in total. The LL choice was always 30 s. The SS choice always resulted in 1 food pellet, and the LL choice always resulted in 2 food pellets.

Euthanasia and Perfusions

Brains were collected to measure cellular activity. After the final session of impulsive choice testing, the rats were euthanized and perfused. Perfusions were timed so that rats were euthanized 120 min after completing the final session of testing (10th session of the impulsive choice task) to capture the peak in c-Fos protein expression produced during testing (Lara

Aparicio et al., 2022). This occurred after the impulsive choice task, so neurobiology reflected neural activity as a result of FI or FT schedules. Any differences between each no training control condition and corresponding intervention condition should be attributable to the receipt of the intervention. For each squad, timing of the final day of testing and euthanasia was offset for the rats within a span of three days, so sixteen rats were euthanized per day. The 10^{th} session of the impulsive choice task occurred over a three-day window, and all variables were counterbalanced such that equal numbers of rats per condition experienced no gap between the 9^{th} and 10^{th} session, one day between the sessions, or two days between the sessions. In addition, operant chamber start times were offset by 15-minute increments per rat so that perfusions followed in the same orderly fashion. This allowed rats to be tested during their normal time of day and allowed for a reasonable number and timing of perfusions each day.

The rats were perfused by administering a fatal dose of sodium pentobarbital. Once the rat was deeply anesthetized, assessed by non-responsiveness to the pedal withdrawal and tail pinch reflexes, the chest of the rat was opened, and the heart was exposed. A needle connected to a line delivering fluid was inserted into the left ventricle; the right atria was cut to allow for drainage. An infusion pump was used to deliver a 0.9% saline solution to flush out the blood initially, then delivered a 4% ice cold paraformaldehyde solution to fix the tissue.

Tissue Removal

Following perfusions, the brains were extracted. The tissue was placed in 4% paraformaldehyde for up to 2 hr. The brains were dehydrated in 20% sucrose solution for up to 48 hr and then immediately frozen in dry ice. The brains were kept at -80°C until they were sliced.

Slicing

The brains were sliced with a cryostat (Leica CM1860) and the Rat Brain Atlas (Paxinos & Watson, 2007) was used to track slices. The regions of interest were depicted in Figure 2.2. There were 3 replicates/slices (40 um thick) for each figure. The sliced brains were kept in wells of cryoprotectant at -20°C until the neurobiological assay was conducted.

C-Fos Assay

The brain tissue was stained in 12-well plates. The slices were rinsed in PBS (phosphate-buffered saline; 1X, pH 7.4) three times for 10 min each. Then the slices were incubated in 10% normal goat serum (NGS), 5% bovine serum albumin (BSA), and 0.5% triton-X in PBS for 1 hr. Then slices were transferred and incubated in the primary antibody (rabbit anti-c-fos monoclonal antibody 1:3200 Cell Signaling Technology no. 2250) with triton-X, NGS, BSA, and sodium azide in PBS for 48 hr at room temperature. The slices were rinsed in PBS three times for 10 min each. Then the slices were transferred and incubated in the secondary antibody (goat anti-rabbit Alexa Fluor 594 1:500 Cell Signaling Technology no. 8889) with triton-X, NGS, and BSA in PBS for 6 hr. The slices were rinsed in PBS five times for 10 min each. Then the slices were transferred into DAPI (4′,6-diamidino-2-phenylindole; Thermo Fisher 62248) in PBS for 10 min. Finally, the slices were rinsed in PBS once for 1 min and then once for 10 min. Slices were stored in PBS and plated. The slices were cover slipped with No 1.5 cover glasses and Prolong Gold Antifade Mountant (Thermo Fisher P36930).

Additional control conditions were completed to confirm that the assay steps resulted in c-Fos protein expression only and not due to non-specific binding or natural autofluorescence often associated with brain tissue (displayed in Figure A.1). In the no primary control condition, the steps described above were completed with the exception of the primary antibody. This

control condition confirmed that the secondary antibody used in the procedure did not bind to the tissue in the absence of the primary antibody. In the no antibody condition, the steps described above were completed with the exceptions of both the primary and secondary antibodies. This control condition confirmed that the blocking steps sufficiently reduced non-specific binding, so that no signal was present in any channel besides DAPI. Altogether, these additional control conditions coupled with well-documented evidence of c-Fos protein production in the rat brain as confirmed by Western Blots (e.g., Bing et al., 1992) confirm that fluorescence captured in the current experiment was the result of specific binding of the antibodies to the c-Fos protein. Expression of the c-Fos protein occurred in the nuclei of cells, and this expression appeared as relatively small circles.

Image Collection and Processing

Images were captured on an Olympus BX63 automated fluorescence microscope equipped with a Hamamatsu ORCA-Flash 4.0 camera. Slides were first scanned using the 4x objective for ease of navigation and to minimize unnecessary tissue exposure to the light sources. Slide scans were also used to ensure there was no overlap of images across regions. Final images of each brain region were obtained with the 10× objective, 204 8 × 2048 pixels, 1.35 × 1.35mm field-of-view, pinhole 1 AU without any averaging or accumulation. The 10× objective allowed for images to capture most of each brain region (75-100% of the brain region), and images were aligned across individual subjects to ensure that images were consistently captured in approximately the same location. Excitation scan settings for the red fluorescence protein (RFP) channel ranged 578–603 nm. Images, or z-stacks, were captured with the same exposure time and depth of field dimensions (32 μm) to control for intensity of the fluorescence signal across samples. Altogether, images across regions were the same size in both field-of-view and depth of

sampling, negating any area standardization. Cells expressing c-Fos were quantified in DMS, DLS, DCS, PL and IL using the automated counting function in FIJI. This image analysis resulted in a single, whole-number count of cells expressing c-Fos in each brain region per subject.

Data Analysis

Analyses conducted on the behavioral and neurobiological data were grouped together, summarized in Tables 2.1-2.5, and detailed in corresponding sections below. Data was imported and compiled with MATLAB 2020a (MathWorks). Repeated measures multi-level analyses were conducted using the *lme4* and *nlme* packages in R (Bates et al., 2015; Pinheiro et al., 2020) for behavioral data. Behavioral data sets were comprised of many observations or replications per individual while neurobiological analyses were conducted on single data point values per brain region. Multi-level models were appropriate for the repeated-measures behavioral data sets, and generalized linear models were deemed more appropriate for neurobiological datasets. These analyses were conducted using the *stats* package in R. 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).

The variables and coding structure were entered into models in a consistent fashion throughout the analyses. Sex was treated as a two-level categorical variable and effects coded (female = 1; male = -1). Group referred to intervention or control assignments during the intervention phase. FI-Exp and FT-Exp rats were in the experimental condition while FI-Con and FT-Con were in the control condition. Group was treated as a two-level categorical variable and effects coded (experimental = 1; control = -1). Schedule referred to FI or FT schedules during intervention and/or choice phases. Schedule was treated as a two-level categorical variable and

effects coded (FI = 1; FT = -1). In all models, fixed effects and their interactions were specified as full factorial models.

Intervention Analyses

Lever presses and head entries to the food cup during the intervention were assessed to examine sex differences in sign-tracking and goal-tracking behaviors in the FI-Exp and FT-Exp groups (Table 2.1). The control groups were not included in these analyses because they did not have an opportunity to lever press during the intervention phase. Separate analyses were conducted for each intervention delay and dependent variable for a total of four models.

Lever presses and head entries during the intervention trials were recorded with 1-ms resolution. Lever presses and head entries per minute were normalized so values ranged from 0 to 1 [(response – minimum response value) / (maximum response value – minimum response value)]. By calculating a proportion of each rat's maximum rate of lever pressing or head entries, the relative response rates account for differences in baseline responding (Meck & Church, 1984). Lever presses and head entries were analyzed as response rates to control for response opportunities.

Lever presses and head entries during the intervention trials were analyzed using repeated-measures multi-level models with a Gaussian distribution and identity link function. Residual values associated with the model fits were examined. In all but one model, residuals were normally distributed. On the 10-s head entries model, residuals were slightly negatively skewed. Across models, schedule (FI or FT), sex (female or male), and seconds were included as fixed effects to determine whether lever pressing and/or head entries increase or decrease as time into the intervention trials increased based on sex or intervention condition. Rat (intercept) was entered as a random effect. Seconds was entered as a continuous variable and mean-centered.

Peak Analyses

All rats received peak interval trials during the impulsive choice task. FI and FT schedules delivered during the intervention and/or choice task were analyzed separately based on the shape of the response distributions. Peak analyses assessed temporal accuracy and precision in the impulsive choice task for rats that received the FI schedule in the current experiment. For the FT schedule, peak analyses assessed initial response rates at the beginning of the intervention trials and the rate at which responding decreases, or the rate of decay, as the trials progress. Separate models were used to analyze SS and LL peak trials (Table 2.2).

Lever pressing was normalized as described in *Intervention Analyses*. In addition, data was transformed into relative rates, so that the starting response rate was at or near 0 and the peak rate was at or near 1. Lever press data was relativized to the group mean responses [(response – min(group mean response))/(max(group mean response) – min(group mean response))]. The goal of the analysis was to compare the most pertinent parameters for understanding timing processes, so normalized relative rates allowed for a simpler nonlinear function.

Nonlinear multi-level models were used to assess peak trial data obtained during the choice tasks (Table 2.2). Rats that received the FI schedule made responses in accordance with Equation 1 where responses increased up to the fixed-interval duration and decreased afterwards. The nonlinear component was specified with a three-parameter modified Gaussian distribution to fit lever presses per minute with the following form:

$$m * e^{-(t-a)^2/2p^2} \quad (1)$$

where *t* represented the time into the peak trial, *a* was the peak time (accuracy), *p* was the standard deviation of the peak (precision), and *m* was the maximum height of responding at peak

time (see Fox et al., 2019 for a similar analysis). Rats that received the FT schedule responded most at the beginning of trials and progressively decreased in responding as time into the peak trial increased. The nonlinear component was specified with a two-parameter exponential decay function to fit lever presses per minute with the following form:

$$b \times e^{(-a \times t)} \quad (2)$$

where *t* represented the time into the peak trial, *a* was response rate decay parameter, and *b* was initial response rate intercept parameter. Across models, the parameters were allowed to vary across group (experimental or control), sex (male or female), and SS delay. SS delay was treated as a continuous variable. Rat (intercept) was included as a random effect.

Impulsive Choice Analyses

All rats received an impulsive choice assessment after the intervention phase, so the impulsive choice analyses included all rats. Head entries and lever presses were recorded during the impulsive choice task as well. Analyses of lever press behavior during forced-choice trials included rats from all groups. Separate analyses were conducted for each dependent measure.

A repeated measures multi-level logistic regression with logit link function was used to analyze impulsive choices (Table 2.3). Schedule (FI or FT), group (experimental or control), sex (male or female), and SS delay were included as fixed effects. Rat (intercept) and SS delay were tested as random effects. SS delay was not significantly correlated with the intercept of the model, so SS delay was included as a random effect. In addition, repeated-measures multi-level models with a Gaussian distribution and identity link function were used for lever press rate analyses (Table 2.3) as a measure of sign-tracking during the forced-choice trials. Schedule (FI or FT), group (experimental or control), sex (male or female), and time into the trial (seconds) were included as fixed effects. Rat (intercept) was included as a random effect.

Neurobiological Analysis

Neural activity for each brain region (DMS, DCS, DLS, PL, and IL) was analyzed using generalized linear models with sex (male or female), group (experimental or control), and schedule (FI or FT) as predictors (Table 2.4). Altogether, there were a total of five generalized linear models. The Poisson distribution with a log link function was specified in each of these models because c-Fos+ cells were counted as whole numbers or discrete outcomes. This distribution accounts for the non-normality in count data, particularly when values are at or near zero because the Poisson distribution has a lower bound of zero.

Exploratory Cluster Analyses

The current experiment yielded dependent measures of SS and LL choices during impulsive choice, timing during impulsive choice, sign-tracking and goal-tracking behaviors during the interventions, and neural activity. Natural clusters in these dimensions may emerge, offering insight into self-control profiles. Exploratory cluster analyses were conducted to determine whether clusters existed within the combined behavioral and neurobiological data set and to further evaluate hypotheses about interval timing and temporal attention versus sign- and goal-tracking (Table 2.5). The primary goal of this project was to evaluate sex differences in the efficacy of time-based interventions and associated neurobiology, so this analysis was treated as a secondary, exploratory goal.

Two clustering techniques were conducted and compared. K-means clustering forms clusters based on distance between each vector and an average vector in each cluster (Forgy, 1965). K-means clusters are ellipsoid in shape and the number of clusters must be specified in advance. There is no theoretical basis for determining number of clusters expected in this data set, so the number of clusters will be specified based on predetermined metrics (see below).

Hierarchical clustering constructs clusters based on pairwise distances between clusters (Alashwal et al., 2019). The number of clusters may equal the number of vectors in a dataset, so the number of clusters does not need to be specified in advance. Hierarchical clustering is not limited to ellipsoid-shaped clusters but is computationally intensive. Given the advantages and disadvantages of each method, both techniques were used to converge on a number of clusters within the dataset.

Across clustering techniques, the number of dimensions specified in the analysis are limited by the number of vectors (Forgy, 1965). In the current experiment, each individual rat was a vector. With 96 rats in the experiment, the clustering techniques were limited to 6 dimensions. To satisfy this constraint, separate analyses were conducted for each brain region of interest (DMS, DCS, DLS, PL and IL) and for competing hypotheses about interval timing processes and sign-tracking versus goal-tracking behaviors. Sign- and goal-tracking behaviors were evaluated during the intervention phase. Control rats did not receive any intervention training, so the control animals did not have any assessments of sign- and goal-tracking during this phase. The dimensions entered into the clustering analyses evaluating timing were the average proportion of LL choices, change in choice between 30- and 10-s delays (slope of the choice function), response rate during the final 3 s of the 30-s LL peak from the choice task, and c-Fos+ cells. In these analyses, average proportion of LL choices and slope of choice functions were summarized raw data values, not model estimates. The dimensions entered into the clustering analyses evaluating sign-tracking and goal-tracking were the head entry response rates during the final 3 s of the 10- and 30-s intervention delays, lever press response rates during the final 3 s of the 10- and 30-s intervention delays, and c-Fos+ cells.

K-means and hierarchical clustering were conducted in JMP 16 Pro (SAS). The number of clusters for the analyses were determined by Cubic Clustering Criterion (CCC) values, visual inspection of the dendrogram, and parsimony. If the CCC value associated with an increase in cluster size did not vastly improve compared to fewer clusters, the more parsimonious cluster solution was selected. The mean and standard deviation values for each dimension were compared across clustering techniques. Also, the rats grouped into each cluster were compared across techniques by labeling each rat with corresponding schedule (FI or FT), group (experimental or control), and sex (male or female) affiliations. Altogether, these exploratory analyses were used to identify possible clusters of behavioral profiles that emerged across impulsive choices, timing, sign-tracking, goal-tracking, and neurobiology.

Predictions

Temporal Processing and Attention Hypotheses

Previous research evaluating time-based interventions suggests temporal processing, or interval timing, and/or temporal attention to delays may promote self-control while research examining sign- and goal-tracking suggests that attending to the lever instead of the food cup may be associated with impulsivity. The current experiment was designed to test these hypotheses using behavioral and neurobiological data. Measuring interval timing with peak interval trials during the impulsive choice task may further elucidate the role of timing and attention. Differences between the abbreviated FI and FT interventions' efficacy in males and females and differences in timing accuracy and precision measured on peak trials in the choice task may offer insights into the importance of timing or attention in addition to sign- and goal-tracking.

The temporal processing and attention hypotheses suggest that the ability to time and/or attend to the delays may increase self-control, particularly in terms of delay sensitivity (Figure 2.3). On one hand, it is possible that the FI schedules may improve interval timing. We may observe this through performance on peak trials with increased accuracy and precision in timing and on forced-choice trials with responses increasing in anticipation of the delay. These patterns may correspond with increased sensitivity to delay on free-choice trials. Rats with poor accuracy and precision on peak trials and responses that decrease in anticipation of the delay may show decreased sensitivity to delay (Figure 2.3A). On the other hand, the FI schedules may require active attention during delays, and interval timing may not be necessary for this process. We may observe similar levels of accuracy and precision on peak trials if timing is not necessarily involved. If temporal attention underlies FI intervention efficacy, responses may increase in anticipation of the delays on forced-choice trials. Where FT schedules may encourage passive attention, responses may decrease as time continues into forced-choice trials. Active attention during delays may result in increased sensitivity to delay on free-choice trials and passive attention during delays may correspond with decreased delay sensitivity (Figure 2.3B). Altogether, we expected that the FI schedules would result in increased delay sensitivity compared to the FT schedules. Across sex, we predicted that the FI intervention would increase self-control to a greater degree compared to the FT intervention particularly evident in increased delay sensitivity in the FI intervention. Within the control groups, we expected the FI choice task to encourage temporal processing and/or attention such that the FI choice task control rats would be more self-controlled than the FT choice task control rats, evident in more LL choices at longer SS delays and increased delay sensitivity (slope; Figure 2.3).

We expected to replicate previous findings by showing that the abbreviated FI intervention successfully promotes self-control in male rats (Panfil et al., in preparation). We also anticipated that the abbreviated FI intervention would increase LL choices in females to a similar degree based on the lack of sex differences in the FI intervention efficacy when the intervention was delivered for a longer period of time (Panfil et al., 2020). It was unclear whether the abbreviated FT intervention can promote self-control in males and females because previous studies delivered the intervention for at least 45 sessions (Rung et al., 2018; Smith et al., under review). Where temporal attention may not be required to the same degree in the FT intervention as in the FI intervention, more sessions may be needed to produce a substantial FT intervention effect. Based on preliminary data obtained from Smith et al. (under review), we expected to see sex differences in choice behavior following abbreviated FT interventions. While the previous study was not designed to examine sex as a biological variable and, therefore, not sufficiently powered to detect sex effects, the trends in the results suggest females were more self-controlled than males after an FT intervention and choice task (Figure 1.4). We expected sex × intervention and sex × intervention × SS delay interactions such that male rats that received the FT intervention would be more impulsive across SS delays and less sensitive to delay. Overall, these predictions suggest that improvements in self-control are a result of increased temporal processing and/or attention, so we expected analyses of timing to differentiate the subcomponents of timing and attention.

If temporal processing, or interval timing ability, is necessary for improvements in self-control, we expected to see differences in peak interval timing analyses between groups and schedules. In Smith et al. (under review), rats that received the FI contingency in choice and/or intervention phases had comparable timing accuracy and precision on peak interval trials, but

rats that received FT contingencies only (FT choice tasks and FT intervention) showed the poorest temporal precision. These results suggest that rats in the FT intervention condition in the current experiment would show poor temporal precision on peak interval trials compared to the FI intervention condition. In addition, rats in the no training control group that received the FI choice task may show more accurate and precise peak timing compared to the rats in the no training control group that received the FT choice task. The FI intervention should enhance temporal processing above and beyond any improvements via the FI choice task, so that rats that received the FI intervention should be more accurate and precise compared to no training control rats that received the FI choice task. These results would suggest that the FI schedule enhanced temporal processing ability, which may increase self-control (Figure 2.3A).

However, it is possible that there will be no differences in accuracy and precision across FI and FT schedules. This result coupled with impulsive choice results may highlight the importance of temporal attention instead (Figure 2.3B). If FI and FT schedules result in similar accuracy and precision in timing but the FI schedule promotes self-control through increased sensitivity to delay more so than the FT schedule, that would suggest that temporal attention learned through the FI contingency affects self-control more so than timing. If the FI and FT interventions result in similar accuracy, precision, and improvements in self-control, that would suggest that an alternative mechanism such as delay tolerance may underlie the efficacy of time-based interventions.

We also expect to observe sex differences across tasks. Based on previous research in mice examining peak interval timing, males responded earlier in a trial while females ramped up in responding closer to the target interval (Gur et al., 2019). This suggests that females may display better temporal precision on peak trials delivered during the choice tasks, evident in

sharper timing functions by decreased standard deviation of the timing functions. Males and females may not differ in accuracy or the overall mean of the timing functions. Taken together, we expected that females should be more precise in their timing across interventions and that sex and schedule may interact so that FI females show the highest degree of precision in timing. However, alternative hypotheses would suggest that sex differences in interval timing and attention may not drive intervention efficacy to promote self-control.

Sign- and Goal-Tracking Hypotheses

An alternative possibility is that differences in self-control after FI and FT schedules may reflect sex differences in sign- and goal-tracking like behaviors (Figure 2.4). Given that previous research showed that female rats attended to and interacted more with stimuli that signal reward availability compared to male rats (Hilz et al., 2021; Hughson et al., 2019; King et al., 2016; Pitchers et al., 2015; Stringfield et al., 2019), females may lever press more while males may check the food cup more. The sign- and goal-tracking hypotheses suggest that the response requirement of the interventions may interact with sex differences in sign- and goal-tracking. The FI schedules may encourage sign-tracking like behavior, and females may be more likely to sign-track than males. Taken together, females in the FI conditions may show increased lever presses leading up to the end of delays in both intervention trials and forced-choice trials, resulting in increased delay sensitivity on free-choice trials (Figure 2.4A). Males in the FI conditions may show decreased lever pressing behavior leading up to the end of delays compared to females on intervention and forced-choice trials. This may correspond to dampened delay sensitivity on free-choice trials. The FT schedules may promote goal-tracking like behavior, and males may be more likely to goal-track compared to females. Altogether, male rats in the FT condition may show more head entries in anticipation of delays elapsing on intervention and forced-choice

trials, resulting in increased delay sensitivity on free-choice trials all when compared to females in the FT conditions (Figure 2.4B).

However, it is also possible that the FI intervention response requirement may influence both males and females to interact with the lever while the FT intervention may encourage goal-tracking behavior in males and females. Previous research also suggests that sign-tracking is related to increased impulsivity. Rat and human sign-trackers display more impulsive actions (Flagel et al., 2010; King et al., 2016; Lovic et al., 2011), make more impulsive choices (Garofalo & di Pellegrino, 2015; Olshavsky et al., 2014; Tomie et al., 1998; but see Flagel et al., 2010 and Lovic et al., 2011 for opposite effects), and make more risky choices (Olshavsky et al., 2014; Swintosky et al., 2021) than rat and human goal-trackers. In this case, the FI intervention females may be most impulsive due to their increased propensity to sign-track. However, this is inconsistent with previous research comparing FI and FT interventions because both time-based interventions promoted self-control to a similar degree (Smith et al., under review).

Sign- and goal-tracking may influence the interventions at the individual level instead of the group level. If this relationship is antagonistic, rats that sign-track may show weaker FI intervention effects and rats that goal-track may show weaker FT intervention effects. Alternatively, this relationship may be synergistic. Rats that sign-track may show stronger FI intervention effects because sign-tracking in the context of the intervention and choice tasks may promote delay sensitivity by increasing attention to delays. While this hypothesis does not align with previous research showing that sign-trackers display high impulsivity, interacting with the levers during the intervention and choice tasks may encourage active waiting. If the FI intervention biases rats toward sign-tracking and the FT intervention biases rats toward goal-tracking, sign-trackers in the FI intervention may show the strongest FI intervention effects and

goal-trackers in the FT intervention may show the strongest FT intervention effects. This may be further influenced by sex differences in sign- and goal-tracking. FI females may sign-track most and show robust FI intervention effects through increased temporal attention while FT males may goal-track most and show robust FT intervention effects but through passive waiting.

Neurobiological Hypotheses

In accordance with previous research investigating the role of brain regions underlying impulsive choice and timing (Dietrich et al., 1997; Kim et al., 2009; Kirkpatrick et al., 2015; Litrownik et al., 1977; Marshall et al., 2014; Meck, 2006; Sackett et al., 2019; Smith et al., 2015; Takahashi, 2005; Wittmann & Paulus, 2008), we expected differences in c-Fos+ cells across brain regions in rats that received the FI versus FT contingencies. After the impulsive choice task, rats were euthanized, so neural activity measured by c-Fos reflected activity during the FI and FT choice tasks. Rats that received the FI schedule in the intervention and choice phases should show higher levels of c-Fos+ cells in the PL and DMS compared to rats that receive the FT intervention and choice tasks (Figure 2.5A). In addition, rats that received the FI intervention may show higher levels of c-Fos compared to rats in the FI control group. The prelimbic cortex and dorsomedial striatum are heavily involved in interval timing (Coull et al., 2011; Finnerty et al., 2015; Matell & Meck, 2004; Tallot & Doyère, 2020), and this functional circuit may underlie the FI intervention efficacy in promoting self-control. If there were no differences in c-Fos+ cells in PL and DMS when comparing the FI and FT schedules, this would suggest that interval timing ability may not be essential to time-based intervention efficacy.

If time-based interventions are not reliant on enhanced interval timing, c-Fos+ cell counts in DCS and DLS may reflect group differences in the current study based on their role in choice behavior (Dunnett et al., 2012; Tedford et al., 2015). FI and FT intervention groups should show

higher levels of self-control compared to FI and FT control groups, so intervention conditions may show higher levels of c-Fos in DCS and DLS compared to controls (Figure 2.5B). Along the same lines, c-Fos expression in the infralimbic cortex may negatively relate with self-control given the relationship between IL and impulsive action (Chudasama et al., 2003; Tsutsui-Kimura et al., 2016). Impulsive action may contribute to impulsive choices if animals high in impulsive action cannot inhibit the impulse to pick the SS choice. The FI and FT intervention groups may show lower levels of c-Fos in IL compared to the FI and FT control groups (Figure 2.5B). Higher levels of c-Fos in IL for the control groups may suggest that they were unable to inhibit the impulse to select the SS choice. Altogether, quantification of c-Fos in PL, DMS, DCS, DLS, and IL may shed light on possible cognitive mechanisms of self-control.

If sex differences in sign- and goal-tracking interacted with the interventions according to the sign- and goal-tracking hypotheses (Figure 2.3), we expect FI-Exp females to show the highest levels of c-Fos expression and the FT-Exp males to show the lowest levels of c-Fos in the brain region related to sign-tracking (Figure 2.6). Based on previous research showing increased c-Fos mRNA in the DMS of sign-trackers (Flagel, Cameron, et al., 2011), we expected higher levels of c-Fos+ cells in DMS in sign-trackers compared to goal-trackers. Based on sex differences in sign-tracking, females should show higher levels of DMS c-Fos+ cells compared to males because they often sign-track more than males (Hilz et al., 2021; Hughson et al., 2019; King et al., 2016; Pitchers et al., 2015; Stringfield et al., 2019). Extending to the intervention and choice contingencies, if FI schedules biased rats towards sign-tracking and FT schedules biased rats towards goal-tracking, rats that experienced the FI schedule may show higher levels of c-Fos+ cells in DMS compared to rats that experienced the FT schedule. Finally, sex differences in response to the intervention and choice contingencies may interact so that intervention conditions

are affected more so than control groups. Altogether, this suggests that sign-tracking, sex, schedule, and group may interact so that FI-Exp females may show the highest levels of c-Fos+ cells in DMS and FT-Exp males may show the lowest levels of c-Fos+ cells in DMS (Figure 2.6). Importantly, this hypothesized relationship differs from the PL and DMS hypotheses displayed in Figure 2.5A because we expect sex to interact with group and schedule, resulting in key differences between male and female conditions.

However, it is possible that differences in c-Fos may occur in other brain regions depending on theories of sign- and goal-tracking. For example, the incentive salience theory of sign- and goal-tracking behavior suggests that sign-trackers attribute motivational value to the conditioned stimulus that precedes a reward while goal-trackers view the conditioned stimulus as a predictor of a reward without imbuing motivational value (Berridge & Robinson, 1998; Colaizzi et al., 2020; Robinson & Flagel, 2009). This theory posits that dopaminergic signaling in the nucleus accumbens is important to incentive salience attribution (Berridge, 2007; Colaizzi et al., 2020; Flagel, Clark, et al., 2011). If behavioral data suggests that sign- and goal-tracking behaviors are related to impulsivity while interval timing and temporal attention are not, future work should focus on brain regions of interest in the incentive salience theory or alternative theories of sign- and goal-tracking.

Cluster Hypotheses

Exploratory cluster analyses were conducted on the current experiment to determine whether behavioral profiles existed within the data based on impulsivity, timing, sign-tracking, goal-tracking, and neurobiology. The clusters may reflect group assignments or particular behavioral and/or neurobiological phenotypes regardless of group assignments. To test the hypotheses centered around the importance of timing, cluster analyses included temporal

processing dimensions in addition to measures of impulsive choice to determine whether clusters emerged based on these features. In these cluster analyses, we expected that the clustering solutions would converge on similar groupings of animals that reflected the temporal processing and/or temporal attention hypotheses. In particular, we expected a cluster of animals that was characterized by high levels of LL choices, a positive choice function slope, high lever press response rates during the final seconds of the 30-s LL peak from choice, and high levels c-Fos+ cells in PL and DMS. We also expected an additional cluster that showed the opposite characteristics, so low levels of LL choices, a flat choice function slope, low lever press response rates during the final seconds of the 30-s LL peak from choice, and low levels c-Fos+ cells in PL and DMS. When overlaid with group, schedule, and sex labels, we predicted that primarily FI-Exp females and FI-Exp males would make up the cluster showing positive relationships between timing, self-control, and c-Fos in PL and DMS while the FT control group would be assigned to the cluster showing low self-control, poor temporal processing ability, and low c-Fos expression in PL and DMS. In the cluster analyses including DCS, DLS, and IL, we did not expect clusters to reflect the importance of timing to self-control facets.

To test the sign- and goal-tracking hypotheses, cluster analyses included indices of these behaviors during the intervention phase with c-Fos. In these cluster analyses, we expected that one cluster of rats would be characterized by high lever press response rates during the final 3 s of the 10- and 30-s intervention delays, low head entry response rates during the final 3 s of the 10- and 30-s intervention delays, and high levels of c-Fos+ cells in DMS. Also, we predicted that another cluster of rats would be best described by low lever press response rates during the final 3 s of the 10- and 30-s intervention delays, high head entry response rates during the final 3 s of the 10- and 30-s intervention delays, and low levels of c-Fos+ cells in DMS. Based on the

hypothesized relationship between sign-tracking and the FI schedule, we expected the cluster of rats with high lever press response rates and high levels of c-Fos in DMS to mostly contain rats that received the FI schedule. We predicted that the cluster characterized by high head entry rates and low levels of c-Fos in DMS may be made up of rats that received the FT schedule. These clusters would suggest that sign- and goal-tracking like behaviors may occur within the context of time-based interventions, which remains to be seen in the time-based intervention literature.

It is also possible that clusters emerged predominantly from one salient feature like impulsive choices or that these hypothesized relationships only occur within certain conditions of the current experiment. Follow-up cluster analyses were conducted to test this directly. Altogether, these exploratory analyses were conducted to describe behavioral profiles that emerged across impulsive choice, timing, sign-tracking, goal-tracking, and neurobiology. This information may be used to tailor time-based interventions even further to treat the phenotypes discovered, and provide future directions to the timing, sign-tracking, goal-tracking, and impulsive choice fields. Time-based interventions are well-established, so that the current research aimed to replicate previous behavioral findings and offer new insights into the cognitive mechanisms of these schedules. Finally, neural activity associated with FI and FT schedules may illuminate potential brain regions and pathways for targeting with advanced neuroscientific techniques to pinpoint mechanisms of these schedules.

Figure 2.1. Timeline of experiment based on age of the rats in days (post-natal day; PND). Rats completed lever training, intervention training, and impulsive choice testing followed by euthanasia. The final session of impulsive choice testing and euthanasia was offset within a span of three days for rats in a squad, so sixteen rats were euthanized and perfused each day. This allowed rats to be tested during their normal time of behavioral testing and for a reasonable number of perfusions per day.

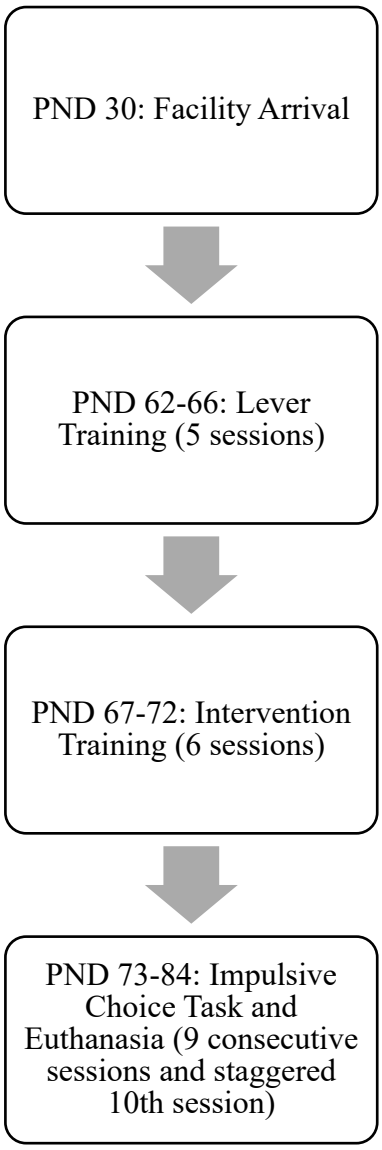


Figure 2.2. Diagrams of coronal sections of the rat brain (from Paxinos and Watson) showing the areas imaged and analyzed. The sections shown are +3.2 (left) and +1.1 (right) anterior to bregma. PL = Prelimbic Cortex; IL = Infralimbic Cortex; DLS = Dorsolateral Striatum; DCS = Dorsocentral Striatum; DMS = Dorsomedial Striatum.

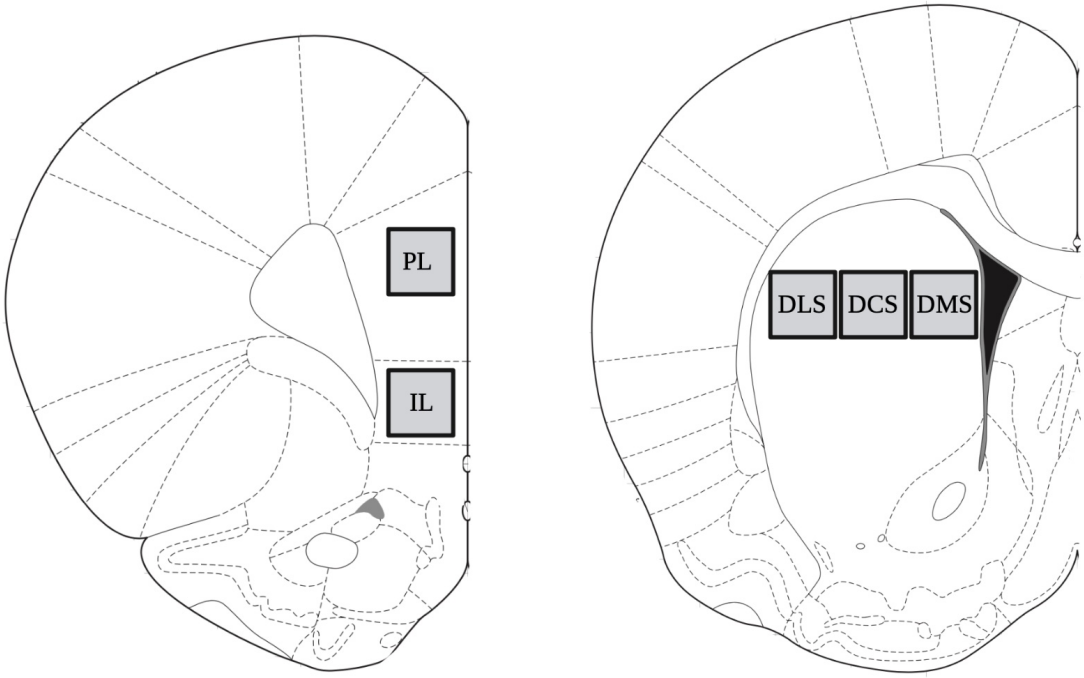


Figure 2.3. Hypothesized effects of temporal processing (A) and attention (B) as possible mechanisms for FI (fixed-interval) and FT (fixed-time) intervention efficacy.

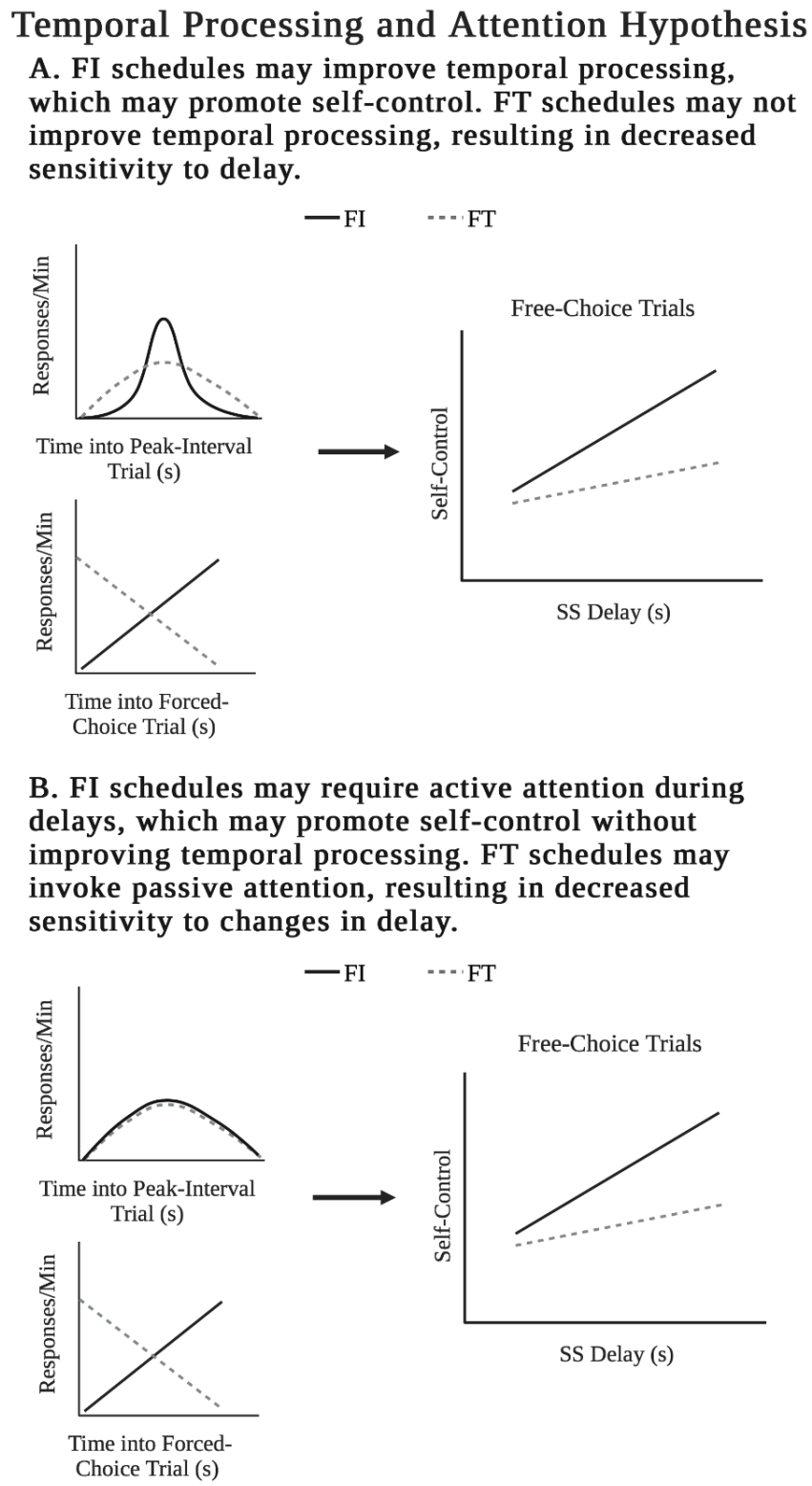


Figure 2.4. Hypothesized effects of sign- and goal-tracking like behaviors on FI (fixed-interval; A) and FT (fixed-time; B) intervention efficacy for females and males.

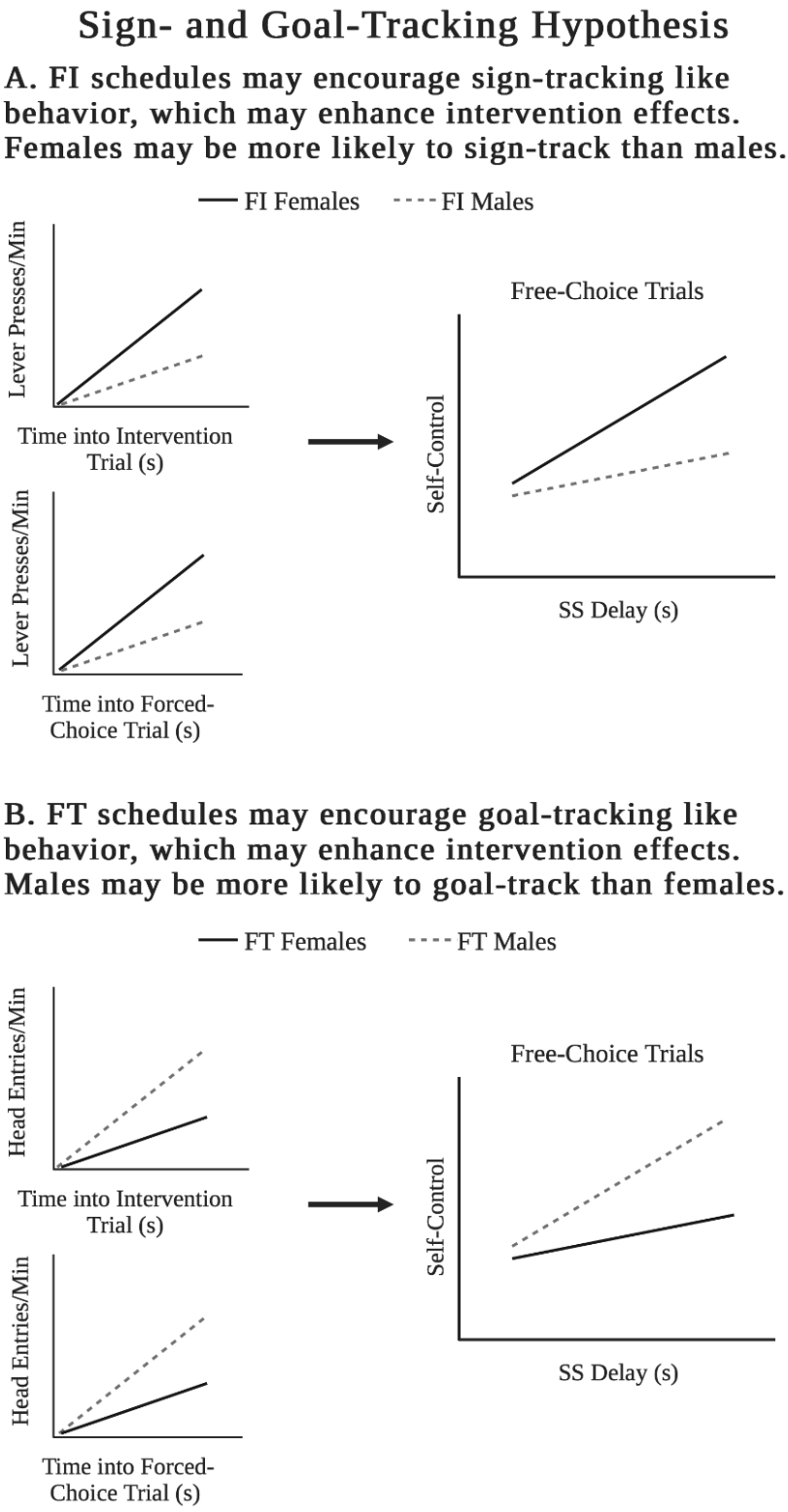


Figure 2.5. Hypothesized effects of c-Fos expression in PL and DMS (A) and DCS, DLS, and IL (B) according to group and schedule conditions. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control; PL = Prelimbic Cortex; IL = Infralimbic Cortex; DMS = Dorsomedial Striatum; DCS = Dorsocentral Striatum; DLS = Dorsolateral Striatum.

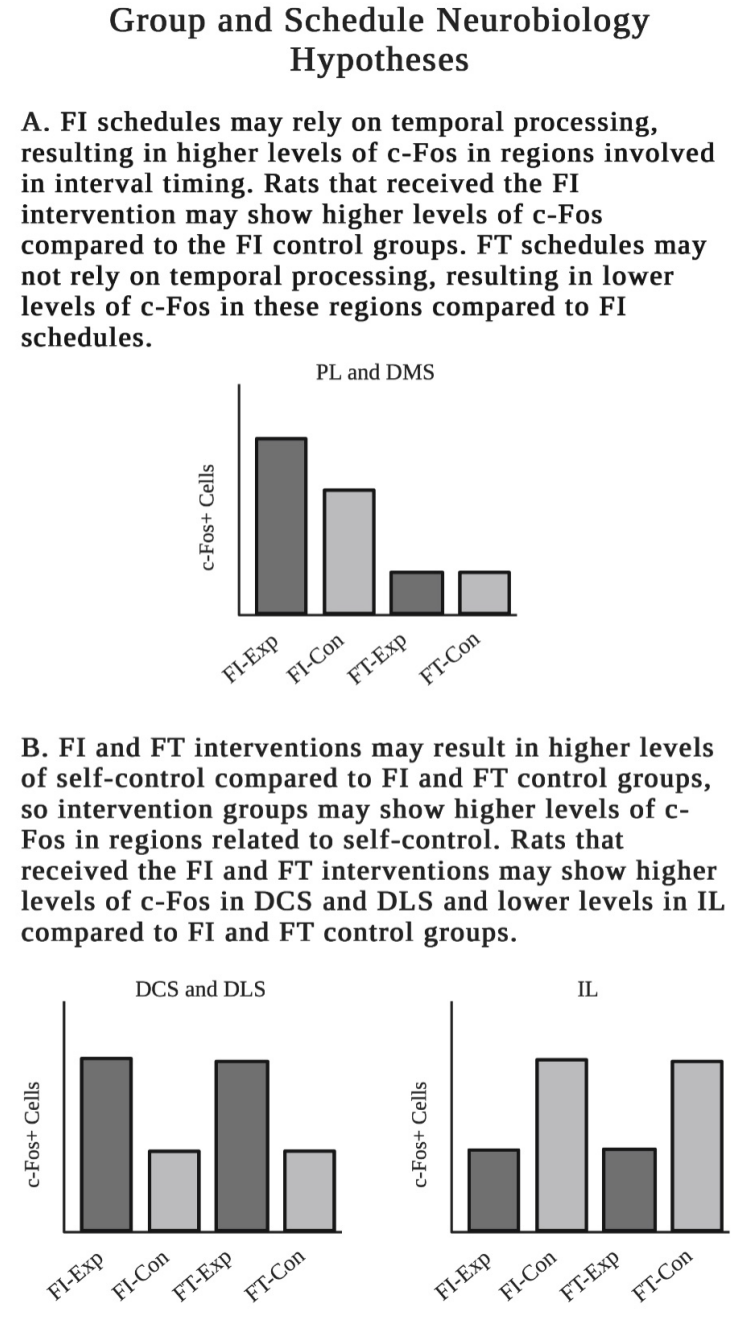


Figure 2.6. Hypothesized effects of c-Fos expression in the dorsomedial striatum (DMS) based on how sex may interact with sign- and goal-tracking like behaviors. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

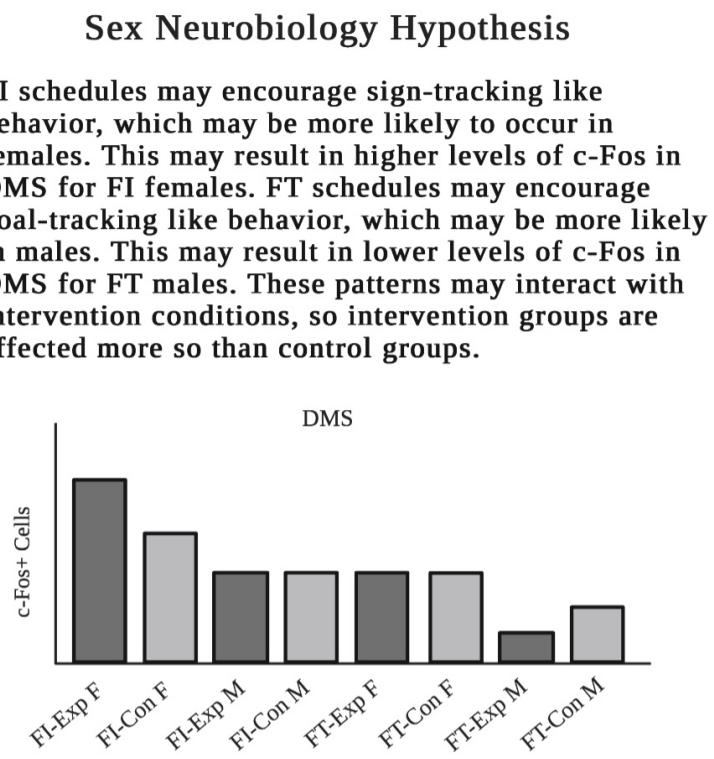


Table 2.1. Intervention analyses examined the FI-Exp and FT-Exp experimental conditions during the six sessions of intervention training. The control conditions (FI-Con and FT-Con) were not included in these analyses. The head entry and lever press response rates were assessed across trials.

	Dependent Variable(s)	Fixed Effects	Random Effects
Sign-Tracking Behaviors	Lever presses per minute, 10-s trials	schedule*sex*seconds	1|rat
	Lever presses per minute, 30-s trials	schedule*sex*seconds	1|rat
Goal-Tracking Behaviors	Head entries per minute, 10-s trials	schedule*sex*seconds	1|rat
	Head entries per minute, 30-s trials	schedule*sex*seconds	1|rat

Note: Both modeling approaches specified a Gaussian distribution. Schedule refers to FI (fixed-interval) or FT (fixed-time) intervention. The fixed effect, seconds, refers to time into trial.

Table 2.2. Peak analyses included peak trial data from the impulsive choice task. FI (fixed-interval) and FT (fixed-time) schedules were analyzed separately.

	Dependent Variable(s)	Fixed Effects	Random Effects
FI SS Peak During Choice Task (Eq. 1)	Lever presses per minute	$a + p + m \sim$ sex*group*SS delay	$a + p + m \sim 1$|rat
FI LL Peak During Choice Task (Eq. 1)	Lever presses per minute	$a + p + m \sim$ sex*group*SS delay	$a + p + m \sim 1$|rat
FT SS Peak During Choice Task (Eq. 2)	Lever presses per minute	$a + b \sim$ sex*group*SS delay	$a + b \sim 1$|rat
FT LL Peak During Choice Task (Eq. 2)	Lever presses per minute	$a + b \sim$ sex*group*SS delay	$a + b \sim 1$|rat

Note: a represents the mean of the distribution (peak time or accuracy), *p* represents the standard deviation of the distribution (peak spread or precision), and *m* represents the maximum rate of responding at peak time according to Equation 1. *a* represents the slope of response rate and *b* represents the initial level of responding at the beginning of the intervention trials according to Equation 2.

Table 2.3. Impulsive choice analyses examined choices (0 = SS, 1 = LL) in the impulsive choice task. All four conditions (FI-Exp, FT-Exp, FI-Con, FT-Con) were included in the analyses and were coded according to group (Exp vs. Con), schedule (FI vs. FT), and sex (female vs. male).

	Dependent Variable(s)	Fixed Effects	Random Effects
Free-Choice Trials	0 (SS) or 1 (LL)	sex*schedule*group*SS delay	1 + SS delay|rat
Sign-Tracking During SS Forced-Choice Trials	Lever presses per minute	sex*schedule*group*seconds	1|rat
Sign-Tracking During LL Forced-Choice Trials	Lever presses per minute	sex*schedule*group*seconds	1|rat

Note: The fixed effect, seconds, refers to time into trial.

Table 2.4. Neurobiological analyses examined c-Fos in DMS, DCS, DLS, PL, and IL. All four conditions (FI-Exp, FT-Exp, FI-Con, FT-Con) were included in the analyses and were coded according to group (Exp vs. Con) schedule (FI vs. FT), and sex (female vs. male).

	Dependent Variable	Predictors
Neural Activity (c-Fos)		
Generalized linear model	c-Fos in DMS	sex*schedule*group
Generalized linear model	c-Fos in DCS	sex*schedule*group
Generalized linear model	c-Fos in DLS	sex*schedule*group
Generalized linear model	c-Fos in PL	sex*schedule*group
Generalized linear model	c-Fos in IL	sex*schedule*group

Note: DMS = Dorsomedial Striatum; DCS = Dorsocentral Striatum; DLS = Dorsolateral Striatum; PL = Prelimbic Cortex; IL = Infralimbic Cortex.

Table 2.5. Exploratory cluster analyses with specified dimensions per analysis. Analyses were conducted using the k-means clustering method and the hierarchical clustering method.

	Dimensions
Timing in DMS	Average proportion (LL choice), slope of choice function, response rate during the final 3 s of the 30-s LL peak from choice, c-Fos+ cells
Timing in DCS	Average proportion (LL choice), slope of choice function, response rate during the final 3 s of the 30-s LL peak from choice, c-Fos+ cells
Timing in DLS	Average proportion (LL choice), slope of choice function, response rate during the final 3 s of the 30-s LL peak from choice, c-Fos+ cells
Timing in PL	Average proportion (LL choice), slope of choice function, response rate during the final 3 s of the 30-s LL peak from choice, c-Fos+ cells
Timing in IL	Average proportion (LL choice), slope of choice function, response rate during the final 3 s of the 30-s LL peak from choice, c-Fos+ cells
Sign- and Goal-Tracking in DMS	Lever press response rate during the final 3 s of the 10- and 30-s intervention delays, head entry response rate during the final 3 s of the 10- and 30-s intervention delays, c-Fos+ cells
Sign- and Goal-Tracking in DCS	Lever press response rate during the final 3 s of the 10- and 30-s intervention delays, head entry response rate during the final 3 s of the 10- and 30-s intervention delays, c-Fos+ cells
Sign- and Goal-Tracking in DLS	Lever press response rate during the final 3 s of the 10- and 30-s intervention delays, head entry response rate during the final 3 s of the 10- and 30-s intervention delays, c-Fos+ cells
Sign- and Goal-Tracking in PL	Lever press response rate during the final 3 s of the 10- and 30-s intervention delays, head entry response rate during the final 3 s of the 10- and 30-s intervention delays, c-Fos+ cells
Sign- and Goal-Tracking in IL	Lever press response rate during the final 3 s of the 10- and 30-s intervention delays, head entry response rate during the final 3 s of the 10- and 30-s intervention delays, c-Fos+ cells

Note: DMS = Dorsomedial Striatum; DCS = Dorsocentral Striatum; DLS = Dorsolateral Striatum; PL = Prelimbic Cortex; IL = Infralimbic Cortex.

Chapter 3 - Intervention Results and Discussion

10-s Trials

Because some animals did not complete all intervention trials every day, a generalized linear model was conducted to examine the number of 10-s intervention trials completed across schedule and sex (data not shown). This analysis did not include rats in the control conditions because FI-Con and FT-Con groups did not receive any intervention trials. There was a significant main effect of sex on the number of 10-s intervention trials completed (b = -0.054, t = -4.98, $p < .001$) such that males completed more trials than females. There were no effects of schedule on the number of trials completed. On average, males completed 93 trials (SE = 1.65) while females completed 83 trials (SE = 1.89) of 100 total possible trials per session. Altogether, males completed more 10-s intervention trials than females across both schedules.

Head Entries

On the 10-s FI and FT intervention trials, rats did not differ in their rate of head entries per minute across schedule or sex. There was a significant main effect of time in trial (b = -0.01, t = -2.74, p = .01) such that head entries decreased as time into the trial increased (Figure 3.1). This was an unexpected finding because we anticipated that head entries would increase over time in the FT conditions, if this schedule biased rats towards goal-tracking like behavior. It is important to note that rats did not enter the food cup very often during 10-s intervention trials (Table 3.1). For both FI-Exp and FT-Exp rats, 35-46% of the trials contained zero responses during the delay periods, indicating that few head entries were made during 10-s intervention trials. Altogether, rats entered the food cup less as time into the 10-s intervention trials progressed regardless of schedule, which was not consistent with our original hypotheses (see

Figure 2.3) positing that the FT schedule would result in significantly higher rates of goal-tracking like behavior compared to the FI schedule.

Lever Presses

Examination of lever presses during 10-s intervention trials disclosed significant differences between intervention schedules. There was a significant Time in Trial × Schedule interaction ($b = 0.03$, $t = 8.87$, $p < .001$) such that rats that received the FI intervention increased lever pressing as time into the trial increased while rats that received the FT intervention decreased lever pressing as time into the trial increased (Figure 3.2). Unlike head entries, rats frequently interacted with the levers regardless of condition. Less than 6% of trials contained zero responses during the delays for each condition, suggesting that rats spent most of their time interacting with the levers during 10-s intervention trials (Table 3.1). Overall, the FI intervention resulted in increased sign-tracking like behavior compared to the FT intervention condition, which was consistent with our original hypothesis. However, there were no differences in lever pressing between males and females, which is inconsistent with our hypothesis suggesting that females would likely display more sign-tracking like behavior than males.

30-s Trials

A generalized linear model was conducted to examine the number of 30-s intervention trials completed across schedule and sex (data not shown). Like the 10-s intervention trials, there was a significant main effect of sex on number of 30-s intervention trials completed ($b = -0.042$, $t = -4.04$, $p < .001$) such that males completed more trials than females. There was no effect of schedule on the number of trials completed. On average, males completed 50 trials (SE = 0.38) while females completed 46 trials (SE = 0.90) of 50 total possible trials per session. In sum, males completed more 30-s intervention trials than females regardless of schedule.

Head Entries

Unlike the analysis of head entries on the 10-s trials, rats differed in their head entries based on schedule and sex on 30-s trials. There were significant Time in Trial × Schedule (b = -0.001, t = -2.95, p = .003) and Time in Trial × Sex (b = -0.001, t = -3.67, p < .001) interactions. In addition, there was a significant Time in Trial × Sex × Schedule interaction (b = 0.001, t = 2.37, p = .02) such that female rats decreased head entries as time into the trial increased across both FI and FT interventions while male FT rats maintained head entries, but male FI rats decreased head entries across 30-s trials (Figure 3.3). Altogether, male rats in the FT intervention condition were stable in head entries across time compared to other conditions. Like the 10-s intervention trials, rats did not enter the food cup often during 30-s intervention trials (Table 3.2).

Lever Presses

Like the 10-s intervention trials, there was a significant Time in Trial × Schedule interaction (b = 0.005, t = 15.04, p < .001) such that rats that received the FI intervention increased lever pressing as time into the trial increased while rats that received the FT intervention decreased lever pressing as time into the trial increased. In addition, there was a significant Time in Trial × Sex interaction (b = -0.002, t = -5.53, p < .001) such that males showed a steeper slope in lever press rates as time into trial increased compared to females (Figure 3.4). This effect appeared to be driven by the males in the FI condition, but the three-way interaction between Sex, Schedule, and Time in Trial was not significant. Overall, the FI intervention increased sign-tracking like behavior compared to the FT intervention condition, which was consistent with our original hypothesis. However, males showed a steeper slope in lever press rates compared to females as time into the 30-s trials increased, which was

inconsistent with our hypothesis suggesting that females would likely display more sign-tracking like behavior than males. When examining lever press behavior, few trials contained zero responses across groups. Altogether, rats in both FI and FT groups interacted with the levers during 30-s intervention trials and their behavior was sensitive to the schedule received.

Discussion

Analyses of lever pressing during the intervention trials showed that the FI schedule promoted lever press rates as a function of time in trial compared to the FT schedule. This suggests that the FI schedule encouraged sign-tracking like behavior in males and females. Particularly on the 30-s intervention trials, males showed increased lever press rates compared to females across schedules. In addition, the FT schedule did not result in exclusive interaction with the food cup. Female and male rats in the FT-Exp condition spent significant time interacting with the levers. This was unexpected based on the differences in response requirements across schedules. Previous research using the FT schedule as a time-based intervention could not measure lever pressing behavior because levers were retracted after initial selection (Rung et al., 2018; Smith et al., under review). The current study suggests that the FT schedule did not bias the rats towards interacting with the food cup when the levers remained available in the chamber.

Instead, the presence of the lever may have resulted in nonselective sign-tracking like behavior. Given that the FT-Exp rats spent time interacting with the levers, they may have learned a simple association between the lever and the food delivery. Previous research has shown that rats acquired lever pressing behavior with reinforcement delayed up to 30 s (Byrne et al., 1997; Critchfield & Lattal, 1993; Escobar & Bruner, 2007; Lattal & Gleeson, 1990; LeSage et al., 1996; Sutphin et al., 1998; van Haaren, 1992; Wilkenfield et al., 1992). Typically, responding occurred more often when the delays were shorter and when the rate of reinforcement

was higher compared to long delays and lower rates of reinforcement (Sutphin et al., 1998). This pattern of lever pressing behavior during FT schedules occurred on the lever associated with food even when presses reset the delay, although responding decreased as the length of delay reset increased (Wilkenfield et al., 1992). Furthermore, lever pressing behavior appeared to be specific to the lever that was associated with food as rats pressed significantly less on a second lever with no programmed responses when both were available (Sutphin et al., 1998) and on six other levers with no programmed responses when all seven levers were available (Escobar & Bruner, 2007). Taken together with previous literature, the FT-Exp rats may have learned an association between the lever and food delivery, resulting in acquisition of lever pressing behavior despite delays to reinforcement.

However, FT-Exp rats did not increase their lever pressing as the intervention delay approached like FI-Exp rats, which may suggest that FT-Exp rats did not learn to anticipate the time of food delivery. Previous research examining the acquisition of lever pressing behavior with delayed reinforcement showed results in terms of cumulative responses in a session, responses rates as a function of session, or response rates as a function of dose of a pharmacological agent instead of responses as a function of time into each delay, so it is unclear whether rats in previous studies learned to anticipate the time of food delivery. In the current study, rats in the FT condition pressed the levers most at the beginning of the intervention trials and decreased in responding as time into the trials progressed. It is possible that the FT-Exp rats associated lever insertion specifically with food delivery, which may be why the rats pressed most at the beginning of trials when the lever was inserted. Lever pressing did not increase as food delivery approached and they pressed minimally immediately prior to food delivery, suggesting that they do not associate the act of lever pressing with food. Altogether, lever

insertion may be associated with food delivery, so rats in the FT condition press the lever at the beginning of trials immediately following insertion.

Analyses of head entries during the 10-s intervention trials showed no differences between schedule or sex. On the 30-s intervention trials, male FT rats displayed a relatively flat slope in head entry rates while other conditions' slopes were negative. However, across delays, head entries did not dramatically increase in anticipation of food reward. Instead, head entries mostly decreased as a function of time into the intervention trials. It is important to note that rats spent little time in the food cup across schedules. This suggests that the availability of the lever during the intervention trials was a salient feature in the operant chambers, which may have decreased goal-tracking like behavior. Particularly in the FT condition, rats still interacted with the levers often, so they may have learned an association between the lever and food delivery, resulting in lever pressing behavior even though there were delays to reinforcement (Byrne et al., 1997; Critchfield & Lattal, 1993; Escobar & Bruner, 2007; Lattal & Gleeson, 1990; LeSage et al., 1996; Sutphin et al., 1998; van Haaren, 1992; Wilkenfield et al., 1992). Overall, whether the lever remains inserted in the chamber likely affects the expression of sign- and goal-tracking behaviors during the intervention. This possibility should be explicitly tested in future studies.

Across intervention delays, males completed more intervention trials than females did regardless of FI or FT schedules. Both sexes completed the majority of trials available, but males may have been more motivated to earn reinforcers. Females could have reached satiation before males during intervention sessions. In the current study, rats were food restricted to 87% of their free-feeding weights based on growth curves obtained from the supplier. Food restriction at or below 85% may interact with dopaminergic functioning (Cabib & Bonaventura, 1997; Costall et al., 1980), which is often associated with reward processing (Cardinal, 2006). Rats may rely on

reward processing information during training and testing, so rats were maintained above 85% to minimize this potential confound. However, mild food restriction at or greater than 90% may reduce rats' motivation to complete behavioral training, so the target weight was 87%. Given the differences in number of trials completed, female and male rats may be differentially sensitive to food restriction levels. Females may need slightly longer sessions to account for the effects of body weight on motivation and satiation.

Altogether, analyses of lever pressing and head entries during the intervention revealed key differences between schedules and sexes. Rats in the FI-Exp condition increased lever pressing behavior in anticipation of food rewards and spent little time in the food cup. Rats in the FT-Exp condition did not increase head entries in anticipation of food rewards and spent a significant amount of time interacting with the levers during the intervention despite no explicit response requirement to collect the reinforcer. Across schedules, males completed more intervention trials than females as well. These differences may directly impact intervention efficacy in timing of delays and promotion of larger-later choices.

Figure 3.1. Normalized (proportion of maximum rate) head entries per minute during 10-s intervention trials for rats in the experimental group. Regardless of schedule (fixed-interval, FI, or fixed-time, FT), rats entered the food cup less as time into the 10-s intervention trials progressed. Markers represent mean responses and lines represent repeated measures multi-level linear regression fits to the data.

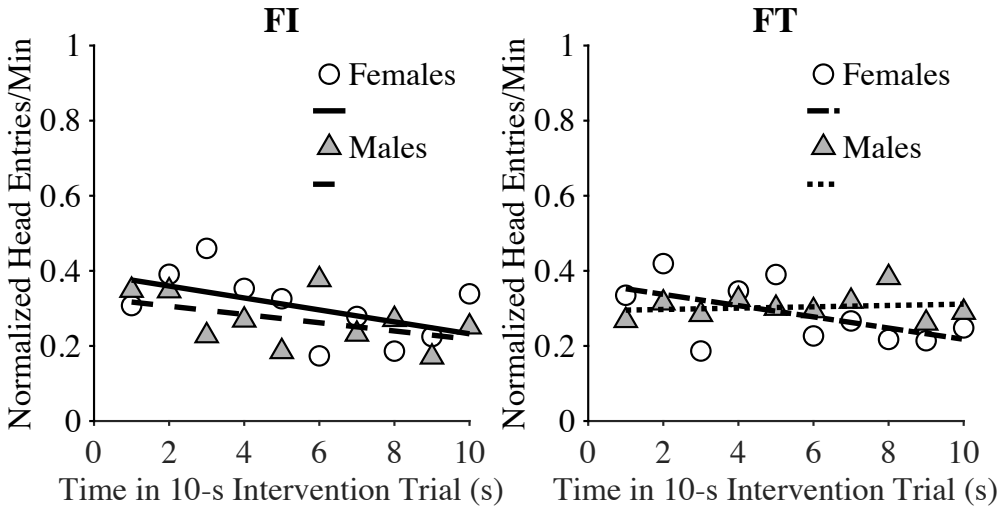


Figure 3.2. Normalized (proportion of maximum rate) lever presses per minute during 10-s intervention trials for rats in the experimental group. Rats assigned to the FI (fixed-interval) schedule increased lever pressing as time into the 10-s intervention trials progressed while rats assigned to the FT (fixed-time) schedule decreased lever pressing as time into the trials increased. Markers represent mean responses and lines represent repeated measures multi-level linear regression fits to the data.

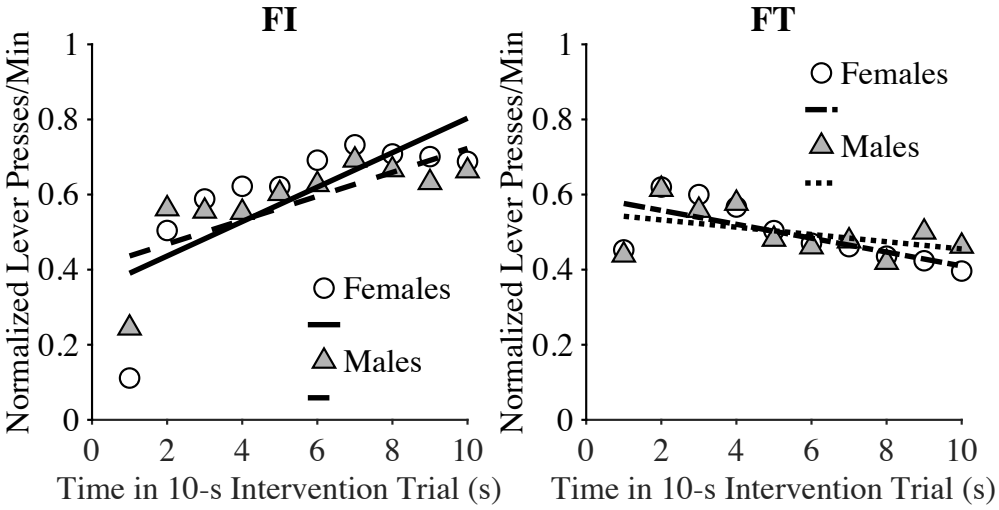


Figure 3.3. Normalized (proportion of maximum rate) head entries per minute during 30-s intervention trials for rats in the experimental group. Female rats decreased head entries as time into the trial increased across both FI (fixed-interval) and FT (fixed-time) schedules. Male FT rats maintained head entries, but male FI rats decreased head entries across 30-s trials. Markers represent mean responses and lines represent repeated measures multi-level linear regression fits to the data.

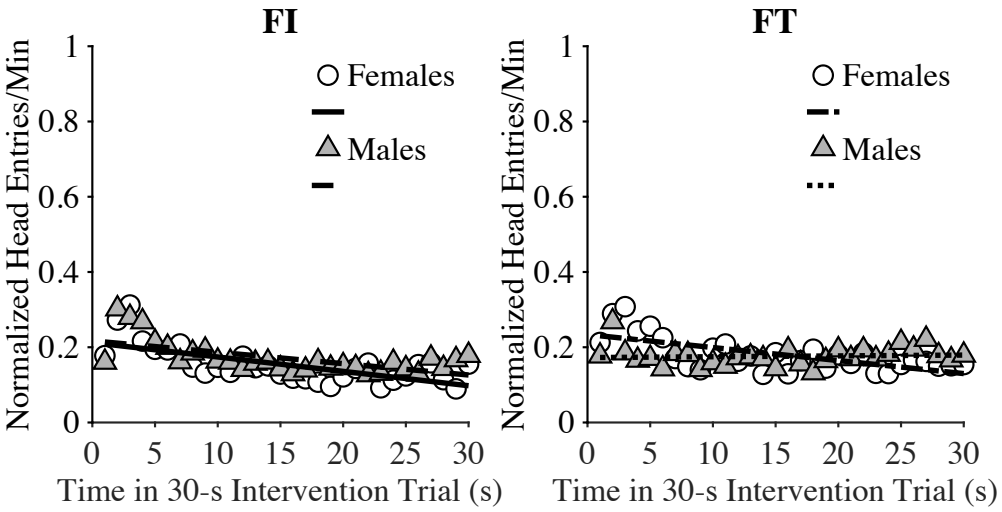


Figure 3.4. Normalized (proportion of maximum rate) lever presses per minute during 30-s intervention trials for rats in the experimental group. Rats that received the FI (fixed-interval) intervention increased lever pressing as time into the trial increased while rats that received the FT (fixed-time) intervention decreased lever pressing as time into the trial increased. Markers represent mean responses and lines represent repeated measures multi-level linear regression fits to the data.

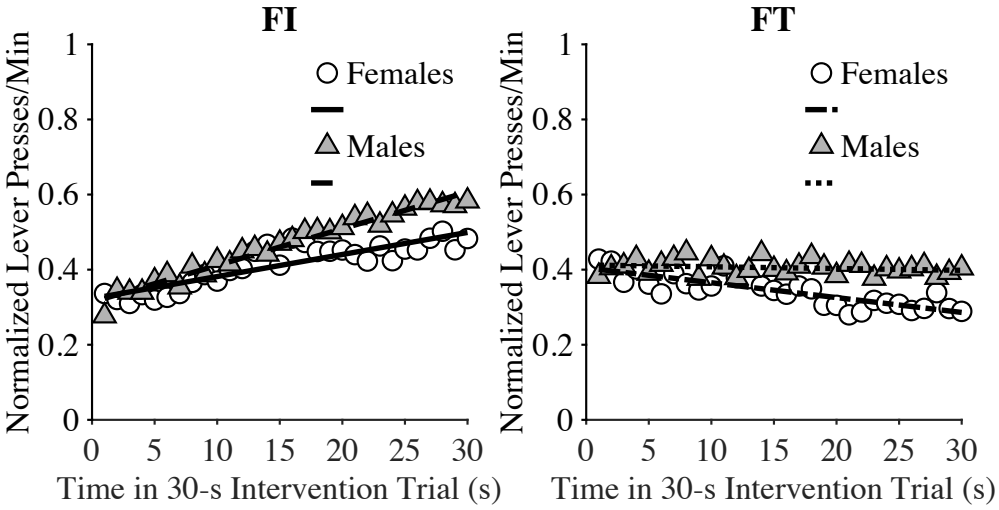


Table 3.1. Percentage of 10-s intervention trials that contained zero lever presses or zero head entry responses during delay periods, indicating no additional lever presses or head entries were made during the trial. The control groups (FI-Con and FT-Con) were not included in this analysis. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental.

Group	Sex	Lever Presses	Head Entries
FI-Exp	Female	5.0%	44.6%
	Male	5.0%	45.8%
FT-Exp	Female	5.0%	37.1%
	Male	5.8%	35.0%

Table 3.2. Percentage of 30-s intervention trials that contained zero lever presses or zero head entry responses during the delay periods, indicating no additional lever presses or head entries were made during the trial. The control groups (FI-Con and FT-Con) were not included in this analysis. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental.

Group	Sex	Lever Presses	Head Entries
FI-Exp	Female	1.2%	48.4%
	Male	0.9%	47.7%
FT-Exp	Female	6.4%	41.7%
	Male	1.4%	42.7%

Chapter 4 - Impulsive Choice Results and Discussion

Peak Timing

Peak trials were delivered during the impulsive choice task on both SS and LL levers to assess timing of the intervals, and lever presses and head entries were recorded for the duration of the trials. These trials were similar to forced-choice trials except no food rewards were delivered and the trials lasted 90 s.

Head Entries

Head entries made during SS and LL peak trials were not analyzed because few entries were made across schedules, groups, and sexes (Tables 4.1 and 4.2).

Lever Presses

When examining lever press behavior on SS and LL peak trials, the FI schedule encouraged more frequent lever pressing behavior. Rats in the FT conditions interacted with the levers less compared to the rats that received the FI schedule (Tables 4.1 and 4.2). Lever pressing response distributions differed based on the schedule received.

Fixed-Interval Schedule

Rats in the FI conditions produced peak-shaped functions with increased responding as the target delay approached, maximum responding near the target delays, and decreased responding after the target delays passed. The model fits to the SS and LL peak functions are shown in Figures 4.1 and 4.2. Multi-level analysis of SS peak trial behavior for the FI schedule revealed significant differences in SS peak time based on experimental group, sex, and SS delay. There was a Group × Sex × SS Delay interaction ($b = 0.12$, $t = 5.00$, $p < .001$) such that female FI-Exp rats' SS peak time increased as SS delay increased at a steeper rate than female FI-Con rats ($b = 0.38$, $t = 4.65$, $p < .001$), male FI-Exp rats ($b = 0.41$, $t = 6.27$, $p < .001$), and male FI-

Con rats (b = 0.29, t = 4.18, p < .001). In other words, female FI-Exp rats' SS peak times shifted more dramatically as SS delay increased compared to all other conditions (Figure 4.3).

There was also a significant Group × Sex × SS Delay interaction (b = 0.10, t = 4.09, p < .001) on SS peak spread. Female FI-Con rats' peak spread decreased as SS delay increased while female FI-Exp rats (b = 0.55, t = 6.74, p < .001), male FI-Exp rats (b = -0.76, t = -10.46, p < .001), and male FI-Con rats (b = -0.62, t = -8.15, p < .001) showed increases in SS peak spread as SS delay increased. To summarize, female FI-Con rats became more precise in SS peak timing with more experience in the task while the other groups showed decreases in precision as SS delay increased (Figure 4.3). In addition, female FI-Exp rats showed smaller increases in SS peak spread as SS delay increased compared to male FI-Exp rats (b = -0.21, t = -3.11, p = .01). In other words, male FI-Exp rats were increasingly less precise in timing the SS delays as the delay increased in the impulsive choice task compared to female FI-Exp rats. However, it is important to note that female FI-Exp rats had larger peak spreads compared to male FI-Exp rats at all SS delays. While the female FI-Exp rats' peak spreads did not increase as dramatically with each new SS delay, they were still more imprecise in timing than male FI-Exp rats.

Multi-level analysis of LL peak trial behavior for the FI schedule revealed no significant differences in LL peak time based on group or sex. There was a significant main effect of SS delay on LL peak time (b = -0.18, t = -5.68, p < .001) such that LL peak times shifted earlier as SS delay increased (Figure 4.4). Across group and sex, rats' LL peak times suggest that they overestimated the 30-s LL delay during earlier sessions of the choice task and then began to underestimate the delay during the final sessions.

There was a significant Group × Sex × SS Delay interaction (b = 0.18, t = 4.01, p < .001) on LL peak spread. Female FI-Con rats' LL peak spread decreased at a steeper rate as SS delay

increased compared to female FI-Exp rats (b = 1.11, t = 7.41, p < .001), male FI-Exp rats (b = -1.43, t = -10.52, p < .001), and male FI-Con rats (b = -1.04, t = -7.05, p < .001). While all conditions showed decreases in LL peak spread with more experience in the choice task, female FI-Con rats showed the largest decreases as SS delay increased (Figure 4.4). In addition, male FI-Exp rats showed significantly smaller decreases in LL peak spread compared to female FI-Exp rats (b = -0.32, t = -3.21, p = .01) and male FI-Con rats (b = 0.39, t = 3.98, p < .001).

The model fits to the SS and LL peak functions were used to generate the parameters shown in Figures 4.3 and 4.4 (see Appendix Figures A.2 and A.3 for alternative views of SS and LL peak timing parameters). Parameter values of SS and LL peak functions are shown in Tables A.1 and A.2 in Appendix A as well.

In sum, female FI-Exp rats showed increased sensitivity to SS delay compared to other conditions during SS FI peak trials based on peak times. Across SS and LL FI peak trials, female FI-Con rats showed the greatest decreases in peak spread as SS delay increased, suggesting significant improvements in temporal precision over time. All other conditions showed increases in peak spread as SS delay increased. Also, across SS and LL peak trials, female FI-Exp rats became more precise in timing SS and LL delays as SS delay increased compared to male FI-Exp rats, although FI-Exp female rats did not show improvements to the same degree as FI-Con female rats. To simplify comparisons of timing across groups and delays, coefficient of variation (CV) values were calculated and are displayed in Figure 4.5 (and see Appendix for Table A.3). CV values were calculated by dividing peak spread by peak time, and lower CV values suggest reduced relative timing errors. Across groups, CV values decreased as delay increased, suggesting that rats made fewer timing errors with more experience in the task. Overall, the current results suggest that female FI-Exp rats made greater improvements in temporal precision

compared to male FI-Exp rats. However, we did not expect significant improvements in interval timing ability of female FI-Con rats. While female FI-Con rats showed the largest improvements in timing across the impulsive choice task, these rats made the most timing errors in earlier sessions.

Fixed-Time Schedule

Rats in the FT conditions did not produce peak-shaped functions like rats in the FI conditions. Instead, maximum responding occurred at the beginning of peak trials and responding decayed as time into peak trials progressed. The model fits to the SS and LL functions are shown in Figures 4.6 and 4.7. Multi-level analysis of SS peak trial behavior for the FT schedule revealed significant differences in initial lever press rates (intercept) at the beginning of SS peak trials. There was a significant Group × SS Delay interaction ($b = 0.002$, $t = 2.48$, $p = .01$) such that rats in the control group decreased initial lever pressing more so as SS delay increased (Figure 4.8). Rats in the experimental group also decreased initial lever pressing as SS delay increased but not as much as the control group. There was a significant Sex × SS Delay interaction ($b = 0.003$, $t = 3.26$, $p = .001$) such that male rats overall decreased more in initial lever pressing as SS delay increased compared to females. Females also decreased in initial lever pressing as SS delay increased but not as dramatically as males.

In addition, there was a significant Group × Sex × SS Delay interaction ($b = -0.0003$, $t = -3.55$, $p < .001$) on the rate of decay in lever pressing as time into peak trials progressed. Female FT-Con rats differed significantly compared to female FT-Exp rats ($b = -0.0013$, $t = -5.52$, $p < .001$), male FT-Con rats ($b = 0.0017$, $t = 6.41$, $p < .001$), and male FT-Exp rats ($b = 0.0017$, $t = 6.71$, $p < .001$). Lever pressing increased as SS delay increased for female FT-Con

rats while lever press response rates decreased as SS delay increased in the other conditions (Figure 4.8).

Multi-level analysis of LL peak trial behavior for the FT schedule revealed significant differences in initial lever pressing at the beginning of LL peak trials (intercept). There was a significant main effect of Sex (b = -0.06, t = -2.86, $p < .01$) such that female rats lever pressed less initially compared to males (Figure 4.9). There was also a significant Group × SS Delay interaction (b = 0.002, t = 2.61, $p < .01$) such that rats in the control condition across sex decreased in initial lever pressing as SS delay increased. Rats in the experimental condition also decreased initial lever pressing with more task experience but not as dramatically as the control condition.

In addition, there was a significant Sex × SS Delay interaction (b = -0.0002, t = -2.63, $p < .01$) on response rates (rate of decay). Lever press response rates stayed relatively stable as SS delay increased resulting in a shallow positive slope for female rats while lever press response rates increased as SS delay increased resulting in a steeper positive slope for males (Figure 4.9).

The model fits to the SS and LL functions were used to generate the parameters shown in Figures 4.8 and 4.9 (see Appendix Figures A.4 and A.5 for alternative views of SS and LL peak function parameters). Parameter values of SS and LL peak functions are shown in Appendix Tables A.4 and A.5.

In short, rats in the FT-Con group decreased in initial responding as SS delay increased on SS and LL peak trials. In addition, males often displayed steeper slopes compared to females, indicating that males decreased responding with time into the peak trials while response rates stayed relatively stable as SS delay increased for female rats. Altogether, differences in lever press response rates during peak trials were unexpected in the FT schedule because we

anticipated greater head entry responses than lever press responses. More specifically, we hypothesized that rats that received the FT schedule would increase in head entries leading up to the target intervals and decrease after the target intervals. However, few head entries were made during peak trials, and lever press responses occurred most often at the beginning of peak trials and tapered off as the trials progressed, indicating an absence of anticipation of the time of food delivery in the FT schedule.

LL Choices on Free-Choice Trials

An analysis of LL choices was conducted on free-choice trials. Rats chose between SS and LL options where the SS delivered one food pellet and the LL option delivered two food pellets. The SS delay increased from 10 to 25 s across sessions while the LL delay was held constant at 30 s. There was a significant main effect of SS delay ($b = 0.17$, $t = 13.02$, $p < .001$) such that all rats made more LL choices as SS delay increased (Figure 4.10). While no other main effects or interactions were significant, the Group × SS Delay interaction approached statistical significance. This interaction was examined at hypothetical 0- and 30-s time points with additional contrast tests. Experimental and control groups were not significantly different when the SS delay was 30 s. At 0 s, the experimental groups ($b = -1.86$) made more LL choices than the control groups ($b = -3.24$; $t = 1.98$, $p = .048$). Altogether, rats in the experimental groups were more self-controlled when the SS delay was tested at 0 s. These results were inconsistent with our original hypotheses suggesting that the FI intervention would be most effective at promoting self-controlled choices in females and the FT intervention would be most effective in males.

Responding During Forced-Choice Trials

During forced-choice trials, only one lever was presented, and rats received reinforcement at the end of the trial.

Head Entries

Like the intervention and peak trials, rats made few head entries during the forced-choice trials delivered during the impulsive choice task (Tables 4.3 and 4.4). This pattern was more pronounced in FI conditions than FT conditions, but rats generally spent little time interacting with the food cup. No analyses were conducted on head entries made during SS and LL forced-choice trials.

Lever Presses

Multi-level analysis of lever pressing during SS forced-choice trials revealed a Group × Schedule × Sex × Time in Trial interaction (b = -0.001, t = -4.66, p < .001), indicating that the slope of the responding as time into forced-choice trials progressed, differed between conditions (Figure 4.11). Further pairwise comparisons showed that female FI-Exp rats had a positive slope while female FT-Exp, female FT-Con, male FT-Exp, and male FT-Con rats had a negative slope (bs $\geq$ 0.02, ts $\geq$ 19.82, ps < .001; see Table 4.5 for specific comparison values). Female FI-Exp rats did not differ from female FI-Con, male FI-Exp, or male FI-Con conditions. A similar pattern occurred where female FI-Con rats differed from female FT-Exp, female FT-Con, male FT-Exp, and male FT-Con rats (bs $\geq$ 0.02, ts $\geq$ 20.36, ps < .001) but not female FI-Exp, male FI-Exp, or male FI-Con conditions. This occurred in male FI rats as well such that male FI-Exp rats differed from female FT-Exp, female FT-Con, male FT-Exp, and male FT-Con rats (bs $\geq$ 0.02, ts $\geq$ 22.21, ps < .001) but not female FI-Exp, female FI-Con, or male FI-Con conditions, and male FI-Con rats differed from female FT-Exp, female FT-Con, male FT-Exp, and male FT-Con rats

($bs \geq 0.02$, $ts \geq 19.35$, $ps < .001$). Altogether, rats that received the FI schedule (female FI-Exp, female FI-Con, male FI-Exp, and male FI-Con) had a significantly more positive slope than rats that received the FT schedule (female FT-Exp, female FT-Con, male FT-Exp, and male FT-Con), but the FI schedule conditions were not significantly different from each other. All FI schedule conditions increased lever press response rates as time into the SS forced-choice trials progressed.

There were differences in response rates between males and females within the FT schedule conditions on SS forced-choice trials (Figure 4.11). Female FT-Exp rats had a shallower negative slope than female FT-Con and male FT-Con rats ($bs \geq -0.003$, $ts \geq -3.28$, $ps < .02$; see Table 4.5 for specific comparison values) but not male FT-Exp rats. Female FT-Con rats were different from both male FT-Exp and male FT-Con rats ($bs \geq -0.007$, $ts \geq -7.13$, $ps < .001$) such that female FT-Con rats had a steeper negative slope than the male FT conditions. Male FT-Exp and male FT-Con rats did not differ. Overall, females and males that received the FT schedule showed negative slopes, indicating that lever press response rates decreased as time into the SS forced-choice trials progressed. In most cases, females in the FT conditions showed steeper negative slopes than the males in the FT conditions.

Like SS forced-choice trials, multi-level analysis of LL forced-choice trial behavior revealed significant differences in lever presses based on experimental group, sex, and schedule (Figure 4.12). There was a Group × Schedule × Sex × Time in Trial interaction ($b = -0.001$, $t = -8.00$, $p < .001$), meaning response rates (slope) differed based on condition. Further pairwise comparisons showed that female FI-Exp rats had a positive slope of their response rate function while female FT-Exp, female FT-Con, male FT-Exp, and male FT-Con rats had negative slopes ($bs \geq 0.01$, $ts \geq 22.10$, $ps < .001$; see Table 4.6 for specific comparison values). Female FI-Exp

rats' slope was steeper than the male FI-Con (b = 0.002, t = 3.37, p = .02) rats. However, female FI-Exp rats did not differ from female FI-Con or male FI-Exp rats. Female FI-Con rats did not differ from male FI-Con rats but showed a significantly shallower slope compared to male FI-Exp (b = -0.002, t = -3.83, p < .01) rats. Female FI-Con rats had a positive response rate slope while female FT-Exp, female FT-Con, male FT-Exp, and male FT-Con rats showed negative response rate slopes (bs $\geq$ 0.01, ts $\geq$ 19.40, ps < .001). Male FI-Exp rats' slope was steeper than male FI-Con (b = 0.003, t = 4.50, p < .01) rats. Male FI-Exp rats also differed from male FT-Exp, male FT-Con, female FT-Exp, and female FT-Con rats (bs $\geq$ -0.01, ts $\geq$ -26.73, ps < .001). Along the same lines, male FI-Con rats also differed from female FT-Exp, female FT-Con, male FT-Exp, and male FT-Con rats (bs $\geq$ -0.01, ts $\geq$ -25.72, ps < .001). In both cases, male FI-Exp and FI-Con rats had a positive response rate slope while the FT conditions had a negative response rate slope. In sum, rats that received the FI schedule (female FI-Exp, female FI-Con, male FI-Exp, and male FI-Con) displayed positive response rate slopes while the FT schedule (female FT-Exp, female FT-Con, male FT-Exp, and male FT-Con) conditions showed negative response rate slopes.

Similar to the SS forced-choice trials, there were differences between males and females within the FT schedules on LL forced-choice trials (Figure 4.12 and Table 4.6). Female FT-Exp rats had a shallower negative slope than female FT-Con and male FT-Exp rats (bs $\geq$ 0.002, ts $\geq$ 3.50, ps < .05). Female FT-Con rats had a shallower negative slope than male FT-Exp (b = 0.002, t = 3.71, p < .01) rats. Male FT-Con rats had a relatively flat slope while male FT-Exp, female FT-Exp, and female FI-Con rats had a negative slope (bs $\geq$ -0.002, ts $\geq$ -3.50, ps < .05). Overall, rats in the FT condition decreased in their response rates as time into LL forced-choice trials increased except for the male FT-Con rats who showed stable response rates with time.

Discussion

The impulsive choice task delivered a mixture of SS and LL peak trials, free-choice trials, and SS and LL forced-choice trials, offering a multitude of data to compare groups, sexes, and schedules. Head entry response rates were insufficient for analysis, but lever pressing was analyzed on SS and LL peak trials and forced-choice trials to assess timing behavior.

On peak trials in the FI condition, female rats demonstrated significant improvements in temporal precision as measured by lever press response rates. In particular, female FI-Con rats' peak spreads decreased dramatically as SS delay increased on SS and LL peak trials, suggesting that experience with the FI choice task improved precision in timing. All other groups' SS peak spreads increased as SS delay increased, which was expected based on the scalar variance property of scalar expectancy theory. Scalar variance suggests that the spread or precision, measured by the variance in responding around a target interval, should increase as the target duration increases (Gibbon, 1977). It is possible that female FI-Con rats' peak spreads did not follow the scalar variance property because their peak spreads were larger than the other conditions at the beginning of the task, indicating uncertainty in the intervals. Both female FI-Con and FI-Exp groups' CV values decreased as SS delay increased, suggesting reduced timing errors with more task experience. More experience with the task may have decreased the uncertainty in timing the delays. It is unclear why the female FI-Con group made more timing errors at first, particularly compared to male FI-Con rats. Female FI-Con CV values decreased at a faster rate than the male FI-Con group. In fact, the female FI-Con rats were the most precise in timing the final SS and LL delays and had the lowest CV values at those delays, indicating fewer timing errors than the other groups. It is possible that the intervention helped the FI-Exp females time more precisely than FI-Con females early in the choice task. Because the FI-Con females

did not receive the intervention, this group showed substantial improvements in and as a result of the choice task. Altogether, these results suggest that the female FI-Con group learned to time at a comparable level to other FI conditions despite making the greatest number of timing errors in earlier sessions of the impulsive choice task.

Along the same lines, female FI-Exp rats showed the greatest sensitivity to delay in terms of accuracy on SS peak trials. However, this delay sensitivity in SS delay timing accuracy did not also occur in terms of timing precision. Female FI-Exp rats had larger peak spreads than male FI-Exp rats on both SS and LL peak trials. Female FI-Exp rats showed smaller increases in peak spread as SS delay increased in SS and LL peak timing compared to male FI-Exp rats, but male FI-Exp rats' peak spreads were still consistently smaller. Altogether, we expected that female FI-Exp rats may show enhanced interval timing ability compared to other conditions, leading to the greatest improvements in self-control. While the female FI-Exp rats did show improvements in temporal processing, they were not significantly more self-controlled than other groups. Female FI-Con rats also showed improvements in temporal processing, but these improvements did not translate into increased self-control compared to other groups as well. Overall, differences in lever press response rates on peak trials within the FI conditions did not directly correspond with differences in self-control, but this remains to be formally tested with clustering analyses (see Chapter 5).

On peak trials in the FT condition, examination of lever press rates was included because rats unexpectedly interacted with the levers often. We expected rats in the FT conditions to spend more time interacting with the food cup than the levers. We quantified the percentage of trials that contained zeros for lever press and head entry behaviors during SS and LL peak delays. These percentages suggest that rats in the FT conditions interacted with both the levers and the

food cup while the rats in the FI conditions interacted with the levers markedly more so than the food cup during peak trials. However, lever press rates did not take the same shape across schedules. Instead, rats that received the FT schedule responded more so at the beginning of peak trials and decreased responding as time into peak trials increased. Both female and male FT-Con rats decreased in initial responding as SS delay increased. Across FT experimental and control conditions, males decreased responding more dramatically than females.

The shape of the lever press responses during peak interval trials suggests that rats in the FT conditions did not learn to anticipate the target intervals as well as the rats in the FI conditions did. Males and females in the FT-Exp groups also did not increase lever pressing as the target interval approached on intervention trials. It is possible that the lever acted as a distractor while timing the delays. In a previous study, head entries were normally-distributed around target intervals (i.e., a peak function) when a mixed-sex sample received peak trials during an FT impulsive choice task following an FT intervention (Smith et al., under review). It is important to note that previous research administered the FT intervention and choice task where levers retracted after the initial response whereas the current experiment delivered the FT intervention and choice task with levers that remained in the operant chambers during the delays. The presence of the lever in the FT schedule may have acted as a distractor for timing behavior. Within the timing field, research has shown that distractors, or unfamiliar stimuli, and gaps, or interruptions, presented during delays affect peak interval functions, and both distractors and gaps can stop or reset subjects' internal clocks or timing of events (Buhusi, 2012; Buhusi & Meck, 2006; Eudave-Patino et al., 2021; Orduna & Bouzas, 2011). If the lever during the FT schedule acted as a distractor then this may have reduced head entry rates that were anticipated to occur based on Smith et al. (under review). Head entries to the food cup were not analyzed on

peak trials in both FI and FT conditions because rats made few head entries compared to lever presses. Altogether, procedural differences in the delivery of the FT schedule may account for the discrepancy in predictions and results, and analyses of lever press response rates during peak trials revealed that FI and FT schedules significantly affected the shape of responding.

On forced-choice trials, FI conditions showed increased lever pressing leading up to the anticipated receipt of rewards while FT conditions decreased lever pressing as time into forced-choice trials increased, consistent with the patterns observed on peak trials. FI conditions may have encouraged sign-tracking but increases in this behavior did not correspond with significant increases in self-control. Rats in the FT conditions decreased sign-tracking like behavior over time in the forced-choice trials. Rats in the FI and FT conditions made similar levels of self-controlled choices regardless of this difference in lever press responding during forced-choice trials. Similar to peak trials, head entry rates were not analyzed on forced-choice trials, so it unclear whether goal-tracking behavior would correspond with self-control levels. Altogether, sign-tracking behavior differed between schedules and goal-tracking occurred only at low rates of responding.

While responding differed between schedules, this was not reflected in choice behavior on free-choice trials. All rats made more LL choices as SS delay increased, suggesting similar sensitivity to delay. A planned comparison at a hypothetical 0-s intercept showed that experimental groups made more LL choices than control groups but there was no effect of schedule on delay sensitivity, which was not consistent with hypotheses suggesting greater improvements for the FI schedule over FT schedule. The current study only partially replicates previous findings showing that the abbreviated FI intervention successfully promoted LL choices in male rats (Panfil et al., in preparation), but there were no differences between FI-Exp and FI-

Con groups in delay sensitivity. Likewise, the current study also only partially replicated previous findings in that there were increased LL choices at the 0-s intercept for rats that received the interventions. Extended FI and FT interventions successfully promoted LL choices in a mixed sex sample as well, but there were differences in delay sensitivity between schedules (Smith et al., under review). We anticipated no sex differences in FI intervention efficacy based on previous research where the intervention was delivered for a longer period of time (Panfil et al., 2020), and there were no differences between males and females in the current study. It is possible that the different control conditions used across experiments may explain the discrepancies in FI intervention efficacy. The current study employed a control condition where rats did not receive any stimuli in the operant chambers. In Peterson and Kirkpatrick (2016) where the same control condition was used but in a pre-/post- design where impulsive choice was measured before and after the no training phase, control rats made fewer LL choices after the no training phase on two of four delays tested and similar LL choices pre- to post- at the two other delays. Along the same lines, control tasks designed to match the intervention based on response requirement and food earnings rates may increase SS choices according to Fox (2022), although this was not the case in Bailey et al. (2018). This suggests that control task comparisons may exaggerate the strength of intervention effects. Likewise, evaluating intervention effects in a pre-/post- design where impulsive choices are measured before and after the intervention phase may result in decreased variance due to the within-subjects design. Decreased variance may improve statistical detection of differences between pre- and post-intervention measures, also resulting in exaggerated strength of intervention effects.

Perhaps a more likely explanation for the relatively weak intervention effects on free-choice trials is the length of intervention. Although we expected that six sessions would increase

self-control in both intervention conditions compared to the no training control conditions, previous research demonstrating robust intervention effects did so with at least 45 sessions of training (Bailey et al., 2018; Panfil et al., 2020; Rung et al., 2018; Smith et al., 2015; Smith et al., under review; Stuebing et al., 2018). Further training may be necessary to produce sizeable improvements in self-control, especially in comparison to the control conditions used in the current study.

Within the control groups, we expected that the FI-Con rats would be more sensitive to delay than the FT-Con rats because the FI schedule should encourage interval timing ability and/or attention. Previous work has shown that the FI contingency results in greater delay sensitivity than the FT contingency (Smith et al., under review). However, there were no differences between the FI and FT choice conditions in delay sensitivity (slope). Again, it is possible that lever availability may explain these discrepancies. Rats in the FT condition may have learned an association between the lever and food delivery despite delayed reinforcement. In this case, the lever availability in the FT schedule may have acted in a similar fashion to the FI schedule to shape delay sensitivity. Altogether, lever availability in the FT conditions may have affected their behavior in a positive manner on the choice task but negatively in terms of interval timing. This suggests that interval timing may not be essential for self-control. This was further tested in cluster analyses conducted in Chapter 5.

Given that rats in the control groups showed similar delay sensitivity and proportion LL choices to rats in the experimental groups (except at the 0-s intercept), the FI and FT impulsive choice tasks may act as interventions by themselves. Impulsive choice tasks are often designed such that the LL choice is the optimal choice to maximize food earnings in each session. In the current experiment, the choice task included SS and LL forced-choice trials in addition to peak

interval and free-choice trials. Forced-choice trials are identical to the trials provided during intervention training, so it is possible that even limited exposure to these trials during the choice task increased self-control. In the current experiment, differences in lever press responding during forced-choice trials did not align with differences in choice behavior. Taken together, these results suggest that impulsive choice tasks may improve self-control through a more general mechanism than interval timing, sign-tracking, and goal-tracking.

Altogether, differences in responding during peak trials and forced-choice trials did not necessitate differences in impulsive choice at the group level. There were no stark differences in self-control between FI and FT schedules in the experimental or control groups. Intervention efficacy may have been affected by a variety of factors including the FT schedule delivery method and the length of interventions. It remains to be seen how these factors influence neural activity and whether these variables interact at the individual subject level.

Figure 4.1. Normalized (proportion of maximum rate) lever presses per minute during SS peak trials for FI (fixed-interval) conditions. Markers represent mean responses and lines represent repeated measures multi-level nonlinear regression fits to the data. Vertical lines denote target intervals. Exp = Experimental; Con = Control.

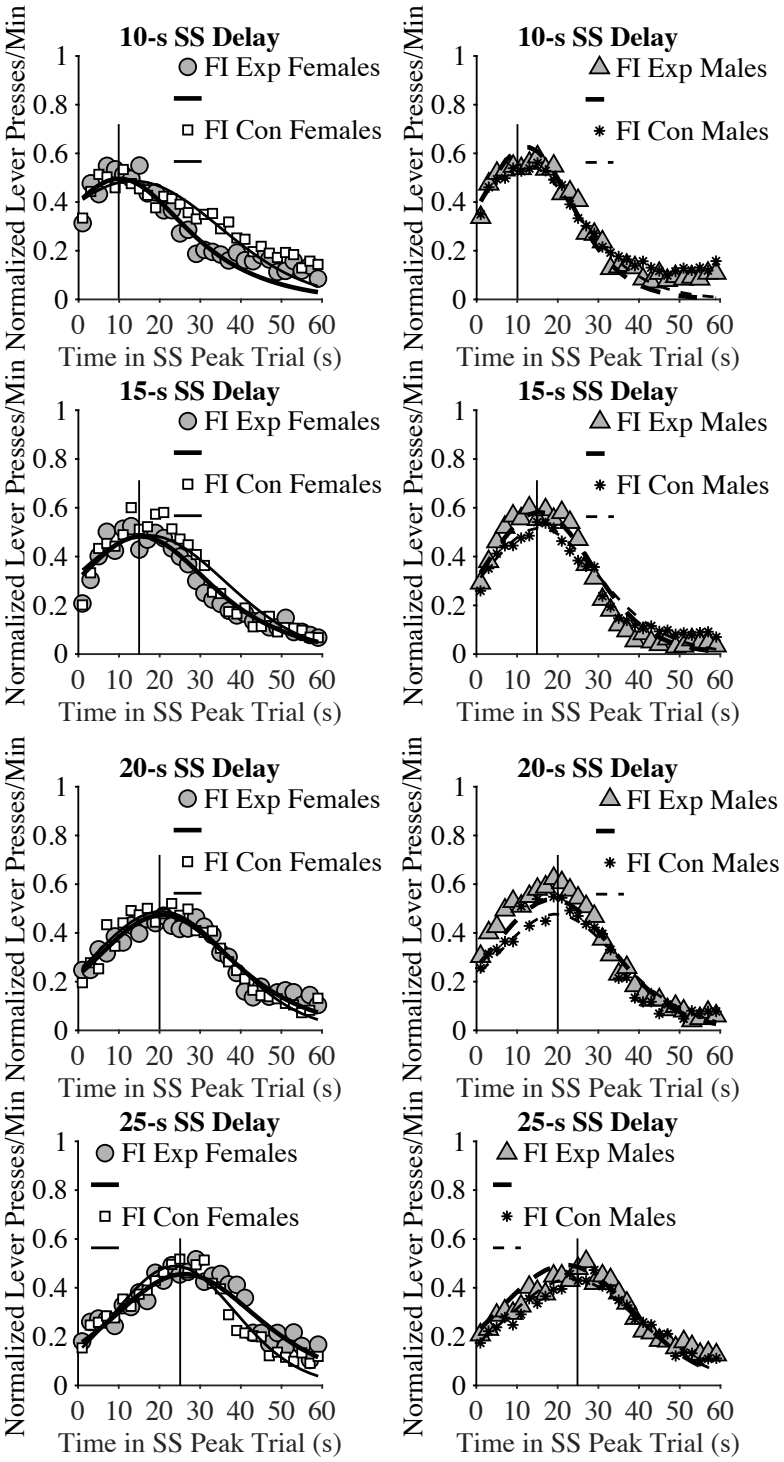


Figure 4.2. Normalized (proportion of maximum rate) lever presses per minute during LL peak trials for FI (fixed-interval) conditions. Markers represent mean responses and lines represent repeated measures multi-level nonlinear regression fits to the data. Vertical lines denote target intervals. Exp = Experimental; Con = Control.

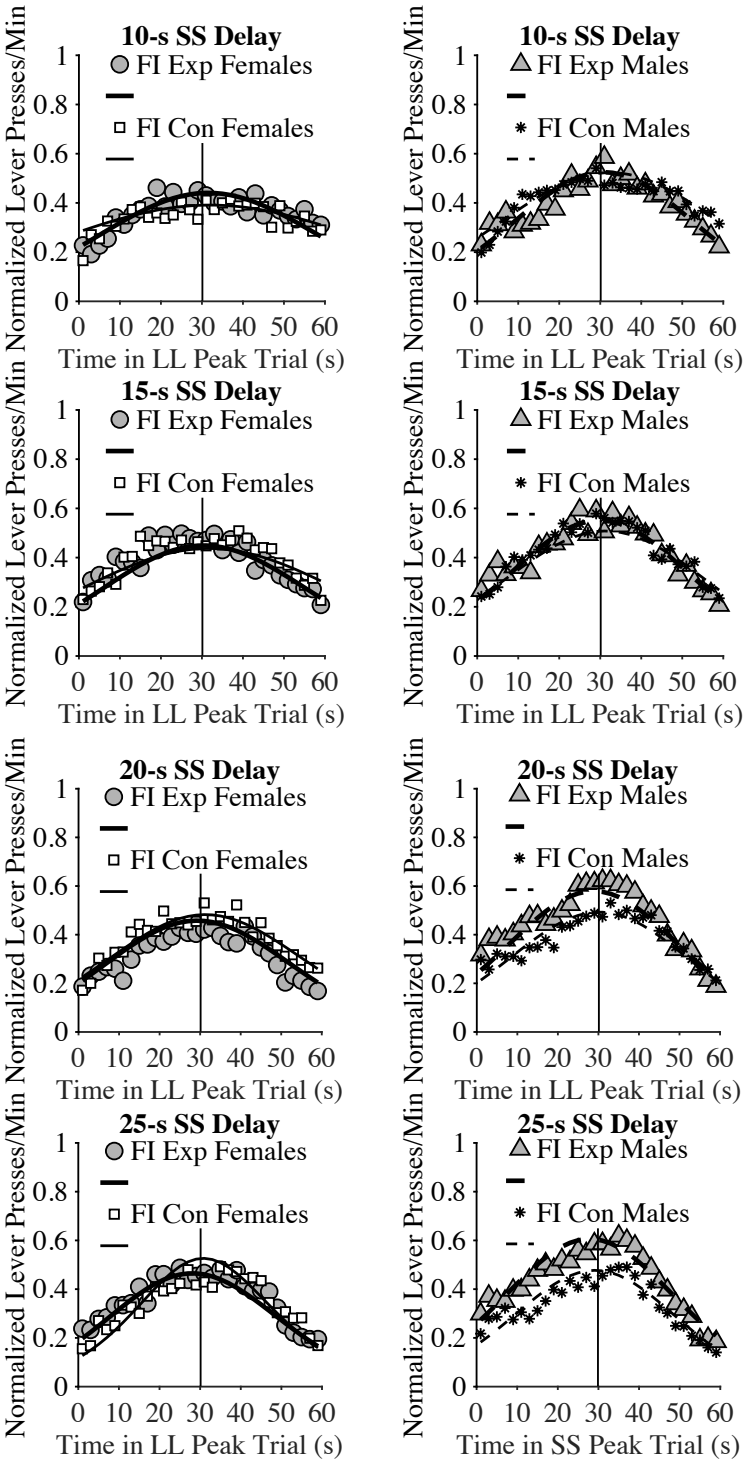


Figure 4.3. Mean peak time and spread as a function of SS delay with error bars (+/− SEM) on SS peak trials for rats in the FI (fixed-interval) conditions. Female FI-Exp rats showed a greater increase in peak time with increased SS delay while female FI-Con rats became significantly more precise as SS delay changed. Note the truncated axes. Points were jittered for readability. Exp = Experimental; Con = Control.

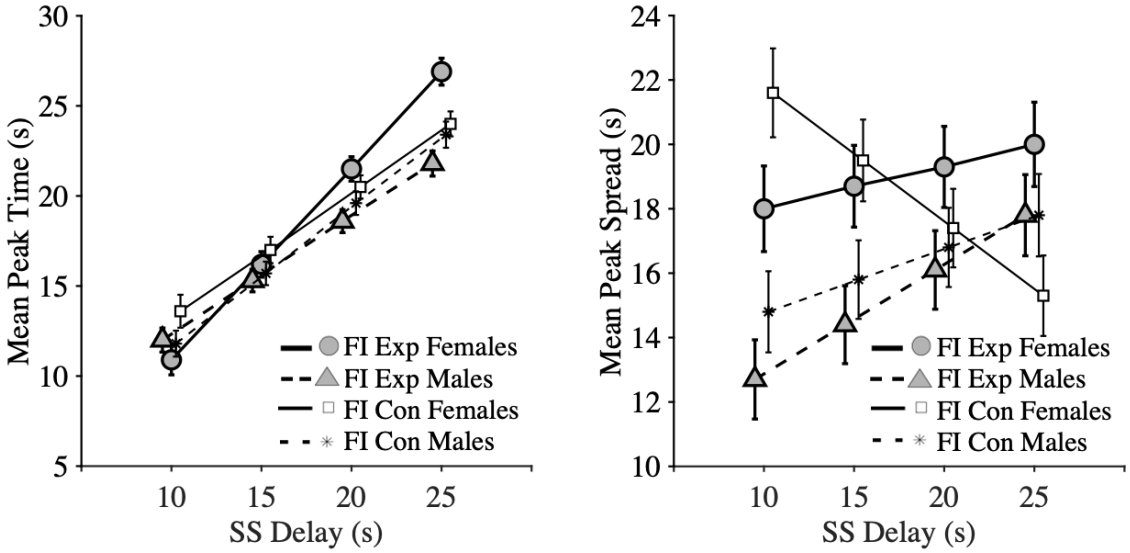


Figure 4.4. Mean peak time and spread as a function of SS delay with error bars (+/− SEM) on LL peak trials for rats in the FI (fixed-interval) conditions. There were no group or sex differences in peak time. Female FI-Con rats showed the largest decreases in peak spread as SS delay increased compared to other conditions. Note the truncated axes. Points were jittered for readability. Exp = Experimental; Con = Control.

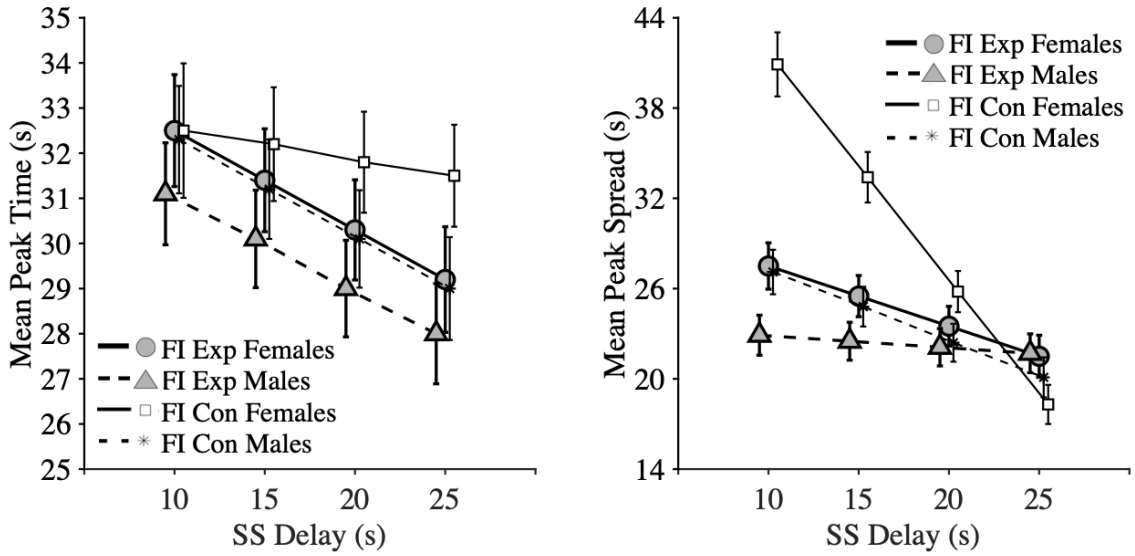


Figure 4.5. CV values (calculated by dividing peak spread by peak time) as a function of SS delay for rats in the FI (fixed-interval) conditions. Lower CV values suggest reduced relative timing errors. Across groups, CV values decreased as delay increased, suggesting rats made fewer timing errors with more experience in the task. Points were jittered for readability. Exp = Experimental; Con = Control.

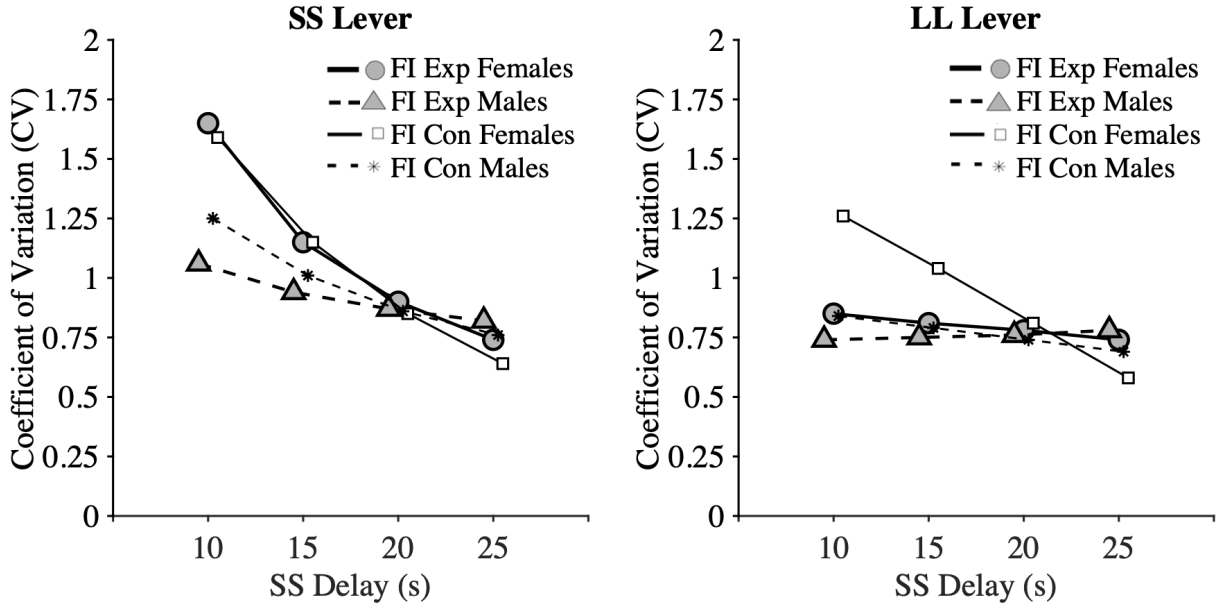


Figure 4.6. Normalized (proportion of maximum rate) lever presses per minute during SS peak trials for FT (fixed-time) conditions. Markers represent mean responses and lines represent repeated measures multi-level nonlinear regression fits to the data. Vertical lines denote target intervals. Exp = Experimental; Con = Control.

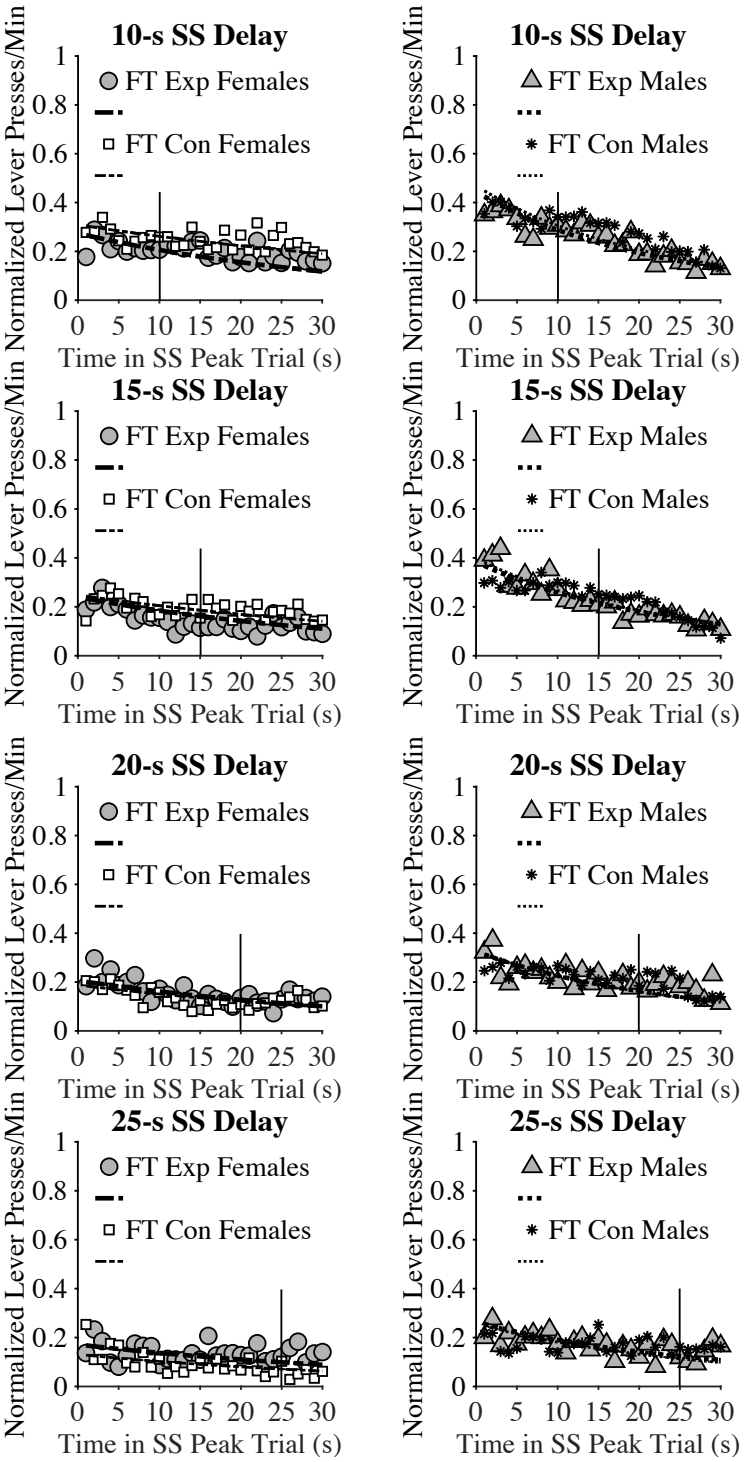


Figure 4.7. Normalized (proportion of maximum rate) lever presses per minute during LL peak trials for FT (fixed-time) conditions. Markers represent mean responses and lines represent repeated measures multi-level nonlinear regression fits to the data. Vertical lines denote target intervals. Exp = Experimental; Con = Control.

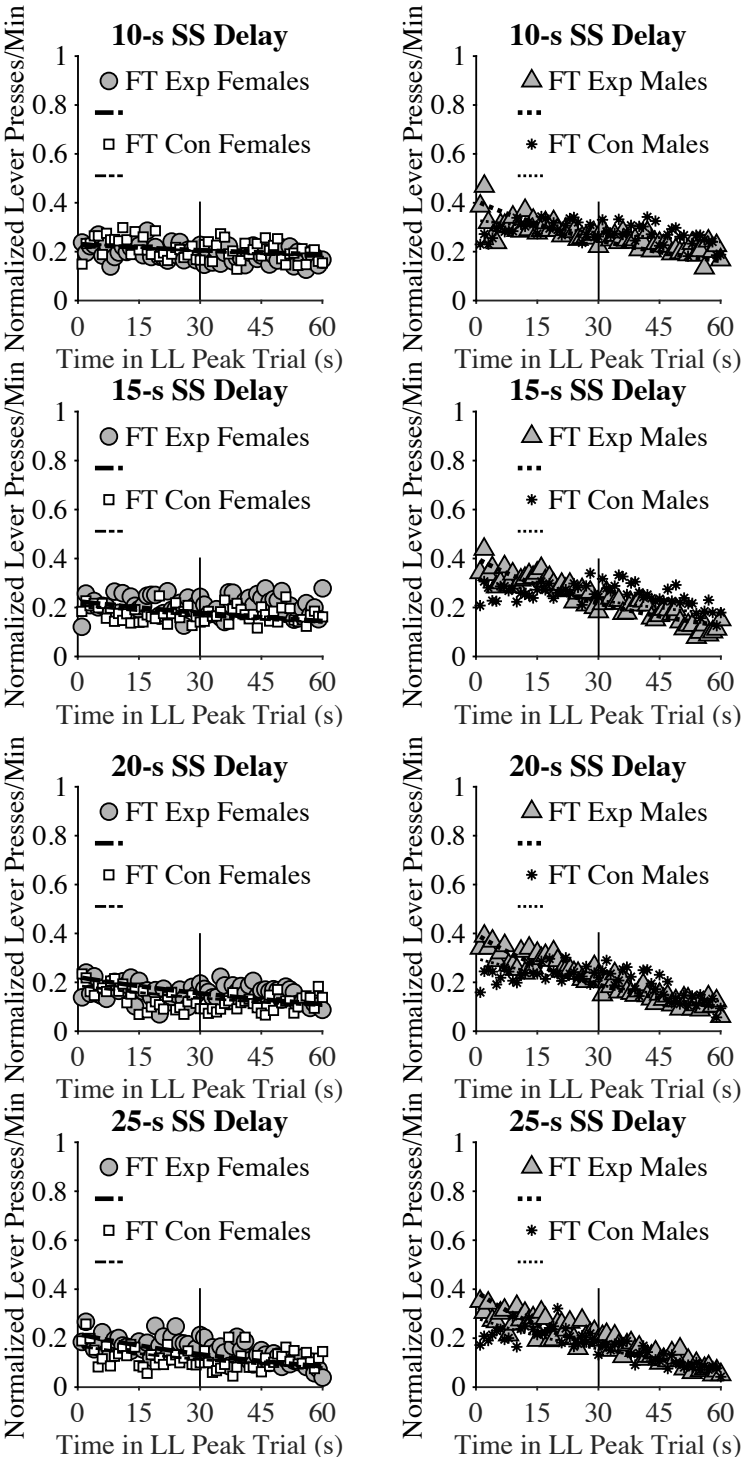


Figure 4.8. Mean intercept and rate of decay values as a function of SS delay with error bars (+/− SEM) on SS peak trials for rats in the FT (fixed-time) conditions. Male FT rats decreased more than females in initial responding as SS delay increased. Response rates were stable as SS delay increased for female FT-Con rats while other conditions had a progressively steeper slope. Points were jittered for readability. Exp = Experimental; Con = Control.

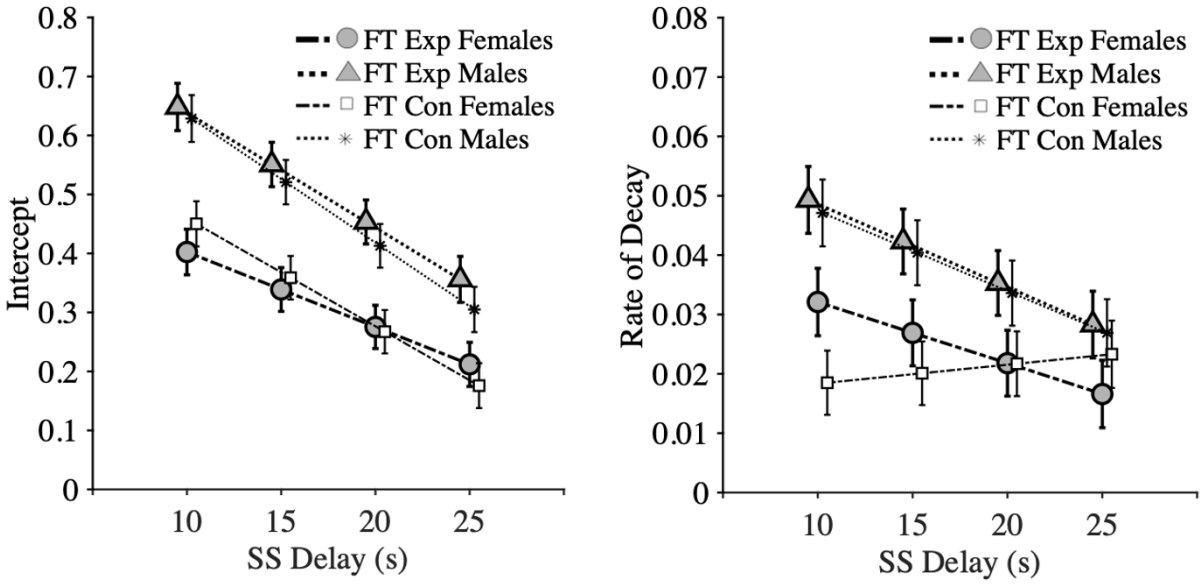


Figure 4.9. Mean intercept and rate of decay values as a function of SS delay with error bars (+/− SEM) on LL peak trials for rats in the FT (fixed-time) conditions. Female FT rats' responding was more stable while male FT rats had a progressively steeper slope as SS delay increased. Con rats decreased in initial responding as SS delay increased. Points were jittered for readability. Exp = Experimental; Con = Control.

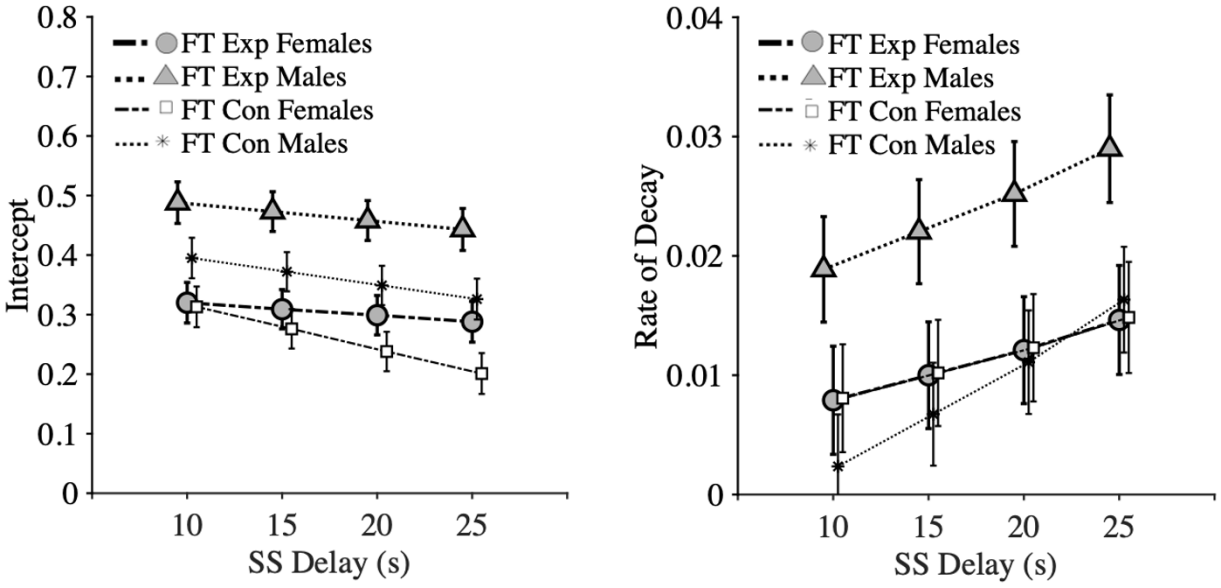


Figure 4.10. Mean proportion of LL choices as a function of SS delay for the male and female rats that received the FI (fixed-interval; left panel) and FT (fixed-time; right panel) schedules. Across group, sex, and schedules, rats made more self-controlled choices as the SS delay increased. Error bars (+/− SEM) were computed with respect to the estimated marginal means of the fitted repeated measures multi-level logistic regression and jittered for readability. Exp = Experimental; Con = Control.

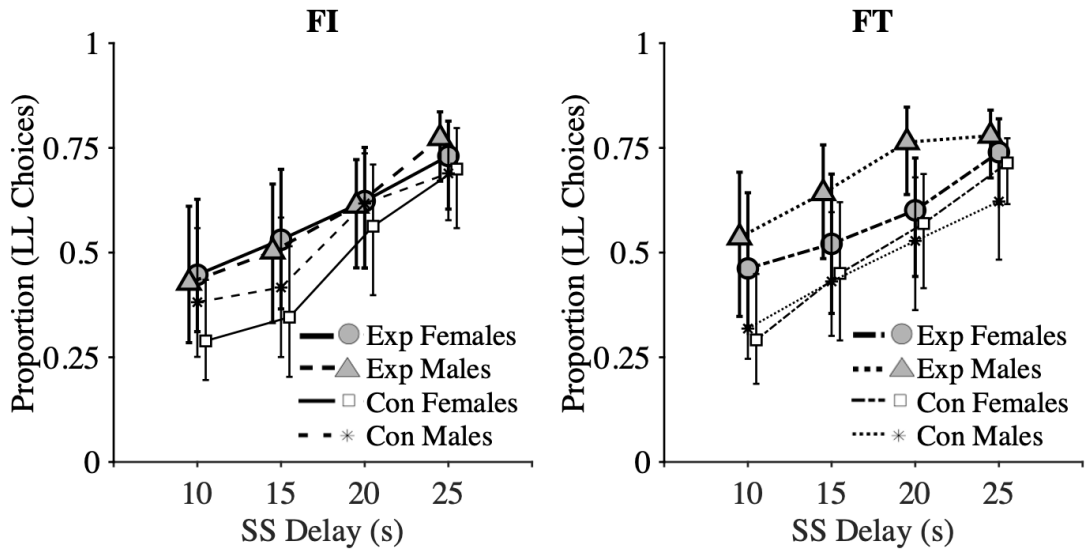


Figure 4.11. Normalized (proportion of maximum rate) lever presses per minute during SS forced-choice trials. Response rates of rats that received the FI schedule (displayed on the left) were significantly different from rats that received the FT schedule (displayed on the right), but there were no differences between the FI schedule conditions as all groups increased lever press response rates as time into the SS forced-choices trials progressed. Response rates for rats receiving the FT schedule decreased over time in the trial. Females in the FT conditions had steeper negative slopes than the males in the FT conditions. Markers represent mean responses and lines represent repeated measures multi-level linear regression fits to the data. Exp = Experimental; Con = Control.

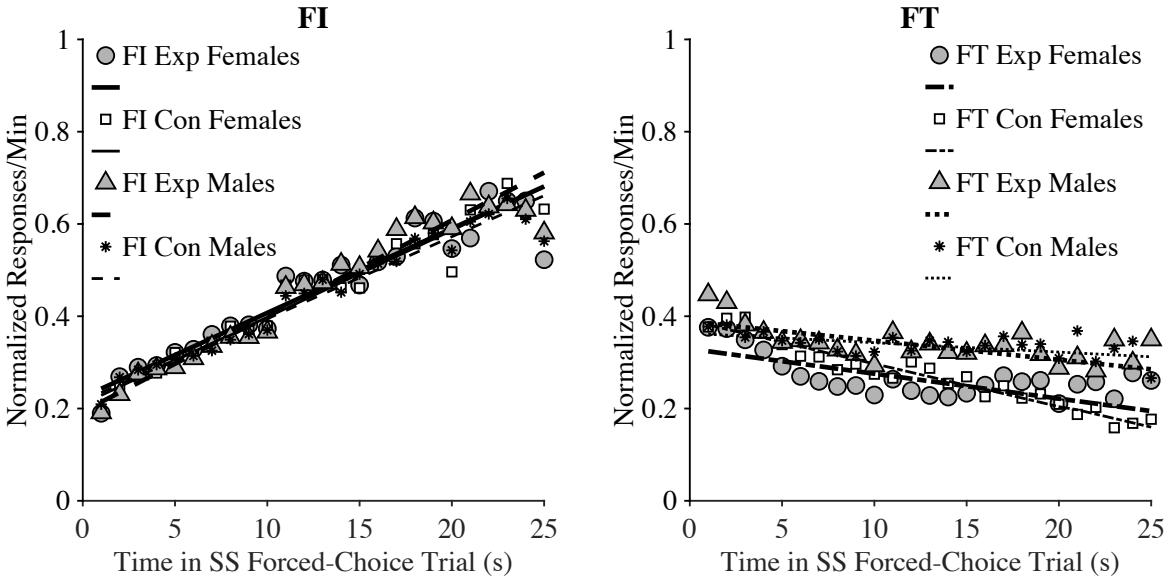


Figure 4.12. Normalized (proportion of maximum rate) lever presses per minute during LL forced-choice trials. Response rates of rats that received the FI schedule (left panel) were significantly different from rats that received the FT schedule (right) with the FI schedule conditions showing increased lever press response rates and the FT conditions showing decreased lever press response rates as time into the LL forced-choices trials progressed. Markers represent mean responses and lines represent fitted repeated measures multi-level linear regression values. Exp = Experimental; Con = Control.

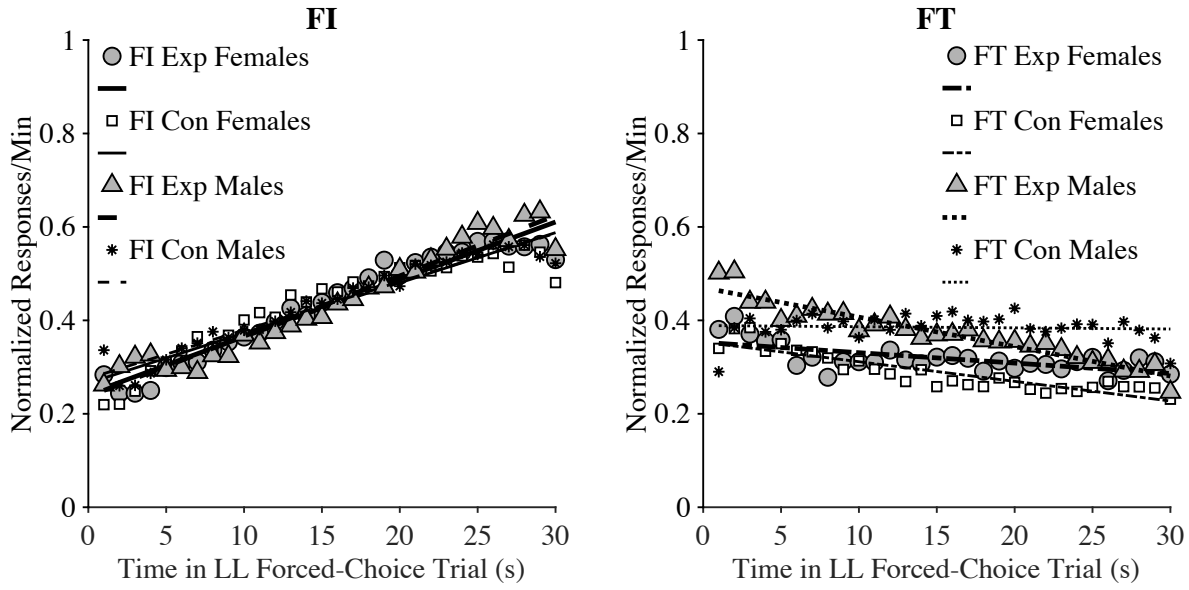


Table 4.1. Percentage of SS peak trials that contained zero lever presses or zero head entry responses during the delay period, indicating no additional lever presses or head entries were made during the trial. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

Group	Sex	Lever Presses	Head Entries
FI-Exp	Female	17.4%	83.9%
	Male	19.1%	81.6%
FI-Con	Female	13.9%	81.9%
	Male	15.5%	78.2%
FT-Exp	Female	50.5%	66.5%
	Male	34.7%	58.9%
FT-Con	Female	42.7%	63.2%
	Male	34.4%	66.5%

Table 4.2. Percentage of LL peak trials that contained zero lever presses or zero head entry responses during the delay period, indicating no additional lever presses or head entries were made during the trial. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

Group	Sex	Lever Presses	Head Entries
FI-Exp	Female	9.7%	89.5%
	Male	2.0%	85.9%
FI-Con	Female	5.3%	72.2%
	Male	3.6%	76.5%
FT-Exp	Female	33.4%	76.8%
	Male	17.0%	60.7%
FT-Con	Female	32.9%	63.8%
	Male	15.8%	59.5%

Table 4.3. Percentage of SS forced-choice trials that contained zero lever presses or zero head entry responses during the delay periods, indicating no additional lever presses or head entries were made during the trial. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

Group	Sex	Lever Presses	Head Entries
FI-Exp	Female	0.8%	76.1%
	Male	0.7%	79.2%
FI-Con	Female	0.9%	67.9%
	Male	0.7%	70.7%
FT-Exp	Female	15.4%	41.1%
	Male	1.7%	41.2%
FT-Con	Female	8.9%	42.3%
	Male	4.8%	53.3%

Table 4.4. Percentage of LL forced-choice trials that contained zero lever presses or zero head entry responses during the delay periods, indicating no additionl lever presses or head entries were made during the trial. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

Group	Sex	Lever Presses	Head Entries
FI-Exp	Female	1.9%	82.5%
	Male	0.5%	86.1%
FI-Con	Female	2.6%	62.4%
	Male	0.7%	74.2%
FT-Exp	Female	16.9%	62.1%
	Male	4.7%	56.8%
FT-Con	Female	12.4%	53.1%
	Male	7.1%	47.9%

Table 4.5. Pairwise comparisons to further probe the Group × Schedule × Sex × Time in Trial interaction when examining lever press responses during SS forced-choice trials. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control; F = Female; M = Male.

Group Comparison	*b*	*t*	*p*
FI-Exp F – FI-Con F	-0.001	-0.54	1.00
FI-Exp F – FT-Exp F	0.024	23.10	<.001
FI-Exp F – FT-Con F	0.028	26.83	<.001
FI-Exp F – FI-Exp M	-0.002	-2.39	.25
FI-Exp F – FI-Con M	0.000	0.47	1.00
FI-Exp F – FT-Exp M	0.022	21.86	<.001
FI-Exp F – FT-Con M	0.020	19.82	<.001
FI-Con F – FT-Exp F	0.024	23.64	<.001
FI-Con F – FT-Con F	0.028	27.37	<.001
FI-Con F – FI-Exp M	-0.002	-1.85	.59
FI-Con F – FI-Con M	0.001	1.01	.97
FI-Con F – FT-Exp M	0.023	22.41	<.001
FI-Con F – FT-Con M	0.021	20.36	<.001
FT-Exp F – FT-Con F	0.004	3.87	.003
FT-Exp F – FI-Exp M	-0.026	-25.49	<.001
FT-Exp F – FI-Con M	-0.023	-22.63	<.001
FT-Exp F – FT-Exp M	-0.001	-1.23	.92
FT-Exp F – FT-Con M	-0.003	-3.28	.02
FT-Con F – FI-Exp M	-0.030	-29.21	<.001
FT-Con F – FI-Con M	-0.027	-26.37	<.001
FT-Con F – FT-Exp M	-0.005	-5.10	<.001
FT-Con F – FT-Con M	-0.007	-7.13	<.001
FI-Exp M – FI-Con M	0.003	2.86	.08
FI-Exp M – FT-Exp M	0.025	24.25	<.001
FI-Exp M – FT-Con M	0.023	22.21	<.001
FI-Con M – FT-Exp M	0.022	21.39	<.001
FI-Con M – FT-Con M	0.020	19.35	<.001
FT-Exp M – FT-Con M	-0.002	-2.04	.45

Note: p values were adjusted with the Tukey method.

Table 4.6. Pairwise comparisons to further probe the Group × Schedule × Sex × Time in Trial interaction when examining lever press responses during LL forced-choice trials. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control; F = Female; M = Male.

Group Comparison	*b*	*t*	*p*
FI-Exp F – FI-Con F	0.002	2.70	.12
FI-Exp F – FT-Exp F	0.014	25.60	<.001
FI-Exp F – FT-Con F	0.016	29.09	<.001
FI-Exp F – FI-Exp M	-0.001	-1.13	.95
FI-Exp F – FI-Con M	0.002	3.37	.02
FI-Exp F – FT-Exp M	0.019	32.80	<.001
FI-Exp F – FT-Con M	0.013	22.10	<.001
FI-Con F – FT-Exp F	0.013	22.90	<.001
FI-Con F – FT-Con F	0.015	26.40	<.001
FI-Con F – FI-Exp M	-0.002	-3.83	.003
FI-Con F – FI-Con M	0.000	0.67	1.00
FI-Con F – FT-Exp M	0.017	30.10	<.001
FI-Con F – FT-Con M	0.011	19.40	<.001
FT-Exp F – FT-Con F	0.002	3.50	.01
FT-Exp F – FI-Exp M	-0.015	-26.73	<.001
FT-Exp F – FI-Con M	-0.013	-22.23	<.001
FT-Exp F – FT-Exp M	0.004	7.21	<.001
FT-Exp F – FT-Con M	-0.002	-3.50	.01
FT-Con F – FI-Exp M	-0.017	-30.23	<.001
FT-Con F – FI-Con M	-0.015	-25.72	<.001
FT-Con F – FT-Exp M	0.002	3.71	.01
FT-Con F – FT-Con M	-0.004	-7.00	<.001
FI-Exp M – FI-Con M	0.003	4.50	.005
FI-Exp M – FT-Exp M	0.019	33.93	<.001
FI-Exp M – FT-Con M	0.013	23.23	<.001
FI-Con M – FT-Exp M	0.017	29.43	<.001
FI-Con M – FT-Con M	0.011	18.73	<.001
FT-Exp M – FT-Con M	-0.006	-10.70	<.001

Note: p values were adjusted with the Tukey method.

Chapter 5 - Neurobiology Results and Discussion

Following the impulsive choice task, all rats were euthanized and perfused, and brains were processed for c-Fos as an indirect measure of neural activity. Representative images of c-Fos expression were shown in Figures 5.1 and 5.2. We quantified c-Fos in the dorsomedial striatum, dorsocentral striatum, dorsolateral striatum, prelimbic cortex, and infralimbic cortex and compared expression across group, schedule, and sex. In addition, we conducted exploratory cluster analyses to probe for possible phenotypes within the data based on impulsivity, timing, sign-tracking, goal-tracking, and neurobiology.

Dorsomedial Striatum

A generalized linear model was conducted to examine the number of c-Fos+ cells in the dorsomedial striatum (DMS). Experimental groups had higher levels of c-Fos in DMS than the control groups ($b = 0.021$, $t = 3.46$, $p < .001$). Rats that received the FI schedule had higher levels of c-Fos expression than rats that received the FT schedule ($b = 0.121$, $t = 19.65$, $p < .001$). Females also showed higher levels of c-Fos expression in DMS than males *(*$b = 0.125$, $t = 20.25$, $p < .001$). There was a significant Group × Schedule × Sex interaction effect ($b = 0.031$, $t = 4.96$, $p < .001$), and pairwise comparisons were conducted to investigate the interaction (see Table 5.1 for specific comparison values). FI-Exp females had higher levels of c-Fos than FI-Exp males, FI-Con females, and FT-Exp females (Figure 5.3). FT-Exp females showed higher levels of c-Fos+ cells in DMS compared to FT-Con females and FT-Exp males. FI-Exp males displayed higher levels of c-Fos compared to FT-Exp males. Both female and male FI control groups had more c-Fos expression in DMS than female and male FT control groups, respectively. However, FI-Exp males showed lower c-Fos expression than FI-Con males. FT-Con females had higher levels of expression than FT-Con males.

In all, rats that received the FI schedule in the intervention and choice phases showed higher levels of c-Fos+ cells in DMS compared to rats that received the FT intervention and choice tasks, which was consistent with our hypothesis. Based on sex differences in sign-tracking, we predicted females would show higher levels of c-Fos+ cells in DMS compared to males, and this prediction was confirmed in the current study. In addition, FI-Exp females had the highest levels of c-Fos expression in DMS while FT-Exp males had some of the lowest levels of c-Fos expression. Although, FT-Exp males were not significantly different from FT-Con males. This was mostly consistent with our hypothesis that sex, group, and schedule may interact so that FI-Exp females show the most c-Fos+ cells in DMS and FT-Exp males shows the least c-Fos+ cells in DMS.

Dorsocentral Striatum

A generalized linear model was conducted to examine the number of c-Fos+ cells in the dorsocentral striatum (DCS). DCS c-Fos expression was higher in experimental groups than control groups ($b = 0.027$, $t = 3.47$, $p < .001$) and higher in the FI schedule conditions compared to the FT schedule conditions ($b = 0.152$, $t = 19.53$, $p < .001$). Females had higher c-Fos+ cells in DCS compared to males ($b = 0.159$, $t = 20.46$, $p < .001$). There was a significant Group × Schedule × Sex interaction effect ($b = 0.037$, $t = 4.77$, $p < .001$). Pairwise comparisons were conducted to examine the interaction (see Table 5.2 for specific comparison values). In particular, females that received the FI schedule had the highest levels of c-Fos expression in DCS (Figure 5.4). FI-Exp females had higher c-Fos expression than FI-Con females, FI-Exp males, and FT-Exp females. FI-Con females had more c-Fos+ cells than FT-Con females and FI-Con males. FT-Exp females had higher c-Fos expression than FT-Exp males. FT-Con females had higher expression in DCS than FT-Con males. FI-Exp males had more c-Fos+ cells than FT-

Exp males. FI-Con males had higher levels of c-Fos expression compared to FT-Con males. However, there were no differences between experimental and control groups in the FT schedule for both females and males.

Rats that received the interventions showed higher c-Fos expression in DCS, and this was consistent with our hypothesis that suggested the interventions would promote self-control leading to higher levels of c-Fos in DCS. However, further comparisons suggest that the FI-Exp females differed from FI-Con females, but this was not the case when comparing the other experimental groups to their controls. In addition, we expected no c-Fos differences between rats that received the FI intervention and the FT intervention. However, FI-Exp females and FI-Exp males had higher c-Fos expression than FT-Exp females and FT-Exp males, respectively.

Dorsolateral Striatum

A generalized linear model was conducted to examine the number of c-Fos+ cells in the dorsolateral striatum (DLS). Rats in the FI schedule had higher c-Fos expression in DLS than rats that received the FT schedule ($b = 0.149$, $t = 10.26$, $p < .001$). In addition, females had more c-Fos+ cells in DLS than males ($b = 0.094$, $t = 6.47$, $p < .001$). There was a significant Group × Schedule × Sex interaction effect ($b = 0.090$, $t = 6.19$, $p < .001$), so pairwise comparisons were conducted to understand the interaction (see Table 5.3 for specific comparison values). Within the experimental conditions, FI-Exp females had more c-Fos+ cells than FT-Exp females (Figure 5.5). Also, FT-Exp females had lower c-Fos expression than FT-Con females and FT-Exp males. FI-Exp males had more c-Fos+ cells than FI-Con males and FT-Exp males. However, FT-Exp males had higher c-Fos expression than FT-Con males. Both female control groups (FI-Con and FT-Con) had more c-Fos expression in DLS than male control groups (FI-Con and FT-Con). In sum, DLS c-Fos expression was highest in FI-Exp females and lowest in FT-Exp females.

Unlike DCS, rats in the experimental groups did not show greater c-Fos expression in DLS, which was inconsistent with our hypothesis that experimental groups would show higher levels of LL choices coupled with higher levels of c-Fos in DLS. FI-Exp males had significantly higher c-Fos expression than FI-Con males, but FI-Exp females did not differ from FI-Con females. FT-Exp males had significantly higher c-Fos expression than FT-Con males, but FT-Exp females had significantly lower c-Fos expression than FT-Con females. Rats that received the FI intervention had significantly higher c-Fos expression than rats that received the FT intervention, which was inconsistent with our hypothesis that the interventions would result in similar levels of c-Fos+ cells in DLS. This effect occurred in both males and females.

Prelimbic Cortex

A generalized linear model was conducted to examine the number of c-Fos+ cells in the prelimbic (PL) cortex. The number of c-Fos+ cells in PL was higher in the experimental groups compared to the control groups ($b = 0.022$, $t = 5.09$, $p < .001$). In addition, c-Fos+ expression was higher in the FI schedule compared to the FT schedule ($b = 0.042$, $t = 9.56$, $p < .001$). Females had more c-Fos+ cells than males ($b = 0.058$, $t = 13.14$, $p < .001$). There was a significant Group × Schedule × Sex interaction effect ($b = 0.056$, $t = 12.81$, $p < .001$). Further pairwise comparisons were conducted to probe the interaction (see Table 5.4 for specific comparison values). Most notably, FI-Exp females had significantly higher levels of c-Fos expression in PL compared to all other conditions (Figure 5.6). FI-Con females, FI-Exp males, and FT-Con males had significantly lower levels of c-Fos expression in PL compared to FI-Con males. Within the FT conditions, FT-Exp females and FT-Con females had higher levels of c-Fos than FT-Exp males and FT-Con males, respectively. FT-Exp males had higher levels than FT-

Con males. There were no differences between FT-Exp and FT-Con females or FI-Exp males and FT-Exp males. In short, FI-Exp females showed the highest levels of c-Fos expression.

Results in PL closely followed those of DMS, which was largely consistent with our hypotheses positing that these regions may work together in the FI intervention. Rats that received the FI schedule showed higher levels of c-Fos+ cells in PL compared to rats that received the FT schedule, matching our hypothesis. Furthermore, females showed higher levels of c-Fos expression than males, which we predicted based on previous literature examining sex differences in sign-tracking. Finally, FI-Exp females had the highest levels of c-Fos expression in PL like in DMS as well. We expected that sex, group, and schedule may interact to produce the highest levels of c-Fos in FI-Exp females and lowest levels in FT-Exp males. However, FI-Exp males and FT-Exp males did not differ in c-Fos expression in PL. This inconsistency with our hypothesis also occurred in DMS.

Infralimbic Cortex

A generalized linear model was conducted to examine the number of c-Fos+ cells in the infralimbic (IL) cortex. Females showed higher levels of c-Fos+ cells in IL compared to males ($b = 0.081$, $t = 13.33$, $p < .001$). In contrast to PL, control groups showed higher levels of c-Fos+ cells in IL compared to experimental groups ($b = -0.023$, $t = -3.75$, $p < .001$). There were significant Group × Sex ($b = 0.024$, $t = 4.01$, $p < .001$) and Schedule × Sex ($b = -0.023$, $t = -3.84$, $p < .001$) interactions. Males in the control groups had higher levels of c-Fos+ cells in IL compared to males in the experimental groups, but there were no differences between female experimental and female control groups (Figure 5.7). Also, males that received the FI schedule had higher levels of c-Fos+ cells in IL compared to males that received the FT schedule. However, there were no differences in c-Fos+ cells in IL between females that received the FT

schedule and females that received the FI schedule. There were no significant Group × Schedule or Group × Schedule × Sex interactions. Altogether, c-Fos expression in IL differed in males based on group and schedule conditions, but there were no group or schedule differences in females.

We hypothesized that c-Fos expression in IL would be higher in control groups than experimental groups because the interventions may promote the ability to inhibit an initial impulsive response. This hypothesis was confirmed, but the effect occurred in males only. There were no differences in females. We also predicted that the interventions would result in similar levels of c-Fos expression in IL. Instead, males that received the FI schedule showed higher levels of c-Fos+ cells in IL compared to males that received the FT schedule.

Exploratory Clustering Analyses

The cluster analyses were planned to test the relationships between impulsivity, interval timing, sign-tracking, goal-tracking, and neurobiology in a way that aligned with our hypotheses about the importance of these cognitive mechanisms to the efficacy of time-based interventions. According to the temporal processing and attention hypotheses, we predicted that temporal processing, or interval timing ability, was essential to the FI intervention, which would result in higher delay sensitivity compared to the FT intervention. In these cluster analyses, we included temporal processing and impulsive choice dimensions with c-Fos expression of each brain region. According to the sign- and goal-tracking hypotheses, we expected that these behaviors may relate to neurobiology instead of timing information. We originally planned to quantify sign- and goal-tracking during the impulsive choice task, but few head entries were made during the task across groups, schedules, and sexes. This limited the cluster analysis of these behaviors to the intervention phase only, so rats in the control groups were not included.

Temporal Processing and Attention Hypothesis

Dorsomedial Striatum

When including average proportion of LL choices, the choice function slope, the lever press response rate during the final 3 s of the 30-s LL peak from the choice task, and the number of c-Fos+ cells in DMS, the two clustering analysis methods did not converge on the same number of clusters. With k-means clustering, the optimal solution was eleven clusters with a CCC (Cubic Clustering Criterion) value of 0.370. With hierarchical clustering, the optimal solution was one cluster. The mean dimension values and their standard deviations per cluster were displayed in Table 5.5.

When examining the k-means clusters with group, schedule, and sex classifications, clusters did not align entirely with any condition (Figure 5.8). For example, female FI-Exp rats were assigned to eight different clusters. In some cases, clusters were comprised of animals from a single schedule. Cluster 2 contained rats that received the FI schedule while cluster 3 was almost entirely rats that received the FT schedule. Assessment of the clusters without classifications suggests that multiple profiles may exist within the four dimensions. For example, cluster 1 was characterized by high levels of LL choices, a shallow positive choice function slope, a relatively high 30-s LL lever press peak rate, and average c-Fos expression in DMS (Table 5.5). However, some clusters captured similar levels of behavioral dimensions and only differed on DMS expression. Clusters 4 and 6 were comparable in terms of LL choice, slope, and peak rate but differed dramatically on c-Fos in DMS. In sum, clustering analyses were conducted to test the hypotheses built on the importance of timing in relation to impulsive choice and neurobiology, and the analyses focused on DMS suggest that clusters did not emerge exclusively based on a combination of these factors or one prominent factor.

Dorsocentral Striatum

Like DMS, clustering methods did not converge on the same number of clusters. The optimal solution was nine clusters with a CCC value of 1.391 for the k-means clustering approach. Like DMS, the optimal hierarchical clustering solution was one cluster. The mean dimension values and standard deviations per cluster were displayed in Table 5.6.

Group, schedule, and sex classifications suggest that the k-means clusters did not systematically align with the current study's conditions (Figure 5.9). Each individual condition (FI-Exp females, FI-Exp males, FI-Con females, FI-Con males, FT-Exp females, FT-Exp males, FT-Con females, FT-Con males) was made up of at least five different clusters. Clusters 4, 5, 7, and 8 appeared to coincide with schedule assignments such that clusters 4 and 7 were made up of rats that received the FT schedule only and clusters 5 and 8 mostly contained rats that received the FI schedule. Regardless of classification, the clusters suggest that multiple phenotypes may exist within the four dimensions but in a complex manner. For example, cluster 8 was characterized by low levels of LL choices, a shallow positive choice function slope, an average 30-s LL lever press peak rate, and high c-Fos expression in DCS while cluster 6 was similar in all regards except for much lower c-Fos expression in DCS (Table 5.6). Altogether, cluster analyses including DCS suggest that clusters did not emerge according to our hypotheses about DCS and self-control, indicating that DCS may not be an essential component of temporal processing as it relates to self-control.

Dorsolateral Striatum

Unlike DMS and DCS, clustering methods converged on five clusters when DLS was included as a dimension. The optimal solution was five clusters with a CCC value of 1.691 for the k-means clustering approach. Based on CCC alone, the optimal hierarchical clustering

solution was ten clusters, but the five-cluster solution was selected because of parsimony. The mean dimension values and standard deviations per cluster were displayed in Table 5.7.

Group, schedule, and sex classifications suggest that the k-means and hierarchical clusters did not match the current study's conditions apart from one cluster (Figures 5.10 and 5.11). In the k-means approach, cluster 3 contained rats that received the FT schedule only, and in the hierarchical clusters approach, cluster 2 was also made up of rats the received the FT schedule only. In particular, these clusters were different from the others based on the lever press response rate during the final 3 s of the 30-s LL peak delivered during the choice task, and all other clusters had a higher lever press response rate. Otherwise, clusters were made up of a variety of conditions across the analysis approaches. Regardless of classification, the approaches detected similar phenotypes within the four dimensions. For example, k-means cluster 1 and hierarchical cluster 1 were both characterized by high levels of LL choices, a shallow positive choice function slope, an average 30-s LL lever press peak rate, and low c-Fos expression in DLS (Table 5.7). However, the number of animals assigned to these clusters differed slightly. In short, clustering analyses including DLS suggest that one subset of rats emerged based on FT schedule and 30-s LL lever press peak rate while other clusters were not specific to experimental conditions. Like DCS, clustering analyses with DLS indicate that clusters did not emerge according to our hypotheses about DLS and self-control. These subregions of the striatum may not be heavily involved in the relationship between temporal processing and self-control.

Prelimbic Cortex

K-means and hierarchical clustering methods suggest that one cluster emerged when c-Fos expression in PL was included as a dimension. One cluster within average proportion of LL choices, the choice function slope, the lever press response rate during the final 3 s of the 30-s

LL peak from the choice task, and the number of c-Fos+ cells in PL may indicate that these dependent measures do not strongly correlate with each other. We expected that PL activity would interact with these dimensions in accordance with the temporal processing and attention hypotheses. However, there were interesting differences in c-Fos expression in PL based on group, schedule, and sex (Figure 5.6). These group-level variables may better explain differences in c-Fos in PL compared to the dimensions included in the clustering analyses.

Infralimbic Cortex

Like PL, k-means and hierarchical clustering methods resulted in one cluster when c-Fos expression in IL was included as a dimension. We anticipated that c-Fos expression in IL may be negatively related with measures of self-control, but there were no phenotypes that emerged based on these factors. There were also no differences in IL c-Fos expression in the FI schedule (Figure 5.7), which may suggest limited variability in IL expression in relation to these facets of temporal processing and attention.

Sign- and Goal-Tracking Hypothesis

To examine the relationship between sign- and goal-tracking like behaviors and c-Fos expression, k-means and hierarchical clustering methods were conducted with the lever press response rate during the final 3 s of the 10-s intervention delay, the lever press response rate during the final 3 s of the 30-s intervention delay, the head entry response rate during the final 3 s of the 10-s intervention delay, the head entry response rate during the final 3 s of the 30-s intervention delay, and the number of c-Fos+ cells in each of the five brain regions. These analyses included FI-Exp and FT-Exp groups only. The control groups did not receive any intervention training, so there were no intervention lever press or head entry rates for these conditions. In four of the brain regions, k-means and hierarchical clustering methods resulted in

one cluster. DMS, DLS, PL, and IL c-Fos expression may not relate with these measures of sign- and goal-tracking like behavior. However, there were k-means clusters detected in relation to c-Fos expression in DCS.

Dorsocentral Striatum

Clustering methods did not converge on the same number of clusters. The optimal solution was nine clusters with a CCC value of 0.374 for the k-means clustering approach. The optimal hierarchical clustering solution was one cluster. The mean dimension values and standard deviations per cluster were displayed in Table 5.8.

Schedule and sex classifications suggest that the k-means clusters did not entirely correspond with the current study's conditions (Figure 5.12). While each individual condition (FI-Exp females, FI-Exp males, FT-Exp females, FT-Exp males) was made up of at least four different clusters, some clusters were specific to experimental conditions. Cluster 3 was comprised of female rats only, and cluster 6 contained male rats only. Cluster 9 also had rats that received the FI schedule only. The clusters suggest that multiple phenotypes may exist within the five dimensions. Relatively high response rates in all four behavioral dimensions were associated with average DCS c-Fos expression while combinations of high and low response rates across behavioral dimensions were associated with more extreme DCS c-Fos values (Table 5.8). However, there were no apparent clusters that suggest sign- or goal-tracking were related to DCS c-Fos expression in accordance with our original hypotheses. For example, cluster 5 was characterized by high lever press response rates and low head entry rates coupled with high c-Fos expression in DCS, yet cluster 7 had the same pattern of responding along with low c-Fos expression in DCS. In all, clustering analyses including DCS suggest that clusters did not coincide with the sign- and goal-tracking hypotheses. However, it is important to note that

control animals were not included in these analyses, and clearer clusters may have emerged if lever press and head entry rates were measurable from the impulsive choice task for all animals in the current study.

Follow-up Exploration

Based on the lack of clear patterns in the planned cluster analyses, we also conducted a series of follow-up analyses to better understand the relationships between behavioral and neurobiological data. We included all brain regions in one cluster analysis with no behavioral data dimensions, and no clusters emerged. This may suggest that there were no circuit-level interactions within the neurobiological data set or that the clustering methods used here were not sensitive to such changes. We also explored clusters that included neurobiological measures and choice measures only to determine whether the data better supported temporal attention hypotheses but not temporal processing hypotheses. These analyses would suggest that timing may be distinct from choice processes. Again, no clusters emerged, so it remains unclear whether timing and choice processes stemmed from separate neurobiological systems. Finally, we also probed for clusters in females only and males only. Specifically, we tested for relationships between timing, PL, and DMS in females only, but no clusters emerged. We also examined choice indices, DCS, DLS, and IL together in males only and found no clusters.

Discussion

The expression of c-Fos in DMS, DLS, DCS, PL, and IL was quantified to understand how neural activity differed based on group, sex, and schedule and how neural activity related to behavioral measures. In DMS and PL, rats that received the FI schedule had higher levels of c-Fos+ cells than rats that received the FT schedule averaged across other conditions, which was consistent with our hypotheses suggesting that FI schedules may rely on temporal processing.

Further pairwise comparisons for PL showed that this was specific to FI-Exp females compared to FT-Exp females and FI-Con males compared to FT-Con males but not FI-Exp males and FT-Exp males nor FI-Con females and FT-Con females. In DMS, the FI schedule resulted in higher levels of c-Fos+ cells than the FT schedule except when comparing FI-Exp males and FI-Con males, which resulted in lower c-Fos expression in the FI-Exp males. We expected that rats in the FT schedule conditions would have lower levels of c-Fos in these regions because the FT schedules may not rely on temporal processing. We saw the same pattern in DCS and DLS where rats that received the FI schedule had higher levels of c-Fos than rats that received the FT schedule averaged across all other conditions. Further pairwise comparisons for DCS showed that this occurred in all cases except for FI-Exp males and FI-Con males, which showed similar levels of c-Fos in this region. In DLS, the FI schedule resulted in higher levels of c-Fos+ cells than the FT schedule but between intervention conditions only. FI-Con females were not difference from FT-Con females, and FI-Con males were not different from FT-Con males. We expected FI and FT intervention groups to have higher levels of c-Fos compared to control groups, assuming that DCS and DLS are related to self-control. We did find this pattern in DCS where rats in the experimental groups had higher levels of c-Fos than rats in the control groups. This effect occurred in DMS and PL as well. DMS and PL results were consistent with our hypotheses and suggest that the interventions produced more neural activity in these regions compared to the control conditions. Rats in the experimental groups had lower levels of c-Fos expression in IL than rats in the control groups but in male rats only, which suggests that the interventions reduced neural activity in this region compared to controls in males as we predicted. Finally, females showed higher levels of c-Fos compared to males in all five brain regions. We expected this sex difference in DMS and PL, according to the hypothesized

interaction between group, schedule, and sex in alignment with the temporal processing and attention hypotheses and influences of sign- and goal-tracking.

According to the temporal processing and attention hypotheses, we expected that the FI schedule in the intervention and choice phases would be associated with higher levels of c-Fos+ cells in the PL and DMS compared to rats that receive the FT intervention and choice tasks. Given that these regions are heavily involved in interval timing (Coull et al., 2011; Finnerty et al., 2015; Matell & Meck, 2004; Tallot & Doyère, 2020), we proposed that a functional circuit between PL and DMS may underlie the FI intervention efficacy in promoting self-control, specifically delay sensitivity. Consistent with this idea, there were higher levels of c-Fos+ cells in these regions when comparing FI-Exp and FT-Exp groups for females and males in DMS but only for females in PL. However, exploratory cluster analyses did not reveal meaningful clusters in accordance with temporal processing and attention hypotheses. We expected that profiles would emerge showing positive relationships between enhanced interval timing ability, increased delay sensitivity, and higher levels of neural activity in PL and DMS. It is important to note that these clusters may have emerged if there were robust differences in delay sensitivity between FI and FT schedules. We also used lever press response rates during the final 3 s of the 30-s LL peak as an index of timing. Clusters may have emerged with alternative timing dimensions such as accuracy and precision values. However, the measures of accuracy and precision in timing were only available as parameters obtained from the nonlinear multi-level models, and we elected to use raw data values in the clustering analyses instead of model fit values. Altogether, temporal attention, not temporal processing, may drive time-based intervention efficacy. Further research is needed to support this hypothesis where both schedules may have promoted temporal attention to a similar degree as evident in similar delay sensitivities.

In the absence of strong evidence for the temporal processing and attention hypotheses, we predicted that self-control may relate to DCS, DLS, and IL. Based on previous research, we hypothesized higher c-Fos expression in DCS and DLS along with increased LL choices (Dunnett et al., 2012; Tedford et al., 2015). There were higher levels of c-Fos expression in experimental groups compared to control groups in DCS but not in DLS. However, these dimensions did not align in clustering analyses. Phenotypes were not detected based on LL choices and delay sensitivity with DCS or DLS. We also found that rats that received the FI schedule had higher levels of c-Fos in DCS and DLS compared to rats that received the FT schedule. However, we did not anticipate differences based on schedule in these regions. Previous literature suggests that the dorsal striatum is heavily involved in interval timing, and lesions to DCS impair temporal processing on peak interval trials (Meck, 2006). However, PL sends few projections to DCS, suggesting that DCS is not part of the PL-DMS circuitry (Cheatwood et al., 2003). In addition, previous research found that synaptic plasticity in DLS corresponded with temporal information, specifically learning new time durations (Yousefzadeh et al., 2021), but this region also does not receive significant input from PL (Balleine et al., 2007). Instead, DCS and DLS may be involved in other functional circuits that contribute to interval timing, as research suggests the neural basis of timing is highly distributed across regions and neurotransmitter systems (Paton & Buonomano, 2018). In all, rats in the FI schedule may have shown higher levels of c-Fos in DCS and DLS compared to rats that received the FT schedule because the FI schedule recruited multiple pathways related to interval timing while the FT schedule did not.

In addition, c-Fos expression in the infralimbic cortex was higher in male control groups, suggesting the interventions may reduce impulsive action, but clustering analyses did not clearly

link IL neurobiology with measures of impulsivity. It is possible that these relationships may emerge with a more direct measure of impulsive action such as the five-choice serial-reaction time task, stop signal task, or go/no-go task where subjects must inhibit responding when specific stimuli are presented. To my knowledge, only one study has examined the effects of a time-based intervention on impulsive action, and researchers found that an FI intervention reduced impulsive action in male but not female mice (Eckard et al., 2023). They also measured serotonin, dopamine, their precursors, and their metabolites in the striatum and prefrontal cortex and found that the intervention was associated with higher levels of serotonin, its precursor, and its metabolite in the striatum of males and females and higher levels of serotonin's precursor in the prefrontal cortex of females only (Eckard et al., 2023). Importantly, the neural analysis did not account for the subregions that make up each of these brain regions, so it remains unclear how IL was affected by the time-based intervention. Taken together with the current study, it is possible that time-based interventions are effective in both sexes but through different cognitive mechanisms. Time-based interventions may improve impulsive action in males and interval timing in females, both resulting in enhanced self-control.

We also examined the relationship between sign- and goal-tracking like behavior and neurobiology. Previous research indicated that c-Fos may be increased in DMS for sign-trackers (Flagel, Cameron, et al., 2011), and that sign-tracking may occur more often in females than males (Hilz et al., 2021; Hughson et al., 2019; King et al., 2016; Pitchers et al., 2015; Stringfield et al., 2019). Clustering analyses of indirect measures of sign- and goal-tracking did detect any clusters in relation to DMS, but we did find that females showed higher levels of c-Fos in DMS compared to males. Taken together, these results may indicate that more direct measures of sign- and goal-tracking may cluster accordingly with DMS as evident in the significant sex difference.

Within the context of the intervention and choice contingencies, we predicted that FI schedules may bias rats towards sign-tracking and FT schedules may bias rats towards goal-tracking, resulting in schedule differences in DMS. Rats in the intervention conditions experienced the FI and FT schedules more than control groups, suggesting group and schedule may interact as well. In the current study, rats that experienced the FI schedule showed higher levels of c-Fos+ cells in DMS compared to rats that experienced the FT schedule. Coupled with group and sex differences in sign- and goal-tracking, we expected FI-Exp females to have the most c-Fos+ cells in DMS and FT-Exp males to have the least c-Fos+ cells in DMS. In the current experiment, FI-Exp females had the highest levels of c-Fos expression in DMS while FT-Exp males had some of the lowest levels of c-Fos expression.

We did not anticipate higher levels of c-Fos expression in all five brain regions for females. One possible explanation for this pattern may be differential receptivity to food restriction levels between males and females. Both sexes were restricted to 87% of their free-feeding body weights based on growth curves obtained from the commercial supplier, but this restriction level may affect males and females differently. In an experiment measuring the effects of stress on c-Fos and other markers, females in the experimental condition showed higher c-Fos expression compared to males in the experimental condition in multiple brain regions associated with stress despite being food restricted to the same percentage of their free-feeding body weight (Lenglos et al., 2013). In the current study, females also had higher levels of c-Fos expression across regions despite being food-restricted to the same percentage level as males, suggesting that further study is needed to determine how levels of food restriction affect neural activity in males and females.

It is also possible that the estrous cycle may have affected the neurobiological results of the current study. The estrous cycle is the recurring cycle of sexual fertility in rodents, and they cycle through four phases (proestrus → estrus → metestrus → diestrus) every 4-5 days (Marcondes et al., 2002; Westwood, 2008). The phases are driven by changes in two major sex hormones, estradiol and progesterone (Marcondes et al., 2002). Previous research has examined effects of female sex hormones on the striatum and the prefrontal cortex as both regions contain estrogen and progesterone receptors (Feng et al., 2004; Fernandez-Ruiz et al., 1989; Kuiper et al., 1997; Shughrue et al., 1997). Estradiol, which is highest during the proestrus phase, increases dopamine release in the dorsal striatum (Mermelstein et al., 1996). Estradiol blocks Ca2+ (calcium) needed to activate the estrogen receptors in this region, affecting the GABAergic regulation of dopamine release (Mermelstein et al., 1996). It is possible that females showed higher levels of neural activity in all five brain regions based on this mechanism of action. Likewise, progesterone may have influenced the current study's results. Progesterone is highest at the beginning of estrus (Marcondes et al., 2002), and administration of progesterone was associated with self-control in a variety of tasks (Llaneza & Frye, 2009; Schneider & Popik, 2007; Swalve et al., 2016; Swalve et al., 2018). Progesterone may also affect self-control and interval timing in freely-cycling female rats (Panfil et al., 2023). Progesterone may affect self-control in the prefrontal cortex by inhibiting dopamine release (Feng et al., 2004). Measurement or manipulation of the estrous cycle during time-based interventions may inform these questions of how neural activity is affected by female sex hormones.

It is important to note some limitations with the measurement of c-Fos as a marker of neural activity. First, the c-Fos protein is not exclusive to neurons, so measures of c-Fos in the current study may reflect activity in both neurons and glia (Lara Aparicio et al., 2022). Follow-

up studies may benefit from additional markers that are specific to these cell types to increase specificity in understanding activity. Second, the c-Fos protein offers little information about the role of inhibition because the marker is specific for activation (Lara Aparicio et al., 2022). In each of the five brain regions examined in the current study, it is possible the inhibition of neurons strongly contributed to the behavioral patterns as well. Cluster analyses may not have detected clusters in the neurobiology data because inhibition was not accounted for within this study. In vivo electrophysiological recordings would be necessary to measure inhibition. Along the same lines, c-Fos expression is a semi-quantitative measure of neural activity because immunohistochemistry is used to label endogenous c-Fos protein expression. Other measures such as in vivo electrophysiological recordings provide more direct evidence of activity in response to stimuli, but it is difficult to obtain these measurements in multiple brain regions in the same animal. In addition, tools such as ELISAs (enzyme-linked immunosorbent assays) offer direct measures of the level of c-Fos protein present in a sample, but samples for this technique are typically obtained with tissue punches, which is challenging to differentiate when dealing with subregions that are proximal to each other. Although indirect, c-Fos offered a highly region-specific approach to measuring neural activity in multiple brain regions in each animal in the current study. Future research may consider using more direct quantification measures in these regions to better understand how time-based interventions affect neural activity.

The c-Fos results may indicate that a complex network of brain regions contribute to impulsive choice, timing, attention, sign-tracking, and goal-tracking. Analyses of c-Fos showed significant differences between group, schedule, and/or sex in all five regions, suggesting that no single brain region adequately explained differences in behavior. The possible cognitive mechanisms underlying time-based intervention efficacy may be a product of both positive and

negative feedback loops that may rely on excitatory or inhibitory signaling. Likewise, these cognitive mechanisms may be specific to sex, as we saw different neurobiological patterns emerge across areas. Based on the differences in peak timing, it is possible that females make choices using different cognitive strategies than males, but in this case, any difference in cognitive processes did not strongly differentiate impulsive choices across sex. Future research may parse apart these possible cognitive processes using a circuit-level approach. For example, chemogenetic techniques such as DREADDs (designer receptors exclusively activated by designer drugs) and optogenetics may be used to target pathways like PL → DMS and IL → to DCS in males and females. In sum, the current results provide insights into possible circuit-level interactions resulting from time-based interventions in male and female rats, but further research is needed to determine how DMS, DCS, DLS, PL, and IL may be synergizing to produce behavioral effects.

Figure 5.1. Representative images of c-Fos expression in the dorsomedial striatum (first row), dorsocentral striatum (second row), and dorsolateral striatum (third row). The first column shows c-Fos+ cells imaged in the red fluorescent protein (RFP) channel. The second column reflects tissue imaged in the DAPI channel, which is used as a counterstain. The third column shows RFP and DAPI merged.

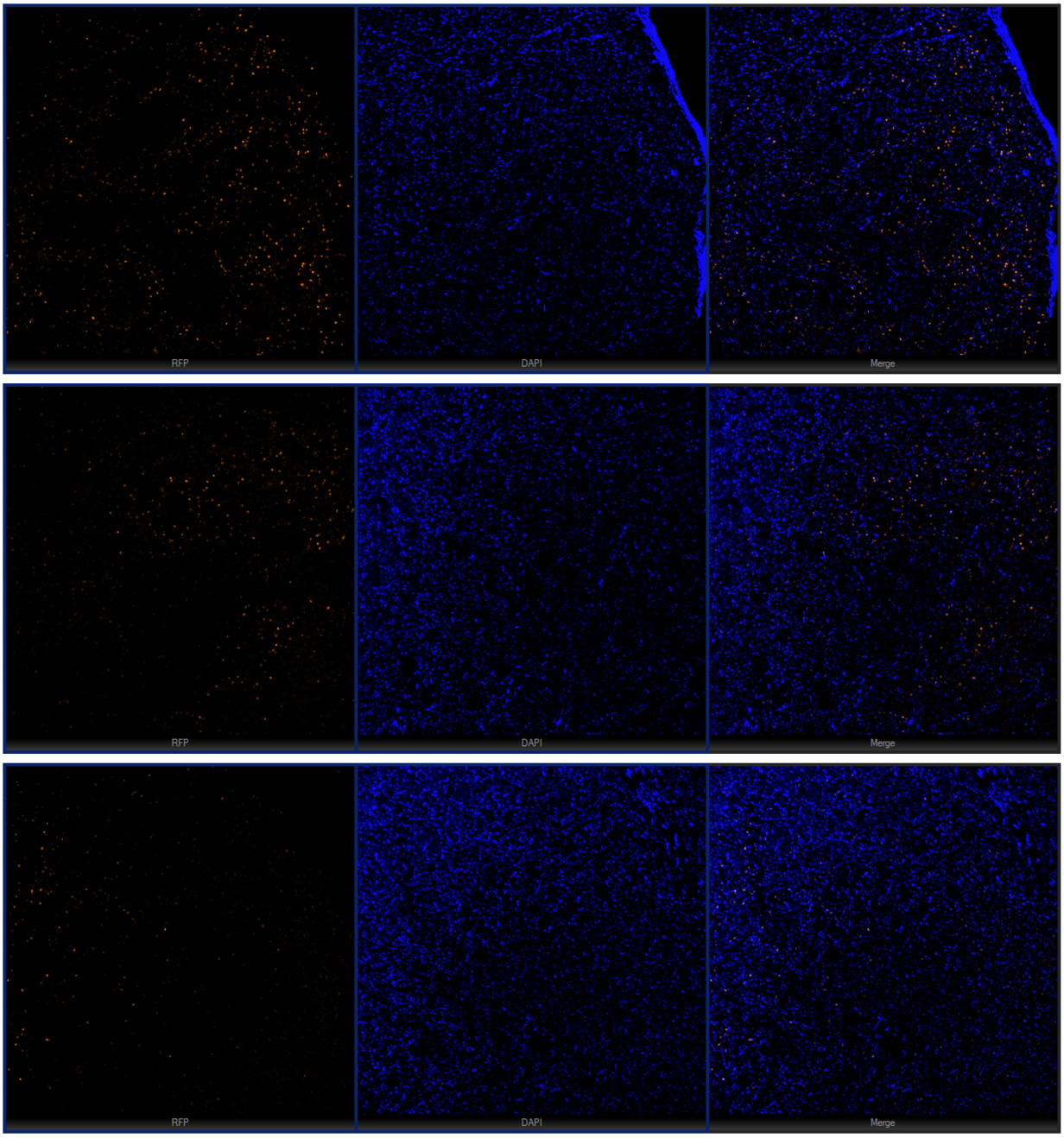


Figure 5.2. Representative images of c-Fos expression in the prelimbic cortex (first row) and the infralimbic cortex (second row). The first column is c-Fos+ cells imaged in the red fluorescent protein (RFP) channel. The second column is tissue imaged in the DAPI channel, which is used as a counterstain. The third column is RFP and DAPI merged.

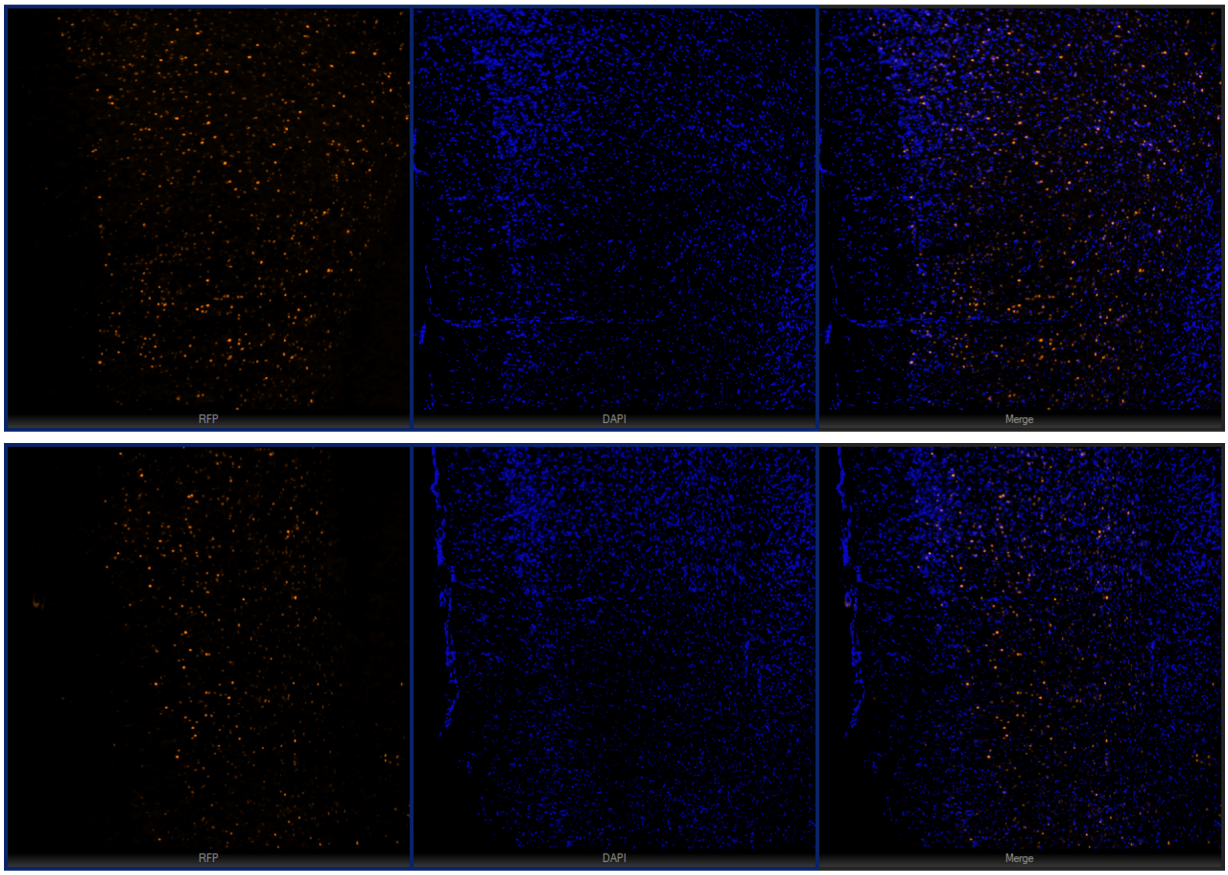


Figure 5.3. Mean number of c-Fos+ cells present in the dorsomedial striatum (DMS) with error bars (+/− SEM). FI-Exp females had the highest levels of c-Fos expression in DMS while FT-Exp males had some of the lowest levels of c-Fos expression. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

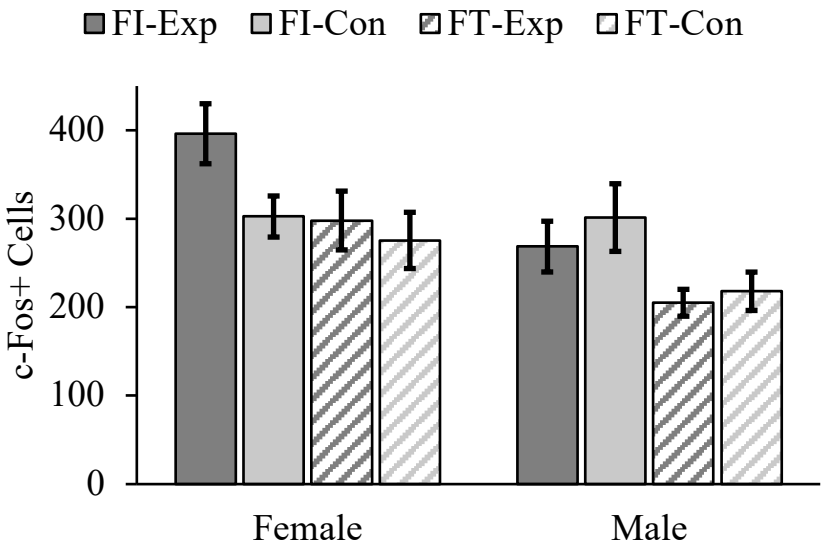


Figure 5.4. Mean number of c-Fos+ cells present in the dorsocentral striatum with error bars (+/− SEM). FI-Exp females and FI-Exp males had higher c-Fos expression than FT-Exp females and FT-Exp males. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

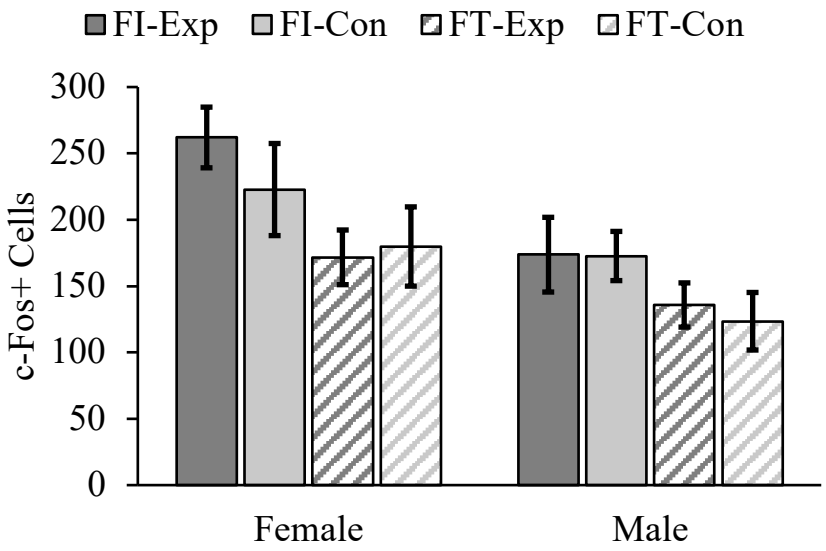


Figure 5.5. Mean number of c-Fos+ cells present in the dorsolateral striatum with error bars (+/− SEM). On average, rats that received the FI intervention had significantly higher c-Fos expression than rats that received the FT intervention. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

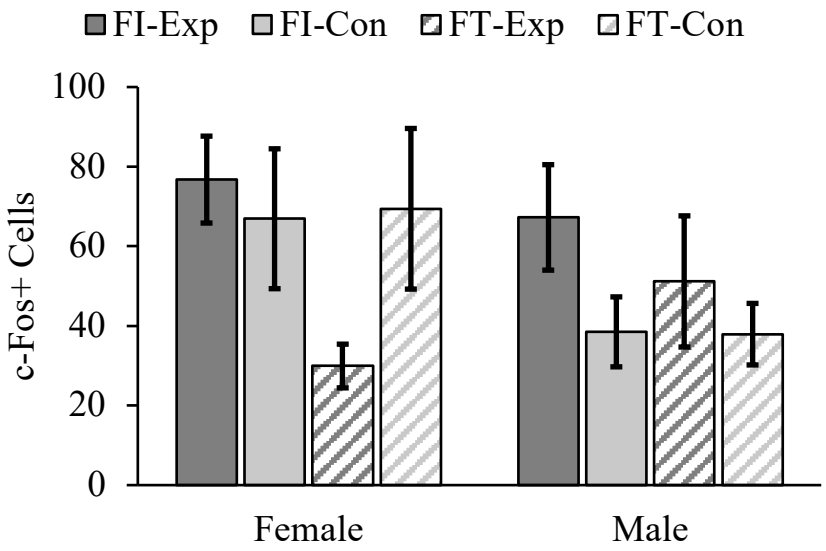


Figure 5.6. Mean number of c-Fos+ cells present in the prelimbic cortex with error bars (+/− SEM). FI-Exp females had the highest levels of c-Fos expression, but there were no significant differences between male FI-Exp and male FT-Exp groups. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

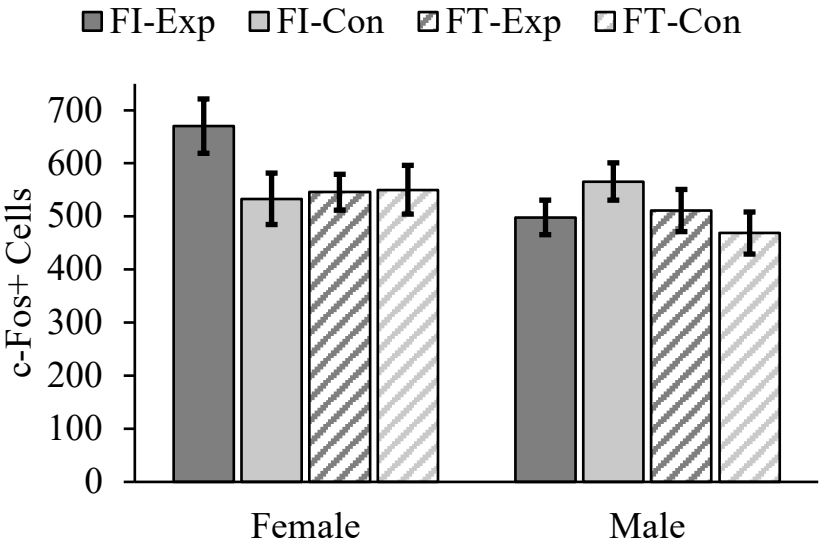


Figure 5.7. Mean number of c-Fos+ cells present in the infralimbic (IL) cortex with error bars (+/− SEM). On average, males that received the interventions showed lower levels of c-Fos+ cells in IL compared to male control groups, and male rats that received the FT schedule had lower levels of c-Fos compared to males that received the FI schedule. There were no differences in female conditions. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

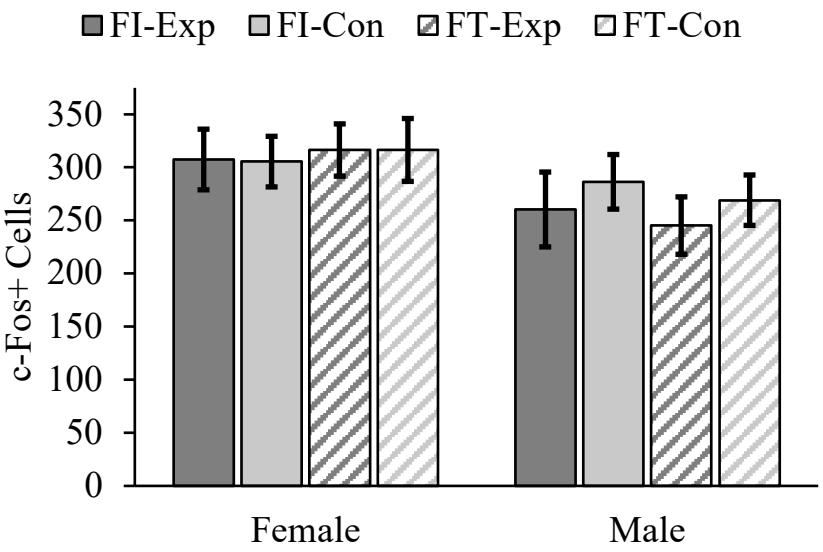


Figure 5.8. K-means clustering solution formed with average proportion of LL choices, choice function slope, the lever press response rate during the final 3 s of the 30-s LL peak from the choice task, and the number of c-Fos+ cells in dorsomedial striatum as dimensions. Clusters were stratified based on group, schedule, and sex with each symbol representing an individual rat. Cluster two contained only rats that received the FI schedule, and cluster three was mostly comprised of rats that received the FT schedule. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

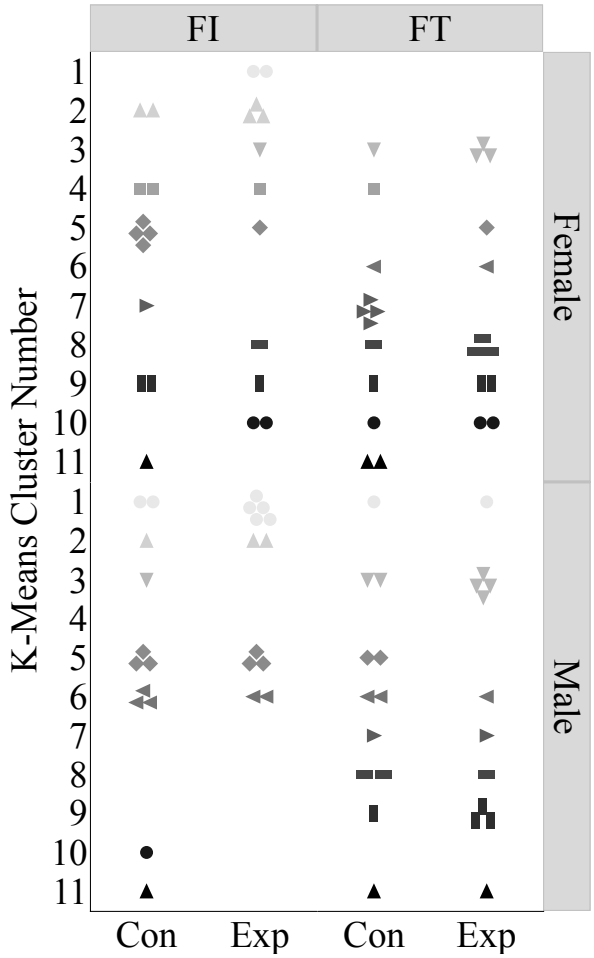


Figure 5.9. K-means clustering solution formed with average proportion of LL choices, choice function slope, the lever press response rate during the final 3 s of the 30-s LL peak from the choice task, and the number of c-Fos+ cells in dorsocentral striatum as dimensions. Clusters were stratified based on group, schedule, and sex with each symbol representing an individual rat. Clusters four and seven were made up of rats that received the FT schedule only and clusters five and eight were rats that received the FI schedule. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

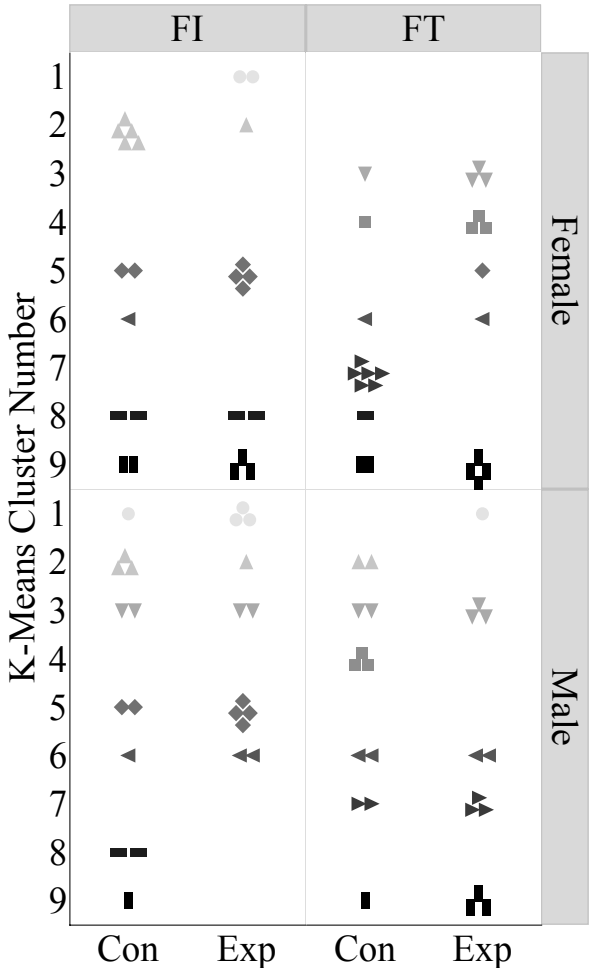


Figure 5.10. K-means clustering solution formed with average proportion of LL choices, choice function slope, the lever press response rate during the final 3 s of the 30-s LL peak from the choice task, and the number of c-Fos+ cells in dorsolateral striatum as dimensions. Clusters were stratified based on group, schedule, and sex with each symbol representing an individual rat. Cluster three contained rats that received the FT schedule only. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

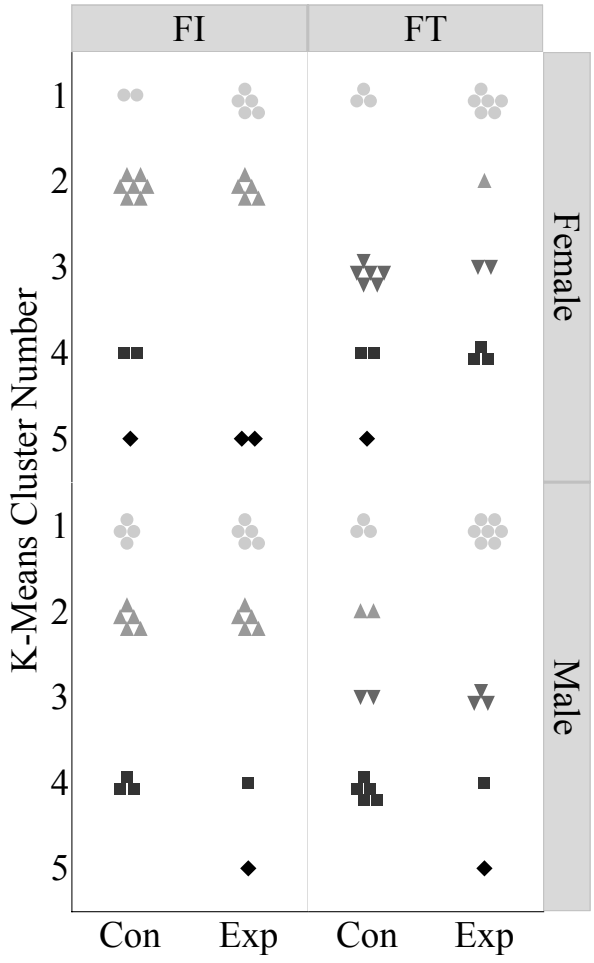


Figure 5.11. Hierarchical clustering solution formed with average proportion of LL choices, choice function slope, the lever press response rate during the final 3 s of the 30-s LL peak from the choice task, and the number of c-Fos+ cells in dorsolateral striatum as dimensions. Clusters were stratified based on group, schedule, and sex with each symbol representing an individual rat. Rats in cluster two received the FT schedule only. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control.

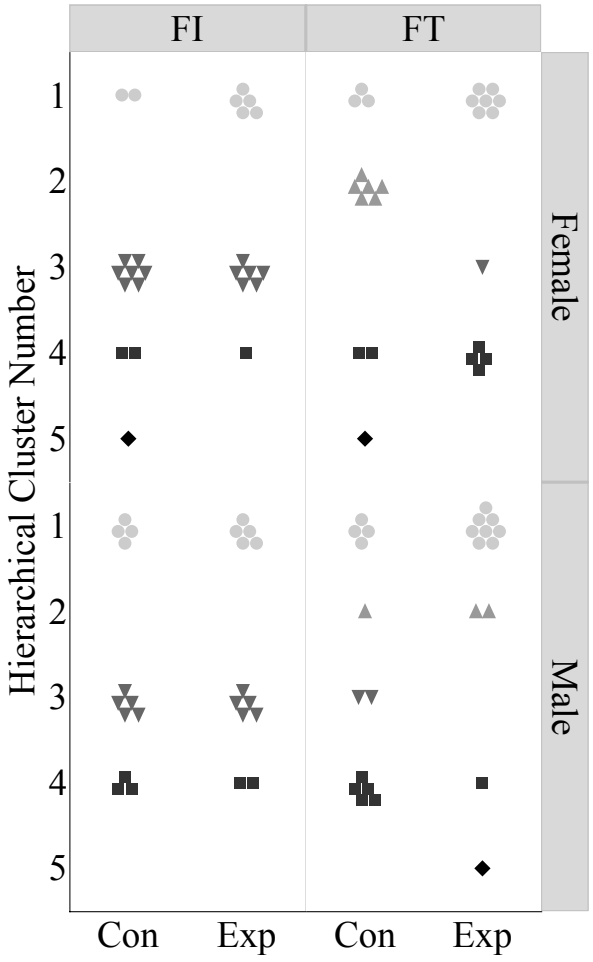


Figure 5.12. K-means clustering solution formed with the lever press and head entry response rates during the final 3 s of the 10- and 30-s intervention delays and the number of c-Fos+ cells in dorsocentral striatum as dimensions. Clusters were stratified based on schedule and sex with each symbol representing an individual rat. Only female rats were in cluster three while only male rats were in cluster six. Cluster nine was comprised of rats that received the FI schedule only. Rats in the control conditions were not included in this analysis because they did not receive the interventions. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental.

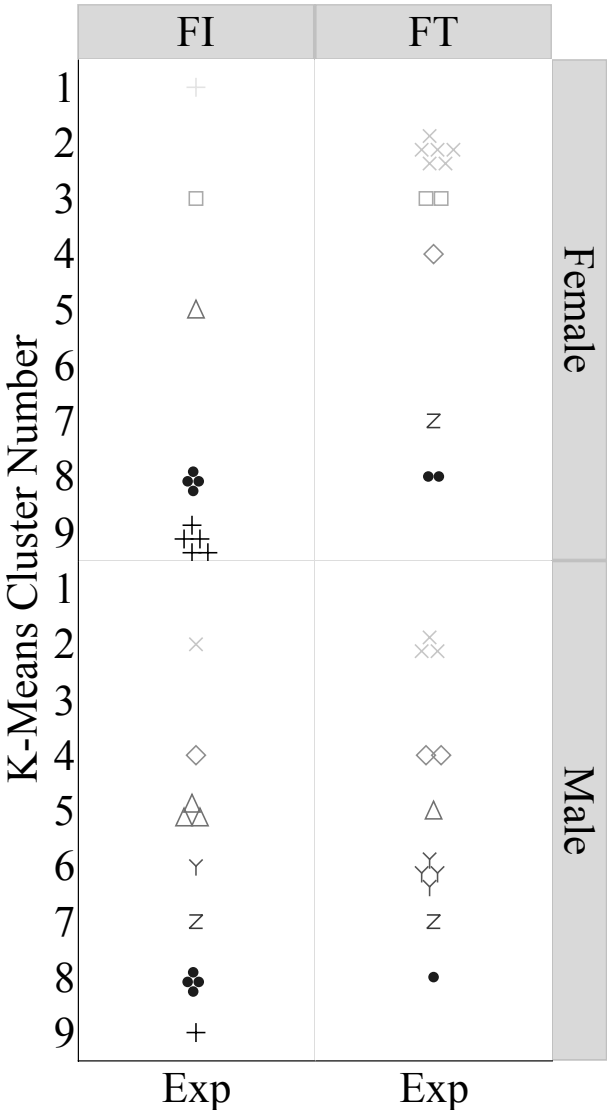


Table 5.1. Pairwise comparisons to further probe the Group × Schedule × Sex interaction when examining c-Fos in the dorsomedial striatum. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control; F = Female; M = Male.

Group Comparison	*b*	*t*	*p*
FI-Exp F – FI-Con F	0.270	12.23	<.001
FI-Exp F – FT-Exp F	0.285	12.85	<.001
FI-Exp F – FT-Con F	0.363	16.03	<.001
FI-Exp F – FI-Exp M	0.389	17.04	<.001
FI-Exp F – FI-Con M	0.273	12.39	<.001
FI-Exp F – FT-Exp M	0.659	26.52	<.001
FI-Exp F – FT-Con M	0.597	24.53	<.001
FI-Con F – FT-Exp F	0.015	0.64	1.00
FI-Con F – FT-Con F	0.093	3.89	.003
FI-Con F – FI-Exp M	0.119	4.93	<.001
FI-Con F – FI-Con M	0.004	0.16	1.00
FI-Con F – FT-Exp M	0.389	14.90	<.001
FI-Con F – FT-Con M	0.328	12.77	<.001
FT-Exp F – FT-Con F	0.079	3.25	.03
FT-Exp F – FI-Exp M	0.104	4.29	.001
FT-Exp F – FI-Con M	-0.011	-0.47	1.00
FT-Exp F – FT-Exp M	0.374	14.28	<.001
FT-Exp F – FT-Con M	0.313	12.15	<.001
FT-Con F – FI-Exp M	0.026	1.04	.97
FT-Con F – FI-Con M	-0.090	-3.73	.005
FT-Con F – FT-Exp M	0.296	11.10	<.001
FT-Con F – FT-Con M	0.234	8.95	<.001
FI-Exp M – FI-Con M	-0.115	-4.76	<.001
FI-Exp M – FT-Exp M	0.270	10.08	<.001
FI-Exp M – FT-Con M	0.208	7.92	<.001
FI-Con M – FT-Exp M	0.385	14.74	<.001
FI-Con M – FT-Con M	0.324	12.61	<.001
FT-Exp M – FT-Con M	-0.061	-2.19	.36

Note: p values were adjusted with the Tukey method.

Table 5.2. Pairwise comparisons to further understand the Group × Schedule × Sex interaction when examining c-Fos in the dorsocentral striatum. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control; F = Female; M = Male.

Group Comparison	*b*	*t*	*p*
FI-Exp F – FI-Con F	0.162	6.17	<.001
FI-Exp F – FT-Exp F	0.423	14.92	<.001
FI-Exp F – FT-Con F	0.376	13.46	<.001
FI-Exp F – FI-Exp M	0.411	14.56	<.001
FI-Exp F – FI-Con M	0.417	14.74	<.001
FI-Exp F – FT-Exp M	0.658	21.54	<.001
FI-Exp F – FT-Con M	0.752	23.87	<.001
FI-Con F – FT-Exp F	0.261	8.89	<.001
FI-Con F – FT-Con F	0.214	7.40	<.001
FI-Con F – FI-Exp M	0.249	8.52	<.001
FI-Con F – FI-Con M	0.255	8.70	<.001
FI-Con F – FT-Exp M	0.495	15.76	<.001
FI-Con F – FT-Con M	0.590	18.21	<.001
FT-Exp F – FT-Con F	-0.046	-1.51	.80
FT-Exp F – FI-Exp M	-0.012	-0.37	1.00
FT-Exp F – FI-Con M	-0.006	-0.19	1.00
FT-Exp F – FT-Exp M	0.235	7.08	<.001
FT-Exp F – FT-Con M	0.329	9.67	<.001
FT-Con F – FI-Exp M	0.035	1.14	.95
FT-Con F – FI-Con M	0.041	1.32	.89
FT-Con F – FT-Exp M	0.281	8.57	<.001
FT-Con F – FT-Con M	0.376	11.14	<.001
FI-Exp M – FI-Con M	0.006	0.19	1.00
FI-Exp M – FT-Exp M	0.246	7.45	<.001
FI-Exp M – FT-Con M	0.341	10.03	<.001
FI-Con M – FT-Exp M	0.241	7.26	<.001
FI-Con M – FT-Con M	0.335	9.85	<.001
FT-Exp M – FT-Con M	0.095	2.64	.14

Note: p values were adjusted with the Tukey method.

Table 5.3. Pairwise comparisons to further examine the Group × Schedule × Sex interaction when analyzing c-Fos in the dorsolateral striatum. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control; F = Female; M = Male.

Group Comparison	*b*	*t*	*p*
FI-Exp F – FI-Con F	0.137	2.84	.09
FI-Exp F – FT-Exp F	0.942	15.14	<.001
FI-Exp F – FT-Con F	0.100	2.10	.41
FI-Exp F – FI-Exp M	0.132	2.74	.11
FI-Exp F – FI-Con M	0.690	12.10	<.001
FI-Exp F – FT-Exp M	0.405	7.78	<.001
FI-Exp F – FT-Con M	0.705	12.31	<.001
FI-Con F – FT-Exp F	0.805	12.68	<.001
FI-Con F – FT-Con F	-0.037	-0.74	1.00
FI-Con F – FI-Exp M	-0.005	-0.10	1.00
FI-Con F – FI-Con M	0.553	9.47	<.001
FI-Con F – FT-Exp M	0.268	5.01	<.001
FI-Con F – FT-Con M	0.568	9.68	<.001
FT-Exp F – FT-Con F	-0.842	-13.33	<.001
FT-Exp F – FI-Exp M	-0.810	-12.77	<.001
FT-Exp F – FI-Con M	-0.252	-3.59	.008
FT-Exp F – FT-Exp M	-0.537	-8.08	<.001
FT-Exp F – FT-Con M	-0.237	-3.36	.02
FT-Con F – FI-Exp M	0.032	0.64	1.00
FT-Con F – FI-Con M	0.589	10.16	<.001
FT-Con F – FT-Exp M	0.305	5.74	<.001
FT-Con F – FT-Con M	0.605	10.37	<.001
FI-Exp M – FI-Con M	0.558	9.56	<.001
FI-Exp M – FT-Exp M	0.273	5.10	<.001
FI-Exp M – FT-Con M	0.573	9.77	<.001
FI-Con M – FT-Exp M	-0.284	-4.62	<.001
FI-Con M – FT-Con M	0.015	0.23	1.00
FT-Exp M – FT-Con M	0.300	4.85	<.001

Note: p values were adjusted with the Tukey method.

Table 5.4. Pairwise comparisons to assess the Group × Schedule × Sex interaction when examining c-Fos in the prelimbic cortex. FI = Fixed-Interval; FT = Fixed-Time; Exp = Experimental; Con = Control; F = Female; M = Male.

Group Comparison	*b*	*t*	*p*
FI-Exp F – FI-Con F	0.228	13.64	<.001
FI-Exp F – FT-Exp F	0.206	12.35	<.001
FI-Exp F – FT-Con F	0.197	11.86	<.001
FI-Exp F – FI-Exp M	0.297	17.37	<.001
FI-Exp F – FI-Con M	0.169	10.27	<.001
FI-Exp F – FT-Exp M	0.271	15.97	<.001
FI-Exp F – FT-Con M	0.357	20.56	<.001
FI-Con F – FT-Exp F	-0.023	-1.30	.90
FI-Con F – FT-Con F	-0.032	-1.80	.62
FI-Con F – FI-Exp M	0.068	3.78	.004
FI-Con F – FI-Con M	-0.059	-3.40	.02
FI-Con F – FT-Exp M	0.042	2.37	.26
FI-Con F – FT-Con M	0.129	7.05	<.001
FT-Exp F – FT-Con F	-0.009	-0.50	1.00
FT-Exp F – FI-Exp M	0.091	5.08	<.001
FT-Exp F – FI-Con M	-0.036	-2.10	.41
FT-Exp F – FT-Exp M	0.065	3.67	.006
FT-Exp F – FT-Con M	0.152	8.35	<.001
FT-Con F – FI-Exp M	0.100	5.58	<.001
FT-Con F – FI-Con M	-0.028	-1.61	.75
FT-Con F – FT-Exp M	0.074	4.16	<.001
FT-Con F – FT-Con M	0.160	8.84	<.001
FI-Exp M – FI-Con M	-0.127	-7.18	<.001
FI-Exp M – FT-Exp M	-0.026	-1.42	.85
FI-Exp M – FT-Con M	0.061	3.28	.02
FI-Con M – FT-Exp M	0.102	5.77	<.001
FI-Con M – FT-Con M	0.188	10.44	<.001
FT-Exp M – FT-Con M	0.087	4.69	<.001

Note: p values were adjusted with the Tukey method.

Table 5.5. Mean dimension and standard deviation (SD) values per cluster of k-means and hierarchical clustering, which included average proportion of LL choices, choice function slope, the lever press response rate during the final 3 s of the 30-s LL peak from the choice task, and the number of c-Fos+ cells in dorsomedial striatum (DMS). The number of animals (*n*) per cluster was displayed as well.

Cluster		LL Choice		Choice Slope		LL Peak Rate		c-Fos in DMS	
K-Means	*n*	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	11	0.920	0.039	0.007	0.007	0.432	0.060	283	68
2	8	0.481	0.172	0.038	0.003	0.455	0.086	459	85
3	12	0.815	0.085	0.021	0.006	0.263	0.072	231	58
4	4	0.127	0.072	0.008	0.006	0.386	0.112	428	56
5	14	0.428	0.150	0.038	0.009	0.393	0.069	249	51
6	10	0.115	0.067	0.010	0.009	0.345	0.075	169	58
7	7	0.408	0.231	0.036	0.008	0.075	0.048	171	33
8	8	0.166	0.141	0.011	0.007	0.127	0.068	325	52
9	10	0.925	0.057	0.001	0.007	0.187	0.077	239	78
10	6	0.839	0.108	0.010	0.010	0.240	0.081	458	58
11	6	0.579	0.162	0.049	0.007	0.181	0.090	300	72
Hierarchical									
1	96	0.556	0.324	0.021	0.017	0.292	0.143	283	112

Table 5.6. Mean dimension and standard deviation (SD) values per cluster of k-means and hierarchical clustering, which included average proportion of LL choices, choice function slope, the lever press response rate during the final 3 s of the 30-s LL peak from the choice task, and the number of c-Fos+ cells in dorsocentral striatum (DCS). The number of animals (*n*) per cluster was displayed as well.

Cluster			LL Choice		Choice Slope		LL Peak Rate		c-Fos in DCS	
K-Means		*n*	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	1	7	0.918	0.038	0.007	0.005	0.448	0.066	216	47
	2	12	0.467	0.156	0.036	0.008	0.389	0.060	131	50
	3	13	0.859	0.098	0.013	0.009	0.328	0.072	88	40
	4	7	0.192	0.135	0.015	0.007	0.092	0.041	214	61
	5	13	0.478	0.180	0.039	0.006	0.410	0.106	275	40
	6	10	0.097	0.067	0.011	0.010	0.304	0.085	89	35
	7	11	0.578	0.196	0.043	0.010	0.103	0.067	133	49
	8	7	0.138	0.061	0.007	0.008	0.367	0.108	335	75
	9	16	0.893	0.084	0.005	0.009	0.200	0.079	206	65
Hierarchical										
	1	96	0.556	0.324	0.021	0.017	0.292	0.143	180	93

Table 5.7. Mean dimension and standard deviation (SD) values per cluster of k-means and hierarchical clustering, which included average proportion of LL choices, choice function slope, the lever press response rate during the final 3 s of the 30-s LL peak from the choice task, and the number of c-Fos+ cells in dorsolateral striatum (DLS). The number of animals (*n*) per cluster was displayed as well.

Cluster			LL Choice		Choice Slope		LL Peak Rate		c-Fos in DLS	
K-Means		*n*	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	1	35	0.889	0.085	0.008	0.009	0.300	0.117	42	34
	2	25	0.473	0.169	0.037	0.008	0.395	0.084	56	32
	3	13	0.584	0.191	0.039	0.012	0.099	0.064	52	36
	4	17	0.112	0.072	0.011	0.010	0.238	0.126	36	21
	5	6	0.152	0.073	0.014	0.011	0.380	0.127	184	52
Hierarchical										
	1	38	0.880	0.089	0.010	0.011	0.288	0.122	42	33
	2	9	0.535	0.203	0.046	0.009	0.090	0.071	56	43
	3	26	0.455	0.192	0.036	0.010	0.406	0.093	64	38
	4	20	0.137	0.107	0.011	0.010	0.236	0.133	41	31
	5	3	0.176	0.019	0.012	0.004	0.328	0.077	231	30

Table 5.8. Mean dimension and standard deviation (SD) values per cluster of k-means and hierarchical clustering, which included the lever press and head entry response rates during the final 3 s of the 10- and 30-s intervention delays and the number of c-Fos+ cells in dorsocentral striatum (DCS). Rats in the control conditions were not included in this analysis because they did not receive the interventions. LP10 = Lever press response rate on the 10-s intervention delay; LP30 = Lever press response rate on the 30-s intervention delay; HE10 = Head entry response rate on the 10-s intervention delay; HE30 = Head entry response rate on the 30-s intervention delay.

Cluster		LP10		LP30		HE10		HE30		c-Fos in DCS	
K-Means	*n*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1	0.791	0	0.022	0	0.167	0	0.231	0	328	0
2	10	0.390	0.108	0.331	0.124	0.185	0.056	0.082	0.026	181	34
3	3	0.460	0.097	0.007	0.004	0.465	0.022	0.183	0.118	270	18
4	4	0.578	0.113	0.459	0.113	0.406	0.058	0.437	0.019	171	30
5	5	0.680	0.045	0.555	0.082	0.166	0.048	0.230	0.116	282	32
6	5	0.343	0.095	0.375	0.125	0.422	0.089	0.137	0.082	80	28
7	3	0.382	0.149	0.594	0.079	0.092	0.064	0.069	0.019	82	51
8	11	0.685	0.088	0.513	0.098	0.199	0.054	0.139	0.077	127	46
9	6	0.769	0.054	0.632	0.062	0.294	0.070	0.063	0.027	306	46
Hierarchical											
1	48	0.558	0.186	0.439	0.194	0.254	0.127	0.152	0.123	186	89

Chapter 6 - General Discussion

Impulsive behavior is associated with Attention-Deficit/Hyperactivity Disorder (ADHD), major depressive disorder, schizophrenia, gambling, substance abuse, and obesity, but these disorders and problematic behaviors affect men and women differently in the prevalence of and progression to disease states (Becker & Hu, 2008; Fattore & Melis, 2016; Hing et al., 2016; Iacono & Beiser, 1992; Kimokoti et al., 2013; Ramtekkar et al., 2010; Randall et al., 1999; Weafer et al., 2015; Williams et al., 2015). Most neuroscience research is conducted in male subjects only, resulting in a paucity of information about sex differences in the brain, cognition, and behavior. The current study aimed to address multiple gaps in the time-based intervention and decision-making fields by comparing two time-based interventions that have successfully promoted self-control when delivered for an extended number of sessions (Panfil et al., 2020; Rung et al., 2018; Smith et al., under review). No previous studies have examined the efficacy of these interventions in males and females when they are delivered for fewer sessions or neural activity following time-based interventions in males or females. We examined multiple behaviors throughout the course of the experiment to better understand the effects of abbreviated time-based interventions.

In Chapter 3, analyses of lever pressing and head entries into the cup where food was delivered during the intervention phase showed that these behaviors differed based on schedule and sex. Rats that received the abbreviated FI intervention pressed the levers more as a function of time in the intervention trials, suggesting this group learned to anticipate reward delivery. The FI-Exp group also spent little time entering the food cup. Unexpectedly, rats that received the abbreviated FT intervention also spent little time entering the food cup. The FT-Exp group pressed the levers often during the intervention trials, but responses did not increase leading up

to reward delivery. Instead, FT-Exp rats maintained or decreased responding as time progressed. While there were key distinctions between intervention conditions, these results did not map on to any robust group differences in the impulsive choice task.

In Chapter 4, lever pressing during peak interval and forced-choice trials was the focus of analysis because rats made few head entries into the food cup across conditions and trial types, curtailing the analysis of this behavior. On FI peak trials, female FI-Exp rats were most sensitive to delay in terms of peak time while female FI-Con rats were most sensitive to delay in terms of peak spread. Although females in the FI condition showed enhanced delay sensitivity, they typically had larger peak spreads than males despite their improvements in timing as the SS delay increased. On FT peak trials, rats responded most at the beginning of the trials and decreased responding as time progressed into peak trials. FT-Con females and males decreased initial responding (i.e., intercept) as SS delay increased compared to FT-Exp conditions. Across experimental and control groups, FT males showed a steeper decrease in responding as time progressed into peak trials compared to FT females. On forced-choice trials, similar responding patterns occurred where rats in the FI conditions increasingly pressed the levers leading up to anticipated reward delivery while rats in the FT conditions decreased in lever pressing leading up to food delivery. Finally, on free-choice trials, all conditions showed similar sensitivity to delay and made more LL choices as the SS delay increased. At a hypothetical 0-s SS delay, the analysis predicted that experimental groups made more LL choices than the control groups, regardless of schedule.

Altogether, examination of behavior across conditions yielded multiple new insights for the time-based intervention and decision-making fields. First and foremost, we expected differences in LL choices and delay sensitivity based on group and schedule. Based on previous

research, we hypothesized that the FI schedule would increase delay sensitivity compared to the FT schedule, and we predicted that the experimental groups would show higher levels of LL choices compared to the control groups. Instead, we found no differences in delay sensitivity between FI and FT schedules, and the experimental groups only differed from control groups in LL choices at the 0-s intercept. While the current study partially replicated previous research, the delay sensitivity observed here in the FT groups was unexpected. Responses made during the intervention and other trial types may provide some clarification to the choice results. We predicted that the FI schedule would encourage lever pressing while the FT schedule would encourage head entries, both of which would increase over time in anticipation of food delivery. However, rats in FI and FT conditions interacted with the levers often and few head entries were made throughout the course of the experiment. A key methodological difference between the current study and previous literature with the FT schedule may provide a possible explanation for this discrepancy. In previous studies, the lever retracted after the lever press that initiated the delay. The current study delivered the FT schedule such that the lever remained extended in the operant chamber during the associated delay and retracted when the delay elapsed immediately prior to the reward delivery, mirroring the FI schedule in that regard. The lever remaining in the chamber may have shaped behavior in the intervention and choice phases for the FT schedule.

Within both the intervention and choice contexts, the lever remaining in the chamber allowed for lever pressing to occur throughout the trials for the FT conditions, but this opportunity to lever press may have acted beneficially or antagonistically depending on the dependent measure. In the intervention, rats did not make any choices between the levers because only one lever was available in a session. Instead, rats likely learned to time 10- and 30-s delays individually. FT rats did not increase lever presses or head entries as the 10- and 30-s

intervals approached. Based on this lack of anticipation, the lever remaining available may have negatively affected interval timing. In the impulsive choice task, we measured interval timing in addition to choices between levers. In both FT-Exp and FT-Con groups, rats did not enter the food cup often but maintained interaction with levers. However, the lever presses did not increase in anticipation of food delivery. Like the intervention, the lack of anticipation suggests that interval timing ability was negatively affected. Examination of lever press and head entry behavior suggests that the lever may have acted as a distractor that pulled attention away from timing. In contrast, in the choice context, the lever availability may have positively impacted delay sensitivity because the FI and FT schedules showed similar slopes. This suggests that the rats in both schedules tracked the choice delays in the choice task even though the FT group did not show anticipatory timing of the delays. In all, the lever remaining in the chamber may have beneficially affected delay sensitivity while impairing interval timing in the FT conditions, suggesting that interval timing ability may not be essential to time-based intervention efficacy.

It is important to note how the FT and FI conditions may differ. Rats were required to press once to initiate the delay and press once more after the delay elapsed to receive a reward. Previous literature has proposed that time-based interventions positively affect both interval timing and choice, and perhaps in a casual manner (Marshall et al., 2014). Improved interval timing ability may lead to increased LL choices and delay sensitivity. Taken together with the FT conditions, the response requirement of the FI schedule for food delivery may positively affect both interval timing and choice. In contrast, the FT schedule with the lever available (but with no second response requirement) may only positively affect choice while negatively affecting timing.

It remains unclear why the rats in the FT conditions continued to press the levers despite no response requirement for food delivery. Previous research suggests that the rats in the current study learned an association between the lever and food delivery despite delayed reinforcement. In past work, rats acquired lever pressing behavior when reinforcement was delayed up to 30 s (Byrne et al., 1997; Critchfield & Lattal, 1993; Escobar & Bruner, 2007; Lattal & Gleeson, 1990; LeSage et al., 1996; Sutphin et al., 1998; van Haaren, 1992; Wilkenfield et al., 1992). In the current study, the FT-Exp rats may have learned an association between the lever and food delivery, but response rates during intervention, peak interval, and forced-choice trials suggest that they did not learn to anticipate the time of food delivery. Rats in the FT conditions responded most on the levers towards to the beginning of trials and tapered off in responding as food delivery approached. This suggests that the FT rats may have associated lever insertion with food delivery, resulting in more lever presses at the beginning of trials than at the end of trials like rats in the FI conditions.

This association may have encouraged more attention towards the lever compared to previous studies where the lever was unavailable during the delays. Greater attention to the levers may have promoted delay sensitivity, as originally hypothesized in the temporal processing and attention hypotheses displayed in Figure 2.2. This seemingly small procedural detail may account for differences in the literature where FI interventions promote temporal perception and choice (Peterson & Kirkpatrick, 2016; Smith et al., 2015; Stuebing et al., 2018), and FT interventions promote choice but not timing (Rung et al., 2018). Further research is required to confirm this possibility. It may be of interest to compare choice and timing measures directly in groups where rats receive an FI schedule with the levers available during delays, an FT schedule with the levers unavailable during delays, and an FT schedule with levers available

during delays. The current study did not include an FT schedule condition with the levers unavailable during the delays. Along the same lines, testing these proposed conditions with a third dummy lever available or where the two levers remain available during the ITI may shed further light on an association formed between the lever and food delivery. If rats do not continue to lever press after food delivery, this could suggest that incidental reinforcement shaped the response patterns in the current study. If lever pressing continues during the ITI after the receipt of food and before the next trial starts, then it is possible the lever may have acted as a source of self-stimulation instead. Likewise, interaction with a third dummy lever may also indicate that the lever may be used for self-stimulation.

While the lever procedural detail may account for FT and FI schedule effects on delay sensitivity in choice behavior, it is also possible that the short duration of the interventions contributed to the relatively weak intervention effects shown in the current study. However, a recent study in our laboratory showed that the abbreviated FI intervention was effective in male rats when comparing pre-intervention to post-intervention choices (Panfil et al., in preparation). It is possible that we did not replicate this effect in the current study because of the high error variance within this data set. An added source of variability in the current study may have stemmed from shipping stress. Rats were ordered from a commercial vendor and shipped to the research facility. For unknown reasons, one of the four shipments of male rats (14 animals total) experienced an environmental stressor that resulted in an animal's death. The remaining male rats that experienced the stressor were randomly assigned across conditions in one squad. Research has shown long-lasting impacts of adolescent transportation on physiology and behavior such as heart rate, corticosterone (a hormone associated with stress), locomotor activity, and social interaction (Arts et al., 2012). Some of these effects dissipate after an acclimation

period, but levels of corticosterone do not. We included squad as a variable in the models (data not shown) and found no significant effects. While there were no effects of squad on our results, it is still possible that the shipping stress may have added to error variance in this study compared to previous studies in our laboratory.

Regardless of added variability in males due to shipping stress, more sessions of intervention training for the FT conditions may be necessary to produce robust increases in LL choice as demonstrated in previous studies (Rung et al., 2018; Smith et al., under review). This may be of particular importance for female rats. In both FI and FT conditions, females completed fewer intervention trials than males, suggesting that female rats may benefit from training sessions that are delivered over a longer period of time or fewer trials per session with more sessions. It is possible that females completed fewer intervention trials because they reached satiation before males did. If females reached satiety earlier than males did, motivation to finish all intervention trials in a session may have decreased.

The estrous cycle, the recurring cycle of sexual fertility in rodents, may have affected satiety during the intervention. Female rats cycle every 4-5 days through four phases during the estrous cycle: proestrus, estrus, metestrus, and diestrus (Marcondes et al., 2002; Westwood, 2008). Two major sex hormones, estradiol and progesterone, fluctuate across the estrous cycle (Marcondes et al., 2002). The proestrus phase, when estradiol is at its highest across the entire cycle, was associated with decreased food intake when offered choices between food and access to sexually mature males (Yoest, 2018). This suggests that FI-Exp and FT-Exp females may have benefited from longer sessions or more sessions to account for the effects of the estrous cycle on satiety when directly compared to males.

In the same manner, the estrous cycle may affect learning of the intervention, choice tasks, and underlying cognitive processes. Previous research reported that female rats were more sensitive to delay during proestrus and made more LL choices at shorter delays during estrus (Panfil et al., 2023). These differences may have occurred based on what stage of the estrous cycle females were in when learning the task. In another study, females in proestrus (or who received injections of estradiol to mimic proestrus) acquired tasks faster than females in the other stages of the cycle or ovariectomized females (Dalla & Shors, 2009). In the current study, rats experienced two sessions of training on the 10-s delay and four sessions of training on the 30-s delay during the intervention phase and ten sessions of testing on an impulsive choice task where the delays to reward changed often. Learning of these intervals during the intervention and/or the choice task may have been affected by estrous cycles. It is possible that female rats may learn delays more efficiently in certain stages of the estrous cycle. In the current study, the female rats' estrous cycles may not have aligned in a beneficial manner with the delay change given that the estrous cycle length is approximately 4-5 days and intervention and choice delays typically changed more often than that. We did not measure estrous cycles in the current study, so it is unclear how this factor contributed to the results. It is possible that estrous cycle effects worked positively or negatively at an individual level regardless of group and schedule assignments, increasing within-subjects variance and decreasing ability to detect effects. Future research is needed to assess how the estrous cycle affects intervention efficacy. In sum, while the length of intervention may be appropriate for males, females may require further sessions to bolster against influences of satiety and the estrous cycle on learning and motivation.

In Chapter 5, we measured c-Fos expression in three striatal subregions and two prefrontal cortical regions and compared this measure of neural activity between group,

schedule, and sex. Many of the brain regions showed differential activity based on conditions but not in relation with other behavioral dependent measures. Exploratory cluster analyses did not strongly correspond with the hypotheses related to interval timing and/or temporal attention. We predicted that the FI schedule may improve temporal processing, resulting in enhanced delay sensitivity compared to the FT schedule, and neural activity in PL and DMS may underlie this relationship. Instead, both schedules resulted in similar delay sensitivities, and timing, choice, and delay sensitivity did not correspond with PL and DMS activity. We conducted further exploratory cluster analyses to see if the time-based interventions promoted LL choices through temporal attention by removing all dimensions related to interval timing. However, these analyses also did not detect any clusters. Taken together, the cluster analyses suggest that a network of regions and circuitry may be involved in timing and temporal attention. Differences in c-Fos at the group, schedule, and sex levels suggest that a more direct assessment of neural circuitry through advanced neuroscientific techniques may be necessary. Modulating activity in the PL → DMS and IL → DCS pathways with DREADDs (designer receptors exclusively activated by designer drugs) or optogenetics may offer more insights into the relationships between choice, timing, and attention.

In addition to temporal attention, sign- and goal-tracking behaviors may relate to c-Fos expression in DMS. Clustering analyses with lever press and head entry response rates did not detect any clusters in relation to DMS, but females showed higher levels of c-Fos in DMS compared to males. Perhaps more direct measures of sign- and goal-tracking would produce clusters in relation to DMS. For example, sign- and goal-tracking behaviors can be measured with recording devices in the operant chambers, so that time spent in and around the lever and

food cup may be quantified. This may be a more sensitive measure of sign- and/or goal-tracking than lever presses and head entries alone.

Like the behavioral results, lever availability, intervention length, and the estrous cycle may have also contributed to neurobiological results. Clusters may have emerged if there were robust delay sensitivity differences between schedules, and lever availability likely affected this aspect. Along the same lines, intervention length may have influenced neurobiology more strongly than behavior. Previous research has examined c-Fos expression during the acquisition of behavioral tasks, and expression changed based on the stage of learning (Anokhin et al., 1991; Anokhin & Rose, 1991; Bertaina-Anglade et al., 2000; Nikolaev et al., 1992). In early training sessions, c-Fos activity was higher in regions related to novelty, and in later sessions, activity was higher in regions related to the task features. After performance stabilized, there were small differences in c-Fos (Anokhin et al., 1991; Anokhin & Rose, 1991; Bertaina-Anglade et al., 2000) or no differences compared to control conditions (Nikolaev et al., 1992). The abbreviated interventions resulted in sizeable differences in c-Fos expression in multiple brains regions related to self-control and timing, but these effects were relatively weak in the impulsive choice task. More training sessions may be needed for neurobiological differences to manifest as behavioral differences. Future research may test the effects of time-based interventions on c-Fos expression in a dose-dependent manner by manipulating the number of sessions received to better understand the time course of neurobiological and behavioral differences.

Altogether, lever availability, intervention length, and the estrous cycle may have impacted behavioral and neurobiological results. Overall, these results suggest that abbreviated time-based interventions were modestly effective at promoting LL choices in male and female rats, and the interventions were associated with differential neural activity in striatal and cortical

regions. Differences in neural activity suggest that experimental conditions may have diverged further with more training. In other words, more sessions of intervention training may result in more robust group differences in intervention efficacy, which could then correspond with clearer patterns in neurobiology. It is likely that lever availability during the FT schedule affected delay sensitivity in a way that promoted temporal attention but may have impaired interval timing. Taken together, the current results suggest that interval timing ability is not essential to the efficacy of time-based interventions but may be one possible cognitive mechanism that underlies fixed-interval interventions in female rats. The current study produces a variety of questions that future research may focus on including the effects of the estrous cycle on time-based interventions and corresponding neurobiology and direct measures of sign- and goal-tracking during time-based interventions and impulsive choice tasks.

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Appendix A - Supplemental Data

The following supplemental materials contain control tissue fluorescent images and alternative views and information about higher order interactions for peak timing results. Figure A.1 shows no primary and no antibody control conditions that were used to confirm specificity of the secondary antibody and level of autofluorescence within the brain tissue. Figures A.2 and A.3 depict SS and LL peak timing for rats that received the FI schedule. Figures A.4 and A.5 show SS and LL peak timing for rats that received the FT schedule. In Tables A.1 and A.2, peak time and spread values are shown for FI SS and FI LL peak timing, respectively. Coefficient of variation values were also included in Table A.3. In Tables A.4 and A.5, peak time and spread values are shown for FT SS and FT LL peak timing, respectively.

Figure A.1. No antibody control tissue in the striatum (first row) and cortex (second row) that received the same treatment as experimental tissue but no primary or secondary antibodies and no primary control tissue in the striatum (third row) and cortex (fourth row) that received the same treatment as experimental tissue but no primary antibody. The first column shows tissue imaged in the red fluorescent protein (RFP) channel. The second column reflects tissue imaged in the DAPI channel. The third column shows RFP and DAPI merged together.

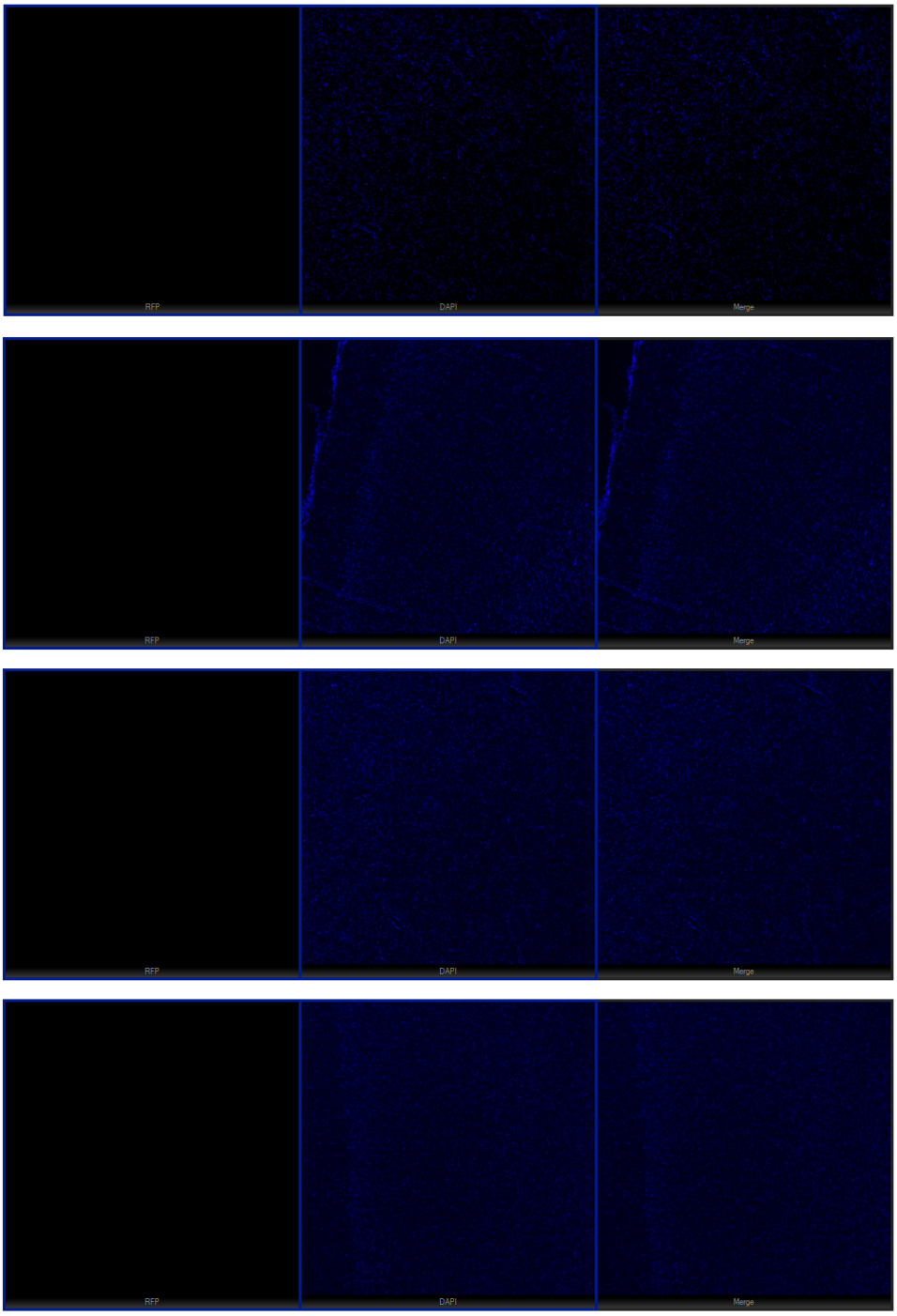

Figure A.2. Alternative views of average SS peak times and spreads with error bars (+/− SEM) for rats in the FI (fixed-interval) conditions. Horizontal lines denote target intervals. Note the truncated axes. Exp = Experimental; Con = Control.

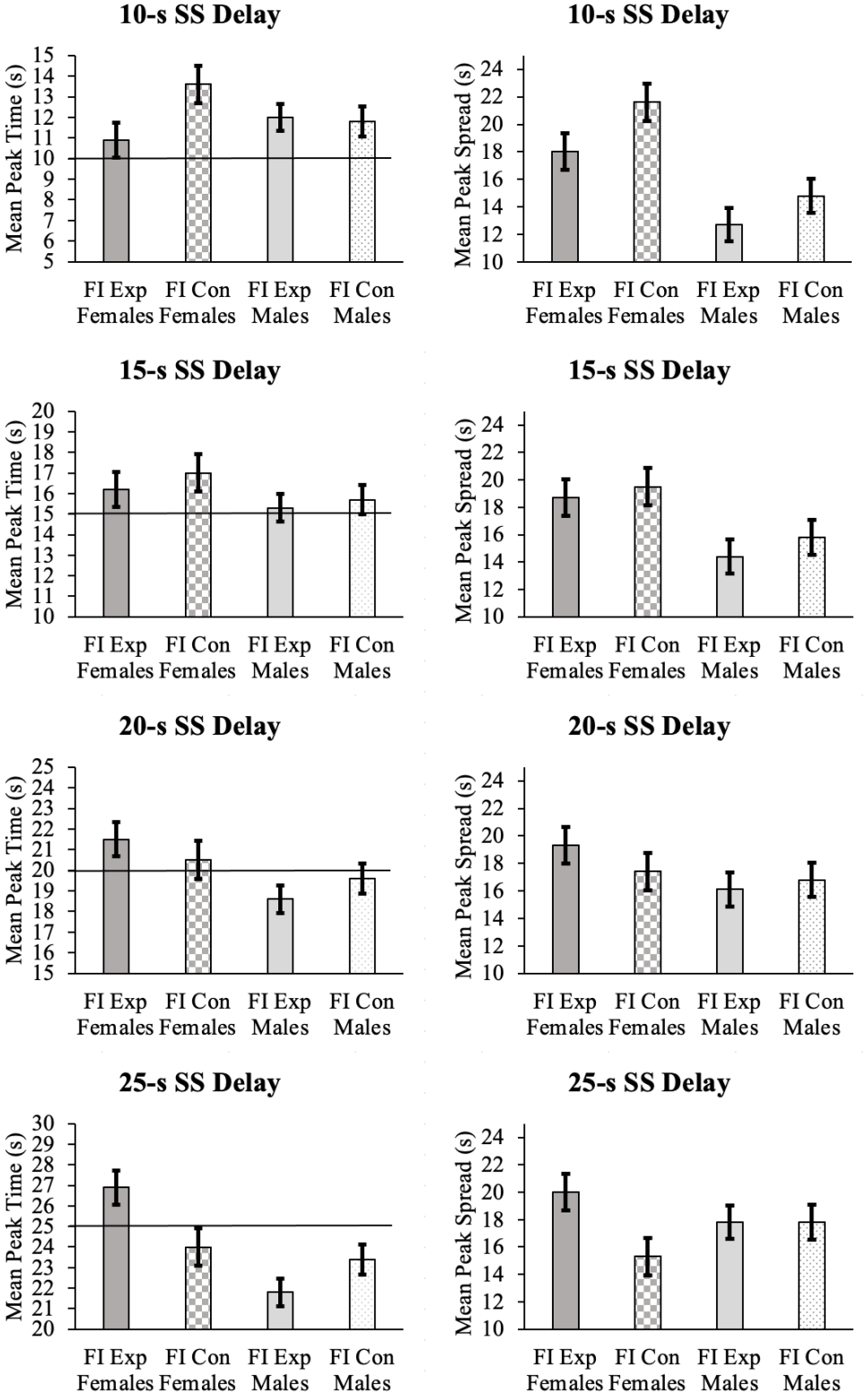

Figure A.3. Alternative views of average LL peak times and spreads with error bars (+/− SEM) for rats in the FI (fixed-interval) conditions. Horizontal lines denote target intervals. Note the truncated axes. Exp = Experimental; Con = Control.

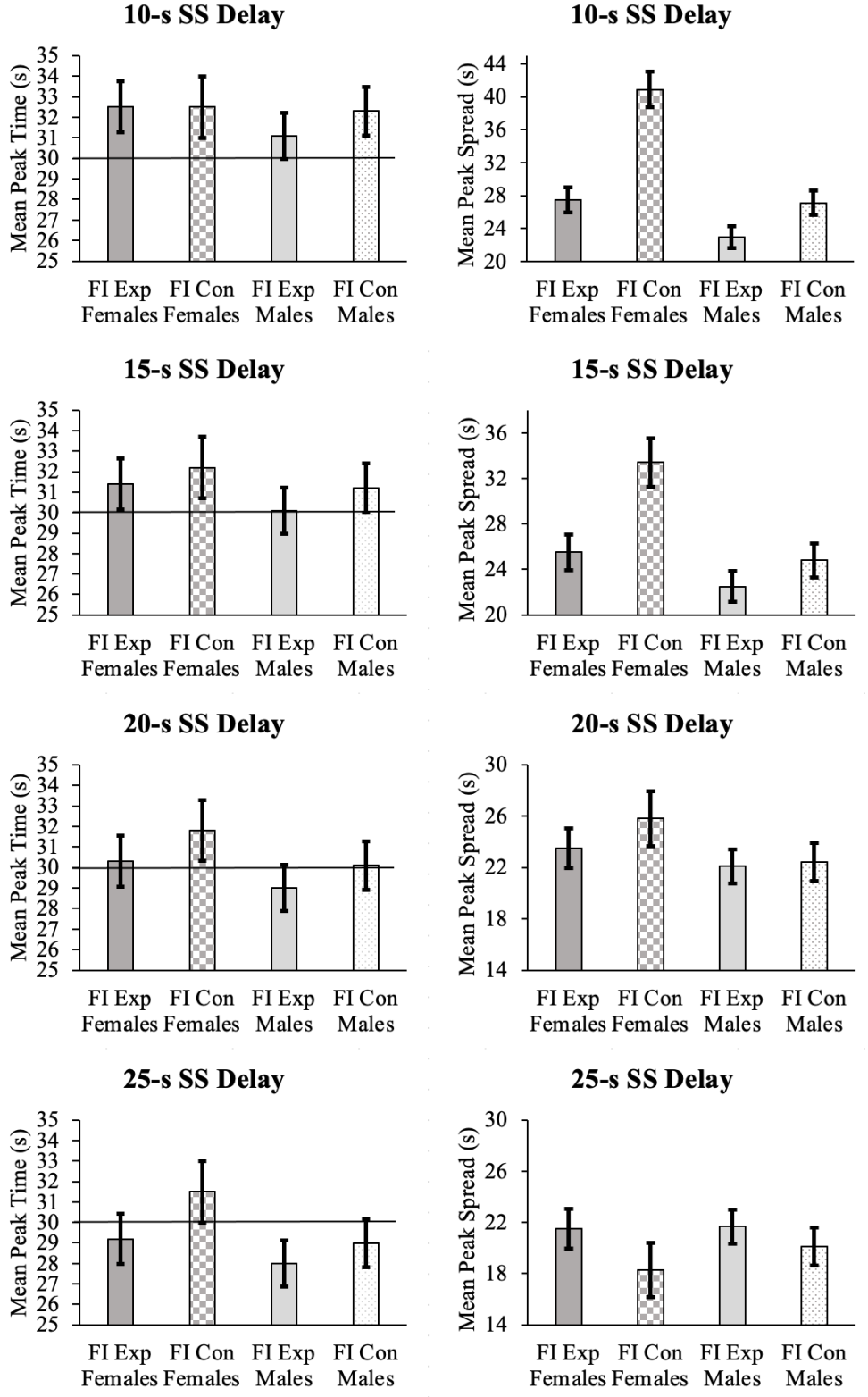

Figure A.4. Alternative views of average rates of decay and intercept values on SS peak trials with error bars (+/− SEM) for rats in the FT (fixed-time) conditions. Exp = Experimental; Con = Control.

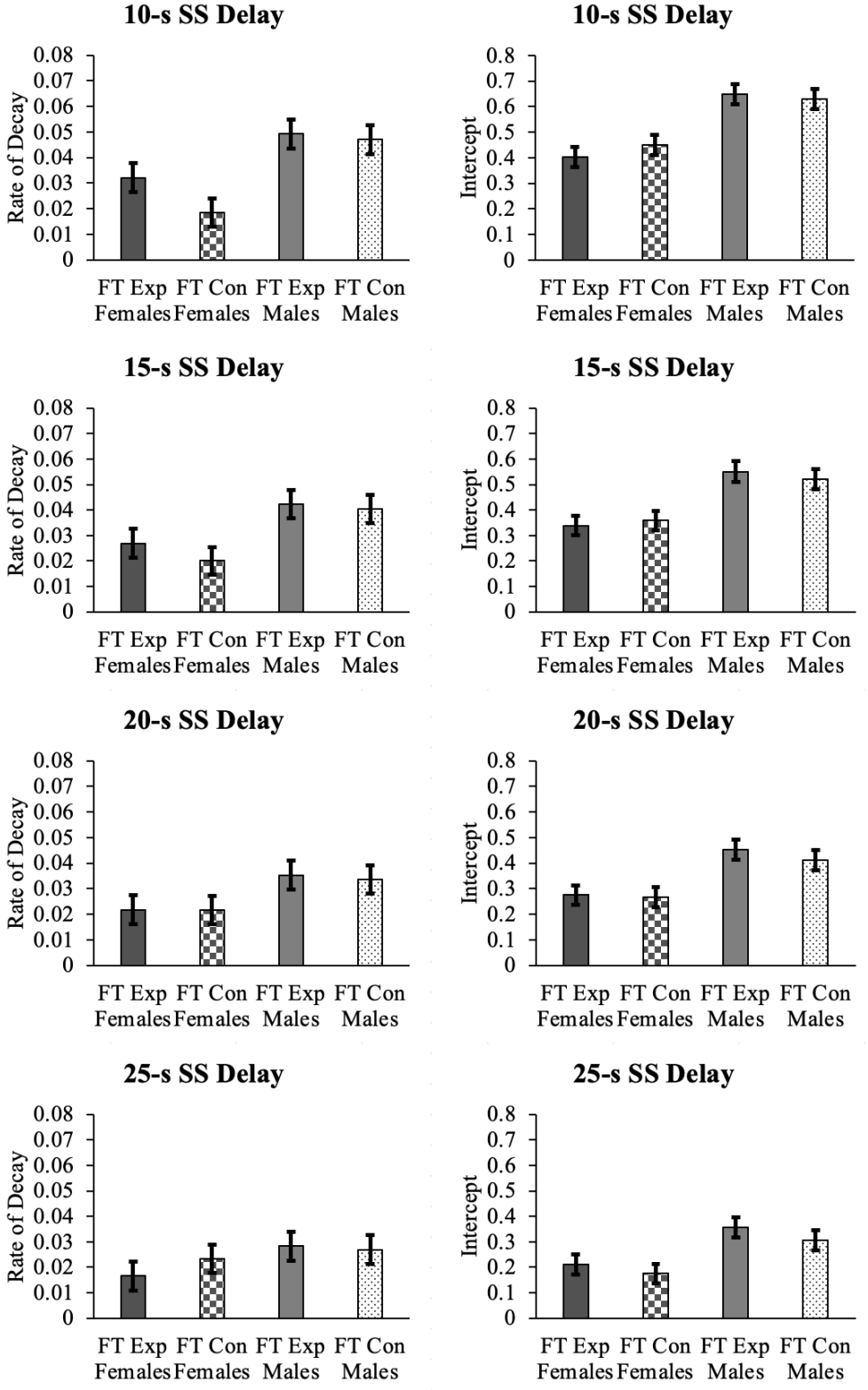

Figure A.5. Alternative views of average rates of decay and intercept values on LL peak trials with error bars (+/− SEM) for rats in the FT (fixed-time) conditions. Exp = Experimental; Con = Control.

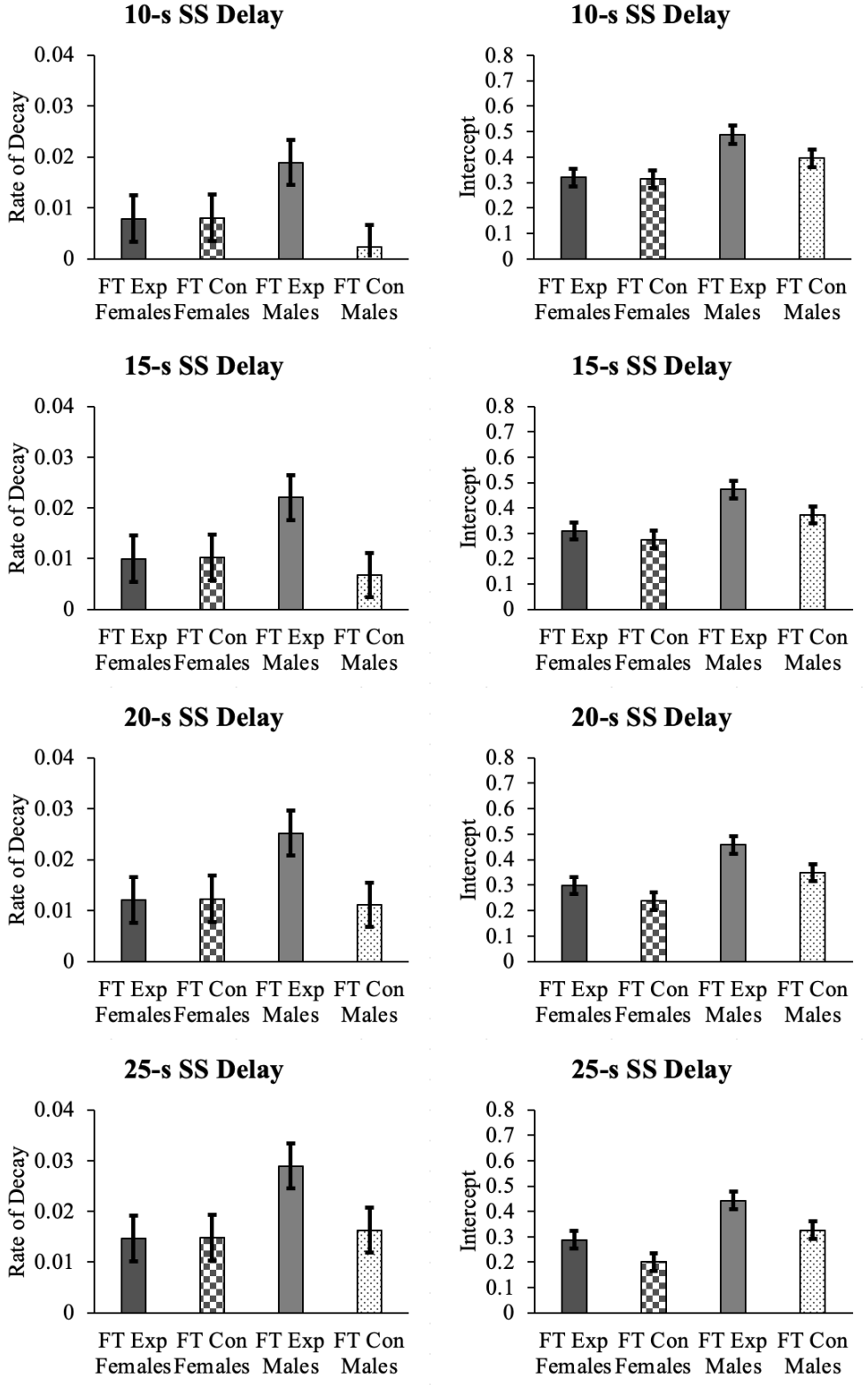

Table A.1. Average peak times and spreads on SS peak trials for rats that received the FI (fixed-interval) schedule. Exp (experimental) represents rats that received the FI intervention while Con (control) represents rats that did not.

Group	Sex	10-s SS		15-s SS		20-s SS		25-SS	
		Time	Spread	Time	Spread	Time	Spread	Time	Spread
Exp	Female	10.9	18.0	16.2	18.7	21.5	19.3	26.9	20.0
	Male	12.0	12.7	15.3	14.4	18.6	16.1	21.8	17.8
Con	Female	13.6	21.6	17.0	19.5	20.5	17.4	24.0	15.3
	Male	11.8	14.8	15.7	15.8	19.6	16.8	23.4	17.8

Note: Rats that received the FT (fixed-time) schedule were not included in this analysis.

Table A.2. Average peak times and spreads on LL peak trials for rats that received the FI (fixed-interval) schedule. Exp (experimental) represents rats that received the FI intervention while Con (control) represents rats that did not.

Group	Sex	10-s SS		15-s SS		20-s SS		25-SS	
		Time	Spread	Time	Spread	Time	Spread	Time	Spread
Exp	Female	32.5	27.5	31.4	25.5	30.3	23.5	29.2	21.5
	Male	31.1	22.9	30.1	22.5	29.0	22.1	28.0	21.7
Con	Female	32.5	40.9	32.2	33.4	31.8	25.8	31.5	18.3
	Male	32.3	27.1	31.2	24.8	30.1	22.4	29.0	20.1

Note: Rats that received the FT (fixed-time) schedule were not included in this analysis.

Table A.3. Coefficient of variation (CV) values for rats that that received the FI (fixed-interval) schedule based on average peak time and spread on SS and LL peak trials. Exp (experimental) represents rats that received the FI intervention while Con (control) represents rats that did not.

Group	Sex	10-s SS		15-s SS		20-s SS		25-SS	
		SS CV	LL CV	SS CV	LL CV	SS CV	LL CV	SS CV	LL CV
Exp	Female	1.65	0.85	1.15	0.81	0.90	0.78	0.74	0.74
	Male	1.06	0.74	0.94	0.75	0.87	0.76	0.82	0.78
Con	Female	1.59	1.26	1.15	1.04	0.85	0.81	0.64	0.58
	Male	1.25	0.84	1.01	0.79	0.86	0.74	0.76	0.69

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

Table A.4. Average rate of decay and intercept values on SS peak trials for rats that received the FT (fixed-time) schedule. Exp (experimental) represents rats that received the FT intervention while Con (control) represents rats that did not.

Group	Sex	10-s SS		15-s SS		20-s SS		25-SS	
		Rate	Intercept	Rate	Intercept	Rate	Intercept	Rate	Intercept
Exp	Female	0.032	0.40	0.027	0.34	0.022	0.28	0.017	0.21
	Male	0.049	0.65	0.042	0.55	0.035	0.45	0.028	0.36
Con	Female	0.019	0.45	0.020	0.36	0.022	0.27	0.023	0.18
	Male	0.047	0.63	0.040	0.52	0.034	0.41	0.027	0.31

Note: Rats that received the FI (fixed-interval) schedule were not included in this analysis.

Table A.5. Average rate of decay and intercept values on LL peak trials for rats that received the FT (fixed-time) schedule. Exp (experimental) represents rats that received the FT intervention while Con (control) represents rats that did not.

Group	Sex	10-s SS		15-s SS		20-s SS		25-SS	
		Rate	Intercept	Rate	Intercept	Rate	Intercept	Rate	Intercept
Exp	Female	0.008	0.32	0.010	0.31	0.012	0.30	0.014	0.29
	Male	0.019	0.49	0.022	0.47	0.025	0.46	0.028	0.44
Con	Female	0.008	0.31	0.010	0.28	0.012	0.24	0.014	0.20
	Male	0.002	0.40	0.007	0.37	0.011	0.35	0.015	0.33

Note: Rats that received the FI (fixed-interval) schedule were not included in this analysis.