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Effects of Procedural Differences on the Performance of Mice on Impulsive Choice Tests

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Psychology

By

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Abstract

Due to its relevance to human diseases and behavioral disorders, delay discounting has become a topic of interest to researchers across a wide range of scientific disciplines. The within-sessions procedure is the most widely used procedure to assess delay discounting in nonhumans (Madden & Bickel, 2015). To date, most within-sessions procedures have been designed specifically for use with rats. However, mice are the organism of choice in biomedical and genetic research. Interdisciplinary research requires the standardization of assessments for mice. Only a few published studies have utilized the within-sessions procedure with mice, these have yielded disparate results.

The present experiment investigated the impact of delay progression, number of trainings and delay length on resultant performance of Balb-c mice on the within-sessions impulsive choice test. The findings suggest that each of these factors influence performance. Mice show greater preference for the large reward and greater sensitivity to delay lengths when delays are gradually increased over time as compared to when they are not. Mice show a greater sensitivity to delay length when delays are kept shorter (maximum delay of 8 s) verses longer (maximum delay of 12 s). Finally, the number of trainings that animals receive impact resultant performance. The present data support the notion that the within-sessions procedure must be specifically calibrated for use with mice and the findings herein can help to guide further development in this important area of research.

Dedication

I dedicate this manuscript and the project that it describes to the following people:

Emily Dunster, your capacity to love is unmatched. You are the strongest and bravest person that I know, and I will always count being your wife as my biggest accomplishment in life. Thank you for making this all possible.

To my sister Tori Haddock and my mother Diane Peters. You always have, and always will be an incredible source of strength for me. From the bottom of my heart, thank you.

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Statement on the Welfare of Animals

All research herein was conducted in accordance with guidelines approved by the University of Nevada, Reno's Institutional Animal Care and Use Committee under protocol #00669.

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Introduction to Delay Discounting

Impulsivity and its converse (self-control) have garnered significant interest from researchers working within a variety of theoretical and methodological frameworks (Duckworth & Kern, 2011). The American Psychiatric Association (APA) has identified several disorders associated with impulsivity including attention-deficit/hyperactivity disorder, substance abuse, kleptomania, pathological gambling, eating disorders and trichotillomania (APA, 2013; Madden & Bickel, 2015; Turturici et al., 2018). According to some, understanding the bio-behavioral variables associated with impulsivity is critical to solving problems on the magnitude of global health and the environment (Madden & Bickel, 2015). While the term impulsivity has been defined in a variety ways by both scientists and non-scientists, it is most often used to describe the tendency to act in a manner which disregards a more rational long-term strategy for success (Madden & Johnson, 2015).

One investigative approach to impulsivity is to study choices between rewards which are larger in size but available only after a delay (rational choice) and those that are smaller in size but available immediately (irrational choice). In these arrangements the rational choice is referred to as the larger-later reward and the irrational choice is referred to as the smaller-sooner reward (Madden & Bickel, 2015). For example, a subject may choose one food pellet available now (smaller-sooner) or three food pellets available after a 20 second delay (larger-later). Patterns of choice that tend towards smaller-sooner (SS) are often characterized as impulsive, whereas those that tend towards larger-later (LL) are characterized as self-controlled (Ainslie, 1974).

Within the field of experimental analysis of behavior (EAB) patterns of choice between SS and LL rewards are analyzed in terms of delay discounting. Delay discounting is the decline in the present value of a reward as a function of the delay to its receipt (Odum, 2011b). To date, a variety of procedures have been designed to assess impulsive choice in humans and non-human animals. Researchers from diverse scientific enterprises, including behavior analysis, psychology, pharmacology and biology have adopted these procedures to assess choice in a wide variety of species and have found the process of delay discounting to be common to every species thus far examined (Madden & Bickel, 2015).

Orderly yet Malleable of Patterns Discounting

Research has revealed orderly effects of both state and trait variables on discounting. State variables are defined as environmental manipulations that affect behavior over a relatively short period time. Trait variables are defined as relatively stable, preexisting characteristics that affect behavior in a protracted manner (Odum, 2011a). State variables found to impact discounting include the type of outcome (Bickel et al., 1999; Charlton & Fantino, 2008; Odum & Baumann, 2015), drug administration (Cardinal et al., 2000) and food deprivation (Eisenberger & Masterson, 1987). Trait variables found to impact delay discounting include age (Olson et al., 2007), IQ (Shamosh & Gray, 2008) and genetic characteristics (Anderson & Woolverton, 2005; Isles et al., 2004).

Recently, some researchers have argued that delay discounting may be understood as trait-like. Support for this view is provided by two types of evidence. First, studies

investigating test-retest reliability have demonstrated similar patterns of discounting with the same subject in assessments conducted across time (Odum, 2011a). Second, some studies have shown that a given individual will respond in a similar manner across delay discounting assessments in which different types of outcomes are used (i.e., different reinforcers are discounted similarly by the same individual; Odum, 2011a). Proponents of the trait argument suggest that while individuals may exhibit a propensity to discount all reinforcers in a similar way across time, patterns of delay discounting are malleable and can be changed through environmental manipulations (Odum, 2011a; Odum & Baumann, 2015).

Mazur and Logue (1978) demonstrated that a delay fading procedure could be used to increase pigeons' choice for a larger amount of grain available after a delay (LL) over a smaller amount available immediately (SS). In this study, a control group was exposed to a choice between 2 s access to grain available immediately (SS) or 6 s access to grain delayed by 6 s (LL). The experimental group was exposed to a delay fading procedure in which both alternatives were initially delayed by 6 s. After this initial training, the subjects in this group experienced a series of sessions during which the SS delay was gradually reduced from 6 s to 0 s. By the end of training, pigeons in the control group continued to select the SS alternative almost exclusively, while those in the experimental group chose the LL alternative significantly more often. Additionally, this pattern of self-controlled responding was maintained by subjects in the experimental group at one year follow up (Logue & Mazur, 1981). Since this seminal study, researchers have successfully used similar delay fading procedures to increase self-control choices in human subjects, including those with attention deficit hyperactivity

disorder (Schweitzer & Sulzer-Azaroff, 1988), autism (Dixon & Cummings, 2001), traumatic brain injury (Dixon & Tibbetts, 2009), and individuals dually diagnosed with intellectual disabilities and mental illness (Dixon & Holcomb, 2000).

Delay exposure training has also been found to be successful in decreasing impulsive choice with rats (Renda & Madden, 2016; Renda et al., 2018; Rung & Madden, 2018; Stein et al., 2013; Stein, et al., 2015). For these experiments, subjects were assigned to one of two groups: immediate reinforcement (IR) or delay exposure (DE). Both groups received initial training for a protracted period. During training, IR rats received immediate food rewards following every lever press. DE rats experienced a delay between each lever press and the delivery of food. At the end of training, all rats were assessed in a delay discounting procedure in which they had a choice between SS and LL rewards. In the assessment, DE rats choose the LL option more frequently than the IR rats, suggesting that pre-exposing subjects to delayed reinforcers ameliorates the extent to which the LL option is discounted during testing.

The studies described above have demonstrated the efficacy of delay fading and delay exposure procedures in establishing more self-controlled choice. Research on why these procedures were effective is still ongoing. Some have hypothesized that core timing processes are fundamental to rational choice-making, thus the accuracy with which an individual is able to predict when a delayed reinforcing event will occur affects measures of discounting (Marshall et al., 2014). However, Rung and Madden (2018) demonstrated that delay exposure training can reduce impulsive choice without impacting interval timing. Furthermore, data have emerged supporting an alternative hypothesis, namely

that delay exposure training may work to decrease impulsive choice by decreasing a subject's aversion to delay. In a recent study, rats that that underwent delay-exposure training showed less discounting in post-tests and made fewer escape responses when presented with signals that had been paired with delay (Peck et al., 2019).

Delay Discounting Assessments

In addition to effects of the aforementioned delay fading and delay exposure procedures, characteristics of the delay discounting assessment itself may affect the extent to which impulsive choice is observed. A variety of procedures have been created to assess delay discounting in nonhumans. These include the adjusting delay procedure (Mazur & Logue, 1978), the adjusting amount procedure (Richards et al., 1997) and the within-sessions procedure (Evenden & Ryan, 1996). All of these share common features. In each, there is a series of trials in which the subject must choose between a SS and a LL alternative. Each procedure employs both forced-choice ("sample") trials and free-choice trials. To prevent adding additional time or effort to one alternative or the other, the procedures all require a single response to produce either the SS or LL consequence. Finally, they all include an adjusting intertrial interval (ITI) following the delivery of the reinforcer. The duration of this ITI adjusts to accommodate different latencies to reinforcement (secondary to latency to respond and programmed delay). This adjusting ITI ensures that the time between choice opportunities remains constant from one trial to the next, and that the maximum reinforcement rate associated with each alternative is held constant (Madden & Johnson, 2015).

While these procedures share the features described above, they differ in other ways, including the independent variables, the organization of trials and the dependent variables that serve as measures of impulsive choice (Table 1). In adjusting delay and adjusting amount procedures, "indifference points" are used to identify the value of the independent variable (i.e., reinforcer delay or amount) at which a subject switches from choosing the LL alternative to choosing the SS alternative (or vice versa) in a consistent fashion. The indifference point is the point at which the subjective value of both rewards is considered to be approximately equal (Odum & Baumann, 2015). From a choice perspective, the subject is said to show indifference between the outcomes. By calculating indifference points at a variety of delays, one can then use nonlinear regression techniques to fit the points using a discounting function (see Madden & Johnson, 2015; Odum, 2011a; McKerchar & Renda, 2012). The discounting function demonstrates how delay decreases the value of a specific amount of an outcome (Odum & Baumann, 2015). Data from the within-sessions procedure do not yield indifference points. Instead, impulsive choice is measured as the percentage of choices on the LL alternative at each of the delay lengths. As the within-sessions procedure is the most widely used procedure to assess delay discounting in nonhumans (Madden & Bickel, 2015), we will consider the features of the procedure in detail in following section.

Within-Sessions Procedure

Within-sessions assessments are typically conducted in operant chambers equipped minimally with a house light, two response operanda (e.g., levers, nose-pokes or lickometers), and a food reward delivery device (e.g., pellet receptacle, liquid dipper,

or sipper tube). During experimental sessions, responding on one operandum produces the SS reward, while responding on the other produces the LL reward. In most preparations, the SS option is available after a 0 s delay while the LL is available after some programmed delay, which varies systematically within an experimental session. Experimental sessions are broken into trial blocks consisting of a combination of forced- and free-choice trials. Each trial block begins with forced-choice trials in which only one option (SS or LL) is available, and the subject is required to respond on the available option to complete the trial. Forced-choice trials are intended to ensure that the subject experiences the programmed consequences associated with each response alternative. Forced-choice trials are followed by a series of free-choice trials. In free-choice trials, both the SS and LL alternatives are available, and the subject can respond on either. The number of trials of each type, the number of trials per block and the proportion of forced-choice to free-choice trials within a trial block are not prescribed and can vary from one experiment to the next. In the original Evenden and Ryan (1996) arrangement, there were two forced-choice trials followed by six free-choice trials within each trial block. Following a response there is an adjusting ITI which ensures that each trial is of equal duration. For example, if trials are scheduled to occur every 45 s, the duration of the ITI will adjust to account for the latency to respond and the programmed delay to the delivery of the reinforcer.

As blocks progress within the session, the delay to the LL alternative changes. For example, in Evenden and Ryan (1996), sessions consisted of five eight-trial blocks (40 trials total). Across the five trial blocks, the delay on the SS alternative remained at 0 s while the delay to the LL reward increased systematically from 0 s in the first block to 60

s in the last block. As noted, the specific delays assigned to each block and the arrangement of these delays vary from experiment to experiment. However, in most within-session preparations, delays are arranged in an ascending order across blocks. For example, the delays for the LL option might be arranged as follows: 0, 10, 20, 40, and 60 s. An important feature of the within-session procedure is that in the first block of any experimental session, the delay to both the SS and LL alternatives is set at 0 s. Thus, the choice in the first block is between a 0 s delay to the LL reward and a 0 s delay to the SS reward. This allows for a baseline measure of the subject's preference for the large reward prior to the imposition of delays. The primary dependent variable is the proportion of choices on the LL and SS alternatives during the free-choice trials. It is anticipated that the proportion of trials in which the LL alternative is selected will decline systematically as a function of delay length (de Wit & Mitchell, 2015). The within-session procedure is favored over others because it provides a measure of sensitivity to reinforcer amount as well as delay in each session. Once stable measures of choice across trial blocks are obtained, the data yielded from this procedure provide a baseline measure which is uniquely suited to the investigation of various manipulations such as drug administration or neurological lesions (Madden & Johnson, 2015).

Despite its widespread use, limitations of the within-sessions procedure have been identified. One limitation relates to response patterns in the first trial block during which the delay to both the LL and SS reward is 0 s. If the subject is sensitive to the reward size, one would assume exclusive preference for the large alternative. However, this is not always observed. It has been hypothesized that carryover effects from previous blocks or sessions might explain this phenomenon (Madden & Johnson, 2015).

Fox et al. (2008) conducted an experiment with rats that examined the effects of arranging delays in either an ascending or descending order across trial blocks by varying the delay to the LL reward across sessions instead of across blocks within a session. In the first four sessions both levers were associated with a 0 s delay. By the conclusion of these four sessions, animals were displaying near exclusive preference for the large alternative. Starting on the fifth session, the delay to the large, now delayed reward (LL) varied across sessions according to the following arrangement: 1, 3, 6, 12, 24, 24, 12, 6, 3, then 1 s. In a second experiment, three changes were made to the procedure. First, the order of the LL delays was mixed, and the 1 s delay was omitted, resulting in the following arrangement: 6, 3, 24 then 12 s. Second, each LL delay value remained in effect for five daily sessions. Third, to minimize the effect of the LL delay from the preceding session on preference, between each change in the LL delay, the animal was exposed to at least two days of sessions in which the delays were reset to 0 s on both alternatives. To move to the next delay in the sequence, an animal was required to respond on the LL alternative for at least 90% of trials for two consecutive sessions. Findings from this study revealed that stable choice percentages were influenced by delays arranged in preceding sessions. Specifically, more impulsive (SS) choices were made across all delay values when those values were arranged in a descending fashion (Fox, et al., 2008).

To ameliorate issues related to carryover effects of this sort, Madden and Johnson (2015) recommended modifications to the within-session procedure. One suggestion was to enhance the salience of the various delays by increasing the number of forced-choice trials and/or adding periodic no-delay control sessions in which the delay to the LL

alternative is set at 0 s across all trial blocks. With respect to the latter, the authors suggested that the no-delay control sessions may increase sensitivity to changing delay lengths during regular sessions. The authors also recommended further research investigating how various procedural details of the within-sessions procedures impact measures of delay discounting.

Species-Related Issues

The species-specific characteristics of the animals being used in assessments of impulsive choice may also affect the outcomes obtained. Most non-human animal delay discounting studies utilize rats as subjects (de Wit & Mitchell, 2015). This homogeneity has been identified as a major limitation as it restricts the generalizability of research findings. For example, studies have examined the impact of d-amphetamine, methamphetamine, cocaine, nicotine, methylphenidate, ethanol, diazepam, and morphine on patterns of impulsive behavior. However, most of these investigations have used only rats as subjects, making it difficult to interpret the general effects of these drugs on discounting (de Wit & Mitchell, 2015).

Isles, et al. (2003) suggested that mice are an ideal candidate for delay discounting research and cite numerous advantages of working with them. Over the last two decades, scientists in fields outside of behavior analysis have transitioned from using rats as subjects to using mice. Due to their genetic and physiological similarities to humans, mice are good model organisms for biological research. They naturally develop many diseases common to humans, and the human diseases that they do not readily develop can be easily induced by manipulating the mouse genome to create a genetic

knockout. These knockouts can be used to study the disease, while also providing a biological context in which therapies and drugs can be tested (NIH, 2002, 2015). Currently, millions of knockout mice are used in biomedical research annually, and the mouse is currently the most commonly used species in biological research (Anft, 2008; NIH, 2002; Rosenthal & Brown, 2007). Given the potential utility of mice to investigate genetic contributions to impulsive choice, as well as the impact of substances on the process of discounting, researchers have advocated for the development of delay discounting assessments that may be used with mice (Isles et al., 2003; Mitchell, 2014; Mitchell et al., 2006). Additionally, operant training procedures have proven useful in efforts to display phenotypic differences between strains of mice (Peters & Hayes, 2020). In addition to helping to study impulsive choice, delay discounting assessments for mice hold promise as phenotyping procedures.

The Within-Sessions Procedure with Mice and Effects of Delay Progression

To date, only a small number of studies have utilized operant delay discounting assessments with mice. Most of these have used the adjusting amount procedure (see Adriani & Laviola, 2003; de Wit Mitchell, 2015; Helms, et al., 2006; Pinkston & Lamb, 2011; Mitchell, 2014), while only a few have attempted to use the within-sessions procedure (Buhusi et al., 2016; Madden & Bickel, 2015). This is notable, as the within-sessions procedure is currently the most widely used procedure to study delay discounting in nonhuman animals (Madden & Bickel, 2015).

A review of those experiments that have utilized a version of the within-sessions procedure with mice suggests that strain, animals' training histories, and the delay

progression used across trial blocks may affect the measures of impulsive choice obtained. Isles et al. (2003) were the first to adapt a within-sessions procedure for use with mice. Testing was conducted with C57Bl/6J mice (n = 16) in nine-hole test chambers adapted for use with mice. A solution of 10% condensed milk was used as a reinforcer. Responses on the nose-poke associated with the LL alternative produced 50 μL of the solution, while responses on the SS alternative produced 25 μL. Sessions were arranged into five blocks of eight trials. Each block consisted of two forced-choice trials (one on each alternative) followed by six free-choice trials. Within the first block, the delay to the LL and SS reinforcers was set at 0 s. Subsequent blocks included a delay on the LL alternative. There were three series of delays across imposed: S1, S2 and S3. Each series was in place for 10 daily sessions. However, some animals received extended training on S3 to achieve stability. The authors did not report how many subjects received additional training, nor did they report how many additional sessions were included. Sessions using the S1 progression were conducted first and consisted of LL delays of 0, 0.5, 1, 2, and 4 s across trial blocks. These were followed by sessions in which the S2 progression was used: 0, 1, 2, 4, and 8 s. The last series of sessions used the S3 progression: 0, 2, 4, 8, and 12 s. An adjusting ITI was used following the presentation of food to ensure that a new trial began every 45 s. Figure 1 depicts responding on the final delay sequence S3 (0, 2, 4, 8, and 12 s). In this figure, we see a characteristic response pattern for the within-sessions procedure demonstrating a pattern of rational choice. There is near-exclusive preference for the LL option in block 1 (indicating a preference for the large reward) followed by a systematic decrease in preference for the LL as the delay to reward receipt increases (demonstrating a sensitivity to delay length). Data from

the preceding sessions in which S1 and S2 delay progressions were in effect were not presented in this paper.

Isles, et al. (2004) replicated and extended the study described above by applying a similar procedure to four strains of mice: C57Bl/6J (n = 6), 129/Sv (n = 7), CBA/Ca (n = 9) and Balb/c (n = 9). In this study, there were only two delay sequences imposed each for 10 daily training sessions. Some animals received extended training on S2 to achieve stability. The authors did not report how many subjects received additional training, nor did they report how many additional sessions were included. The series used were equivalent to S1 and S2 in the 2003 study (S1: 0, 0.5, 1, 2, 4 s; S2: 0, 1, 2, 4, 8 s). Figure 2 depicts the mean proportion of LL choices per session (± SEM) for all groups in three consecutive sessions after stable baseline had been achieved on S2. The data show clear strain-dependent differences in the proportion of choices on the large when delay to both alternatives was 0 s delay, demonstrating difference in terms of preference for the large reward. Nevertheless, all data paths show a similar decrease in the proportion of LL choices as the delay is increased, demonstrating sensitivity to delay length. As in the previous study, data showing S1 patterns of responding were not presented.

Mori, et al. (2018) employed a within-sessions procedure with mice based on the aforementioned procedure used by Isles et al. (2003, 2004). In addition to using the procedure to measure the impact of 5-HT_3 antagonists on discounting, the investigators attempted to optimize the task for use with mice by carefully investigating the impact of specific delays used on assessment results. The subjects were male C57Bl/6J mice (n = 27). The configuration of experimental chambers and the arrangement of trials and blocks (five blocks consisting of two forced- and six free-choice trials) was identical to that used

by Isles and colleagues. One deviation from the Isles et al. studies was the use of 20mg dustless precision pellets in quantities of one (SS) and four (LL). Subjects were separated into three groups, mice in each group were exposed to a different within-session delay progression across the five trial blocks. The first group of mice (n = 6) was exposed to a LL delay progression of 0, 0.5, 1, 2, and 4 s (S1). The second group of mice (n = 9) was exposed to a progression of 0, 1, 2, 4, and 8 s (S2). The final group (n = 9) was exposed to a progression of 0, 2, 4, 8, and 12 s (S3). Mice in each group received 10 sessions of training. Figure 3 depicts the percentage of LL choice for each group. This value was calculated as the grand mean for all subjects within a group for the last two training sessions.

S1 mice (exposed to a maximum delay 4 s) exhibited a characteristic response pattern for the within-sessions procedure. For this group, the percentage of choice for LL was 94% in block 1 indicating a strong preference for the large reward when there was no delay imposed. Subsequently, preference for the LL decreased systematically as a function of the delay indicating sensitivity to delay length. These same patterns were not observed for subjects in S2 and S3 which were exposed to maximum delay lengths of 8 and 12 s respectively. Mori and colleagues hypothesized that the lack of characteristic response pattern was related to the maximum delay lengths imposed. The authors observed that during the first training session, S2 animals showed the characteristic pattern of responding. Over subsequent training sessions this pattern began to degrade, suggesting that over time, exposure to maximum delay lengths of 8 s or greater may result in less characteristic patterns of responding for mice on the within-sessions

procedure. While not identified by the authors, what is described by More et al. appears to be the impact of carryover effects as described above.

To further investigate the impact of delay length on response patterns, Mori et al. conducted two follow-up experiments. In the first, 18 mice were divided into three groups. After preliminary nose-poke training, animals were exposed to a forced-delay task in which they were required to respond on a lit nose-poke to access one reward pellet following a fixed delay. Delays were varied across the groups and consisted of 0, 4 and 8 s. Unlit nose-pokes were counted but produced no programed consequence. All animals received 10 training sessions. The number of lit nose-pokes (i.e., "correct" responses) and the percentage of accuracy (number of lit responses/number of total responses x 100) were reported. Figure 4 depicts correct responses per session for all groups, and Figure 5 depicts percentage of accuracy. As depicted, there was little difference in both measures between the 0 s and 4 s delay groups, but animals in the 8 s delay consistently made fewer correct responses and exhibited less accuracy in responding.

In the second follow-up conducted by Mori et al., mice were first exposed to 10 training sessions in which they experienced the forced-delay task described above with a fixed 0 s delay. Subjects were then exposed to 10 additional sessions in which the fixed delay was changed to 8 s. Figure 6 depicts correct responses on day 10 (after 10 sessions of training with 0 s delay) and day 20 (after 20 sessions with the 8 s delay). Figure 7 depicts the percentage of accuracy on day 10 and day 20. The change to the 8 s delay reduced both the number of correct responses and response accuracy.

Based on these findings, Mori et al. concluded that longer delays impair learning of the response-reward contingency in B57BL/6 mice. Suggesting that a characteristic response pattern (indicative of rational choice) cannot be obtained on the within-session procedure if maximum delays exceed 4 s. However, this finding conflicts with that of Isles et al. (2003) as a characteristic response pattern was observed even when B57BL/6 mice were exposed to a maximum delay length of 12 s.

There are some possible explanations for the disparate results of the above described studies. The first is that the training history of mice and the delays to which they had been exposed prior to the final sessions of the impulsivity assessment affected the results obtained. Isles et al. (2003, 2004) exposed animals to LL delay progressions composed of smaller delays (maximum of 4 s or 8 s) before conducting final assessments utilizing maximum delays of 12 s. On the other hand, Mori et al. (2018) included no such pre-exposure to delays. It is possible that pre-exposure to shorter delays before assessing impulsivity with longer delays has effects like delay exposure training, increasing tolerance delays. However, because data from the preliminary training sessions were not reported in the Isles et al. studies, it is not possible to determine if this is the case.

Another possibility is that the Isles et al. and Mori et al. studies obtained different results because animals were exposed to different amounts of training. Subjects received 30 or more training sessions in the Isles et al. (2003) study and 20 or more sessions in the Isles et al. (2004) study. By contrast, the animals in Mori et al. (2018) received only 10 training sessions. It is possible that more training (i.e., a “practice effect”) is responsible for the different patterns of responding observed in the Isles and Mori studies.

Nevertheless, this would not explain why the S1 delay progression group in Mori et al. (2018) exhibited the expected pattern of choice following only 10 training sessions while the other two groups did not, unless the maximum delay length used had an impact on response patterns (as indicated my Mori et al.).

Purpose

The immediate purpose of the present study was to further investigate the effects of several procedural details of the within-sessions procedure on measures of impulsive choice in mice. Specifically, this work examined the effects of delay length, delay progressions and the number of training sessions on measures of impulsive choice, with the aim of accounting for the disparity of results in the existing literature. The specific details of the within-sessions procedure described below were based upon the studies described above and findings from previously conducted pilot research in our laboratory.

In addition to clarifying the effects of certain features of the within-sessions procedure, this study aimed to make larger contribution. From a methodological standpoint, the project aimed to support the continued development of a standardized impulsive choice assessment for mice. Such a procedure could have utility as a standalone phenotyping strategy. From a conceptual standpoint, this project aimed to demonstrate that historical events have an impact on how subjects respond, even in highly controlled experimental settings.

Experiment

Subjects & Housing

23 experimentally naïve Balb/c mice (Charles River) approximately 9 weeks of age (PND 61) served as subjects. Subjects were randomly assigned to one of three groups (G): G1 (N=8), G2 (N=8) and G3 (N=7). Each group was further divided into subgroups "A" and "B" and subjects were housed according to subgroup; G1A (N=4), G1B (N=4), G2A (N=4), G2B (N=4), G3A (N=4) and G3B (N=3). Outside of experimental sessions, subjects were housed in Techniplast SEALSAFE ® PLUS GM500 Mouse IVC Green Line home cages. Home cages were equipped with corn cob bedding, one red plastic Bio-Serv™ Mouse Hut™ Rodent measuring 7.62 x 9.5 x 4.5cm, nestlets measuring 5.08cm x 5.08cm and wooden gnawing sticks measuring 3.99 x 0.99cm. Home cages were maintained in an Innorack® IVC Mouse Rack located in a temperature-, light-, and humidity-controlled room. Lights were on a 12 h cycle with lights on at 07:00 and off at 19:00. Outside of experimental sessions and timed deprivations, animals had free access to Purina Rodent LabDiet and filtered water.

Food Deprivation Regimen

Subjects were exposed to experimental sessions every other day between the hours of 09:00 and 12:00. Food was removed from the subject's home cage 24 hours prior to experimental sessions. Following scheduled sessions subjects had a 24-hour recovery period during which they had unrestricted access to food. These procedures were in compliance with the University of Nevada, Reno Institutional Animal Care and Use Committee (IACUC) guidelines under the approved IACUC protocol #00669.

Apparatus

All experimental sessions were conducted in one of four identically equipped Med Associates Classic Modular Test Chambers (ENV-307A) measuring 15.24 x 13.34 x 12.7 cm. Each chamber was housed in a Standard MDF Sound Attenuating Cubical (ENV-022MD) with a ventilation fan to mask ambient noise. A Switchable Dipper (ENV-302RM-S) with a square access opening measuring 2.54 x 2.03 cm was placed centrally on the front wall of the chamber. The dipper was outfitted with a cup capable of delivering 0.02 cc of liquid per activation. A Head Entry Detector for Liquid Dipper for Mouse (ENV-302HD) was fitted to the dipper to record data on head entries into the access opening. Two Illuminated Nose-Poke for Classic Mouse Chamber response devices (ENV-313M) with a circular access opening measuring 1.27 x 1.03 cm were mounted to either side of the food receptacle. Entry of the animals' nose at least 0.64 cm into the nose-poke access opening broke a photobeam and constituted a response. A house light (ENV-307A) was mounted in the center of the back wall of the chamber nearest to the celling. Under the house light was a speaker (ENV-324M). The presentation and recording of all experimental events were controlled via MED-PC IV (Med Associates) programming software. Access to a 1:3 solution of Borden® Eagle Brand Sweetened Condensed Milk (SCM) and filtered water was used as a contingent reinforcer following operant responses as designated in the procedure below.

Pre-Training

Phase I: Weighing & Gentling

Subjects were exposed to daily weighing and gentling sessions for two weeks prior to training. The purpose of these sessions was two-fold; 1) to acclimatize subjects to the transport tube and handling procedures and 2) to obtain measures of baseline ad libitum weights prior to implementing deprivation procedures. Transport tubes consisted of clear, polyethylene terephthalate measuring 13 cm tall by 5.75 cm in diameter, with one closed end. Tube handling was used as it has been demonstrated to reduce handling-induced stress and inter-handler variability (Hurst & West, 2010). Subjects were ushered into the handling tubes and gently moved to a scale for weighing before being returned to the home cage. Upon return, the tube was placed on the floor of the cage and subjects could exit the tube on their own. Once all subjects in the home cage had been weighed, a small amount of SCM was dripped into the handling tube and the tube was placed back into the home cage for the mice to explore. Tubes with SCM were left in home cages for approximately 20 minutes before being removed.

Following this phase of training, the laboratory in which the research was being conducted was closed secondary to COVID-19 mitigation efforts. The research project went on hold for 77 days. Over this period subjects remained in their respective home cages and were cared for by Office of Animal Resources staff. Upon return to the laboratory, new ad libitum weights were obtained, however the gentling process was not repeated as subjects readily entered the handling tubes when presented.

Phase II: Pre-Experimental Dipper Training

This procedure was used to help acclimatize naïve mice to the operant conditioning chamber and to teach the association between the sound of the dipper operating and the presence of a liquid food reward in the receptacle. Dipper training was conducted using an automated program. Each training session lasted 20 minutes or until subjects consumed the reinforcer from the dipper 15 times. The dipper arm was outfitted with a 0.02cc cup and lifted to allow 4 seconds access to drink per presentation. Both nose-pokes were illuminated during these sessions. Concurrent schedules of reinforcement were in place: the dipper cup was presented periodically on a variable-time 45 s (VT-45 s) schedule, but responses made on either lit nose-poke were reinforced on a fixed-ratio 1 (FR-1) schedule. Measures recorded during these sessions included responses made on the left and right nose-pokes, session duration (in seconds), number of head entries when the dipper was up and number of head entries when the dipper was down. Animals were exposed to one training session every other day for a total of ten sessions.

Phase III: Pre-Experimental Switching Discrimination Task

The purpose of this phase was to teach the subjects the discrimination of responding on illuminated nose pokes. Training sessions lasted 20 minutes and began with the illumination of one of the nose-pokes, with a 0.5 probability of it being the left or right nose poke apparatus. Responses on the lit alternative produced four seconds of access to the 0.02cc dipper cup on a FR-1 schedule of reinforcement. Following reinforcer delivery, there was a programed blackout during which both nose-pokes went dark for 10 s. At the conclusion of the blackout, a new trial began with the illumination of

one nose-poke (0.5 probability of it being left or right). Responses on an unlit nose-poke were counted but had no programed consequence. Measures included opportunities to respond on the left and right nose-poke, number of lit and unlit responses, latency to respond on a lit nose-poke and duration of head entry when the dipper was up and when the dipper was down. Animals were exposed to one training session every other day for a total of eighteen sessions.

Experimental Sessions

Phase I: Within-Sessions Procedure

The prior phases of training served to prepare the subjects for the within-sessions adjusting delay procedure. Figure 8 is a schematic of the procedure. The dipper arm was outfitted with a 0.02 cc cup. One nose-poke was assigned to a SS reward (a single 3 s lift of the cup) and the other nose-poke was assigned to a LL reward (three 2 s lifts of the cup). Assignment of the left and right nose-poke to the small and large reward was counterbalanced within groups. An auditory stimulus (a tone LL or a click for SS) was associated with each of the alternatives. The relevant auditory stimulus was presented immediately following a choice response and remained on until the end of the reinforcer presentation. Additionally, an auditory stimulus (a tone LL or a click for SS) was associated with each of the alternatives. A response on a lit nose-poke associated with the SS alternative, extinguished the nose-poke light and resulted in the immediate presentation of the reward. However, a response on a lit nose-poke associated with the LL alternative resulted in the nose-poke light blinking (0.5 s on/off cycle) thought the programmed delay.

Sessions were divided into five eight-trial blocks. Blocks consisted of two forced-choice and six free-choice trials. Each block began with two forced-choice trials in which only one nose-poke was illuminated and a response on that nose-poke produced the associated consequence. Following the completion of the first forced-choice trial on one response alternative (large or small), the other nose-poke was illuminated and a response on that alternative produced the associated consequence. Forced-choice trials did not end until a response was made. This arrangement ensured that the subject experienced both programmed consequences (and the associated delays, if any) for the current block before moving on to free-choice trials. After the two forced-choice trials at the start of each block, the remaining trials in a block were free-choice trials. In free-choice trials, both nose-pokes were illuminated and a response on either produced the associated consequence. Failure to respond within 20 s of the onset of a free-choice trial was counted as an omission. Following a choice response and the delivery of the reinforcer (in forced- and free-choice trials) or an omission (in free-choice trials), there was an ITI. The duration of the ITI adjusted on a trial-per-trial basis such that a new trial began every 50 s. This ensured that responding on the SS alternative did not yield a greater rate of reinforcement than responding on the large alternative (Madden & Johnson, 2015).

In the first trial block, the delay to both the large and the small reinforcer was 0 s. In the subsequent blocks, delays for the LL increased systematically across blocks. Subjects in each group were exposed to a unique series of LL delays across trial blocks as depicted in Table 2. Each group was exposed a different series of delays. To examine the impact of delay length on performance, groups were exposed to different maximum delay lengths. Subjects in G2 and G3 experienced a maximum delay of 12 s while subjects in

G1 experienced a maximum delay of 8 s. In order to assess the impact of delay progression, delays either gradually increased in duration (e.g. 0, .5, 1, 2, 4 s then 0, 1, 2, 4, 8 s) or the terminal delay sequence was introduced from the beginning of training. Subjects in G3 were exposed to three delay series which gradually increased in duration while subjects in G1 and G2 had the same series of delays in place throughout training. Finally, to investigate the impact of the number of training sessions received, training was broken into 10 session blocks which allowed for comparison across groups at various points in the training process. As is standard for the within-sessions procedure, the primary dependent variable was the percentage of responses on the large alternative within each block. We also took measures of the number of omissions within trial blocks.

Phase II: Reversal and Zero Second Delay Probes

Following thirty days of training as described above, subjects were exposed to two probes, both of which were designed to test the subjects' sensitivity to the imposed delays. The first was a reversal probe. In this condition each group was exposed to their respective terminal delay sequence; however, the delays were presented in reverse (descending) order across the blocks. This probe is specifically designed to test for sensitivity to delay length. The delays that were presented to each group are depicted in Table 3. The final probe consisted of a 0 s delay probe in which all delays to the large alternative were eliminated and responding on either nose-poke produced either a large or small reward immediately. This probe is designed to test for preference for the large reward. Each probe was in place for one daily session.

Results

Data Analysis

As indicated above, S1 included training sessions 1-10, S2 included training sessions 11-20, and S3 included training sessions 21-30. The delay progression remained consistent across series for G1 (exposed to LL delays of 0, 1, 2, 4, 8 s) and for G2 (exposed to LL delays of 0, 2, 4, 8, 12 s). For G3 the LL delay progression varied across series including longer terminal delays as the training progressed (S1: 4 s, S2: 8 s and S3: 12 s). Table 2 depicts the delay progressions in place for each group across each series. The dependent variable was the average percentage of choice on the LL alternative per trial block. These values were obtained via the following calculations. First, the percentage of choice on the LL alternative was calculated for each subject, within each block, for each training session using the following equation: Number of LL choices divided by six (i.e., the number of free-choice trials in each block). These calculations produced 160 data points per animal (32 sessions x 5 blocks per session). Data for each group of subjects was then averaged per block, per series (i.e., S1, S2, and S3). For example, to calculate the average percent choice for LL for G1 during S1, the percentage of choices on LL for each of the G1 subjects was summed and then divided by 80 (10 block 1 values [1 per session] x 8 subjects). To account for variation within these data sets, the standard error of the mean (SEM) was calculated for each value according to the following formula: σ / standard deviation / $\sqrt{n}$. Table 4 depicts the specific comparisons that allowed for the assessment of the impacts of the three primary independent variables on choice for the LL alternative: LL delay progression, the number of training sessions, and LL delay length. These are considered in detail below. For each comparison, depicted

on the table a line graph was constructed with the percentage of choice on the LL alternative (calculated as described above) scaled to the y-axis and blocks (1-5) scaled to the x-axis. Each data point was fitted with error bars representing ± SEM.

Delay Progression

The right panel of Table 4 depicts comparisons that allowed for the evaluation of delay progression. The effect of this variable can be seen by comparing G1 and G3 performance during S2 and by comparing G2 and G3 performance during S3. In these comparisons, the number of trainings were the same across the groups compared, but the delay progression across S1, S2 and S3 varied. Figure 9 (upper panel) shows the data for the first of these comparisons: G1 and G3 over S2. Visual inspection of this graph reveals disparate data paths for both groups. In block 1, when the delay to both alternatives was 0 s, G3 showed a higher percentage of choices on the LL (53%) as compared to G1 (28%). Both data paths depict a decreasing trend, indicating that the percentage of choices on the LL reward alternative decreased as the delay increased. However, the change in responding between blocks 1 and 5 was greater for G3 then it was for G1. A steeper declining trend for the G3 data path as compared to G1 was observed via visual inspection of the graph. This decline is reflected mathematically, by subtracting the average percentage of choice on the LL in block 5 from that in block 1. As calculated, the change between blocks 1 and 5 for G3 is -44%, while the change for G1 is -25%. A Mann-Whitney test demonstrated that the percentage of choice on the LL alternative was greater over S2 in block 1 for G3 (*Mdn = 0.55*) than for G1 (*Mdn = 0.22*), *U = 11.50, p*

= 0.05 and in block 5 for G3 (*Mdn = 0.08*) than for G1 (*Mdn = 0.03*), *U = 8.00, p = 0.02*.

The lower panel of Figure 9 depicts the second comparison assessing the impact of delay progression: G2 and G3 over S3. As with the comparison described above, visual inspection of this graph reveals disparate data paths for both groups over the first four blocks. In block 1, when the delay to both alternatives was 0 s, G3 showed a higher percentage of choices on the LL (47%) as compared to G2 (15%). Both data paths depict a decreasing trend, indicating that the percentage of choices on the LL alternative decreased as the delay to the larger reward increased. However, the change between blocks 1 and 5, was greater for G3 (43%) then it was for G2 (15%). A Mann-Whitney test demonstrated that the percentage of choice on the LL alternative was greater for G3 than for G1 over S3 for all blocks but block 4 (see Table 5 for individual test results).

Taken together, visual inspection and statistical analysis demonstrate a difference in patterns of responding on the same delay sequence, following the same number of trainings, when the delay progression across S1-S3 varied. Specifically, when delays were increased gradually across training sessions for G3, subjects in this group made a larger percentage of choices on the large alternative in the 0 s delay condition, demonstrating increased preference for the large reward. Additionally, when delays were increased gradually over time, subjects showed a more systematic decline in percentage of choice on the LL, demonstrating an increased sensitivity to delay length. Taken together, this pattern suggests less impulsive choice will be observed for animals for which LL delays are gradually increased across training sessions. The practice of

gradually increasing delays over the course of the within-sessions assessments shares similarities with delay exposure training (described above), and this may help to explain why subjects first exposed to shorter delays appear to show less impulsive choice when later subjected to longer delays.

Number of Trainings

The center panel of Table 4 depicts comparisons that allowed for the evaluation of the effects of the number of training sessions on measures of impulsivity. Figure 10 (upper panel) shows the percentage of LL choice for G1 across S1, S2 and S3. While responding during block 1 remains largely consistent across series, responding in subsequent blocks appears to change as the number of trainings increases. This is particularly evident when we isolate S1 and S3 (see Figure 10, lower panel). The most pronounced change appears when comparing responding over S1 and S2, with little additional change evident between S2 and S3. Notably, change between blocks 1 and 5 increased as the number of trainings increased; S1: -21%, S2: -25% and S3: -29%. A non-parametric Friedman test of differences among repeated measures was conducted to examine differences in responding over S1, S2 and S3 for each block. This test revealed significant difference for block 3 with a Chi-square value of 6.867 which was significant ($p = 0.032$).

Figure 11 (upper panel) shows the percentage of LL choice for G2 across S1, S2 and S3. As with G1, response patterns across blocks change as the number of trainings increases, with the most notable change occurring when comparing responding across S1 and S2. G2 subjects had differentiated response patterns in block 1 with the percentage of

choice on the large decreasing systematically as the number of trainings increased from 30% (S1) to 21% (S2) to 15% (S3). Additionally, the change between blocks 1 and 5, decreased as the number of trainings increased; S1: -23%, S2: -21% and S3: -15%. A non-parametric Friedman test of differences among repeated measures was conducted to examine differences in responding over S1, S2 and S3 for each block. This test revealed significant differences for all blocks (see Table 6 for individual test results).

The results of G1 and G2 across S1-S3 demonstrates a difference in patterns of responding, as the number of trainings increases while the delay sequence and delay progression are held constant. For both groups, the greatest change in responding was between performance over S1 and S2, with little additional change occurring during S3. As the number of trainings increased, there was little change in block 1 for G1 (exposed to a maximum delay of 8 s) demonstrating little change in preference for the large reward when the delay to this alternative was 0 s. However, for G2 (exposed to a maximum delay of 12 s), percentage of choice for the large reward in block 1 decreased systematically as the number of trainings increased, demonstrating a decreased preference for the large alternative. For G1, with additional training, responding became more differentiated across blocks showing an increased sensitivity to delay length. For G3, responding became less differentiated over time, indicating a decreased sensitivity to delay length. These findings suggest that number of training sessions impacts measures of impulsivity on the within-sessions procedure, and the direction in which this change occurs may be mediated by LL delay length in the terminal trial block in sessions; an issue which will be addressed in more detail below.

Delay Length

The right panel of Table 4 depicts comparisons that allowed for the evaluation of the effects of LL delay lengths on measures of impulsivity. The effect of this variable can be seen by comparing G1 and G2 performance across sessions during S1-S3. In these comparisons, the number of trainings were the same across the groups compared, and the delay progression did not change across S1-S3, but the LL delay values during sessions varied across groups (G1: 0, 1, 2, 4, 8 s; G2: 0, 2, 4, 8, 12 s). Figure 12 (top panel) depicts the first comparison examining the possible effect of delay length: G1 and G2 over S1. While responding in blocks 2, 3 and 4 appear to differ, responding during blocks 1 and 5 was equivalent across groups. Figure 12 (middle panel) depicts the second comparison: G1 and G2 over S2. The data paths for G1 and G2 become more distinct with G1 demonstrating a larger percentage of choice on the LL alternative across all blocks. Additionally, the change between blocks 1 and 5 is greater for G1: -25% than for G2 -21%. Figure 12 (lower panel) depicts the third and final comparison for delay length: G1 and G2 over S3. Over the final training sessions, G1 continues to show a larger percentage of choice on the LL alternative as compared to G2. Additionally, the change between blocks 1 and 5 continues to be greater for G1 then G2 with this value increasing for G1 for S2 (-25%) to S3 (-29%) and decreasing for G2 for S2 (-21%) to S3 (-15%). The Mann-Whitney test demonstrated that percentage of choice on the LL alternative was greater for G1 (*Mdn* = 0.20) then for G2 (*Mdn* = 0.08) in block 4 over S1, $U = 12.50$, $p = 0.038$ and for G1 (*Mdn* = 0.03) then for G2 (*Mdn* = 0.00) in block 5 over S2, $U = 10.00$, $p = 0.021$.

The results from these comparisons demonstrate differences in measures of impulsivity as a function of specific within-session LL delay lengths. These differences became more evident as the number of trainings increased. Figure 13 (top and middle panel) depict response patterns across all training series for G1 and G2, respectively. As the trainings progressed G1 (exposed to a maximum delay of 8 s) showed stable preference for the larger reward and increased sensitivity to delay length. G2 (exposed to a maximum delay of 12 s) showed less preference for the larger reward and decreased sensitivity to delay length. The present findings replicate those of Mori et al. (2018) in demonstrating that mice show more characteristic response pattern on the within-sessions procedure when the maximum delay is shorter (8 s) verses longer (12 s). These findings also replicate the authors' observation that with additional trainings, characteristic response patterns appear to degrade. It is possible that this gradual decrease in LL choices across training sessions is due to carryover effects which have been shown to produce a decreased preference for the LL alternative in rats following exposure to longer delays (Fox et al., 2008).

Omissions

Omission data were analyzed in terms of number of omissions, per group, per series. First, the number of omissions for each subject, within each block, for each training session was ascertained. Data for each group of subjects was then averaged per block, per series. For example, to calculate the average omission count for G1 (S1) the number of omissions for each of the G1 subjects was summed and then divided 80 (10

block 1 values [1 per session] x 8 subjects). To account for variation within these data sets, the standard error of the mean (SEM) was calculated for each value.

Figure 14 depicts the average omissions for each group across each series. Data paths for all groups depict a systematic increase in omissions as delay to the reinforcer increases. However, as the number of trainings increase, subjects made fewer omissions overall. Notably, subjects in G3 make the most omissions of all groups. This is particularly interesting given that over S2, subjects in G3 were exposed to the same delay series as subjects in G1 and over S3, subjects in G3 were exposed to the same delay series as subjects in G2.

Omissions are a secondary measure of sensitivity to delay length. It is assumed that if subjects are sensitive to delay length, as delays increase percentage of choice on the LL alternative will decrease and omissions will increase. The present data follow this pattern. However, notably the pattern is more pronounced for subjects in G3 as compared to G1 and G2. These results indicate a greater sensitivity to delay length when subjects are exposed to gradually increasing delays as compared to when they are not.

Reversal and Zero Second Delay Probes

As described above, a single session reversal and 0 s delay probes occurred following the completion of S3. Typically, these probes are repeated and are interspersed throughout the experiment. This arrangement was not utilized in the present study, as adding probe sessions would have introduced confounds to measures related to the number of trainings and exposure to different delay series. The present study found that

increased exposure to a specific delay sequence resulted in changes to patterns of within-session preference. It may be the case that if more probes had been conducted, different response patterns may have emerged. Thus, probe data results must be interpreted (and compared to results of previous studies) with caution.

Figure 15, Figure ***16*** and

Figure *17* depict the average percentage of choice on the large alternative during probes for G1, G2 and G3, respectively. For all groups, during the reversal probe, a lower percentage of responses on the LL alternative is seen relative to that of S3. During blocks 1 and 2 there is an initial drop in responding followed by a steep decline to zero percent choice on the LL by block 5. It could be argued that failure to see an immediate reversal in response pattern during a reversal probe indicates that subjects are not sensitive to the prevailing delays. However, if there was no sensitivity to delay length, there would be no difference in response patterns during the probe. The data pattern observed during this one-session probe may be further evidence of carryover effects: once subjects were exposed to longer delays in the first block, responses on the LL alternative remained low for the remaining trial blocks.

During the 0 s delay probe, for all groups there is a lower percentage of responses on the LL alternative in blocks 1 and 2 as compared to over S3. However, for G1 and G2, the percentage of choice on the LL rises as the delay increases with subjects displaying a slight increase in preference for the LL in blocks four and five. While findings from the single probe may not be conclusive, this pattern suggests that subjects showed more

consistent preference for the large alternative when the delay to either alternative was 0 s across all blocks. The results of this (and previous mouse related studies) demonstrate that mice do not always show exclusive or near-exclusive preference for the large alternative making it difficult to interpret the 0 s probe findings. However, during this probe, the relative preference for the larger option did not decrease systematically as delay increased as was observed during all prior training sessions.

Discussion

The immediate purpose of the present study was to further investigate the effects of methodological features of the within-session procedure on measures of impulsivity with mice. Our results show that delay length, delay progressions across series of training sessions, and the number of training sessions all affect the measures of impulsive choice that are obtained. Specifically, when exposed to gradually increasing LL delays across sessions (i.e., something similar to a delay fading procedure), subjects show a greater preference for LL and a greater sensitivity to delay length. There is emerging evidence that delay exposure training can help to ameliorate patterns of impulsive choice by decreasing aversion to delay (Peck et al., 2019). This phenomenon may help explain why mice that are gradually introduced to delays of increasing length show a more characteristic (rational) pattern of choice on the within-sessions procedure than those that are not gradually introduced to delays. Subjects also show greater propensity to choose the LL option and a greater sensitivity to delay length when the maximum delay in the terminal trial block in the within-session procedure is shorter. Finally, the number of training sessions to which subjects are exposed results in different patterns of choice, and

this appears to be related primarily to the effects of the LL delays imposed during the final trial block of the within-session procedure. Longer LL delays in the final trial block appear to produce more impulsive choice in subsequent sessions. This pattern may emerge over time secondary to carryover effects from previous sessions. As training progresses, the operanda associated with the LL reward is repeatedly paired with a long delay to reward, and this may serve to suppress responding on this alternative. As more responses are allocated to the SS alternative, the overall pattern of responding appears to demonstrate greater impulsivity.

Due to its relevance to human diseases and behavioral disorders, the construct of impulsivity has become a topic of great interest to researchers across a wide range of scientific disciplines. Impulsivity is often measured in terms of choice between two reward options using procedures such as those investigated here. Interdisciplinary research exploring the relation between discounting and its environmental and genetic determinants will be facilitated by the standardization of assessments for mice. These assessments have value as a phenotyping procedure, but only if the effects of methodological features are fully recognized. Comparisons across strains of animals are only valid when their performance has been assessed under equivalent conditions. Methodologically, the findings from the present study have contributed towards this end. The results suggest that the differences across existing mouse studies may be due to small but important procedural details. These small details can have a meaningful effect on the overall outcome of the within-sessions procedure. Researchers hoping to produce characteristic response patterns (depicting more rational choice) with Balb-c mice can accomplish this by using shorter delays, introducing delays systematically over time and

by carefully considering the number of training sessions that a subject is exposed to. While not demonstrated in the present study, there is evidence that rational choice may also be enhanced by including frequent 0 s delay conditions throughout the training period (Madden & Johnson, 2015). While patterns of rational choice may be produced through the above-mentioned manipulations of the within-sessions procedure, the results of these studies must be considered carefully. From a conceptual standpoint, the present study has elucidated the important distinction between constructs and events when interpreting the results of one specific experimental circumstance. Small procedural adjustments to any given assessment can have a large impact on the resultant data. Thus, researchers must always carefully consider their interpretation of findings; particularly when using these findings to consider complicated theoretical constructs such as impulsivity. This is particularly important when you consider that in recent months, scholars within psychology have started to question whether impulsivity should be considered a psychological construct at all. Secondary to emerging evidence that impulsive traits and behaviors may in fact be largely uncorrelated, the status of impulsivity as a unified construct is currently in question (Strickland & Johnson, 2021). By remaining measured in our interpretation of findings from choice studies, researchers can continue to expand the existing literature base while not overstating the applicability of their results.

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References

Adriani, W., & Laviola, G. (2003). Elevated levels of impulsivity and reduced place conditioning with d-amphetamine: Two behavioral features of adolescence in mice. *Behavioral Neuroscience*, *117*(4), 695–703. https://doi.org/10.1037/0735-7044.117.4.695

Ainslie, G. W. (1974). Impulse control in pigeons. *Journal of the Experimental Analysis of Behavior*, *21*(3), 485–489.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.).* American Psychiatric Publishing.

Anderson, K. G., & Woolverton, W. L. (2005). Effects of clomipramine on self-control choice in Lewis and Fischer 344 rats. *Pharmacology Biochemistry and Behavior*, *80*(3), 387–393. https://doi.org/10.1016/j.pbb.2004.11.015

Anft, M. (2008, September). Of mice and medicine. *Johns Hopkins Magazine*, *60*(4). http://pages.jh.edu/jhumag/0908web/mice.html

Bickel, W. K., Odum, A. L., & Madden, G. J. (1999). Impulsivity and cigarette smoking: Delay discounting in current, never, and ex-smokers. *Psychopharmacology*, *146*(4), 447–454. https://doi.org/10.1007/PL00005490

Buhusi, M., Olsen, K., Yang, B. Z., & Buhusi, C. V. (2016). Stress-induced executive dysfunction in GDNF-deficient mice, a mouse model of Parkinsonism. *Frontiers in Behavioral Neuroscience*, *10*. https://doi.org/10.3389/fnbeh.2016.00114

Cardinal, R. N., Robbins, T. W., & Everitt, B. J. (2000). The effects of d -amphetamine, chlordiazepoxide, α-flupenthixol and behavioural manipulations on choice of signalled and unsignalled delayed reinforcement in rats. *Psychopharmacology*, *152*(4), 362–375. https://doi.org/10.1007/s002130000536

Charlton, S. R., & Fantino, E. (2008). Commodity specific rates of temporal discounting: Does metabolic function underlie differences in rates of discounting? *Behavioural Processes*, *77*(3), 334–342. https://doi.org/10.1016/j.beproc.2007.08.002

Davies, W., Humby, T., Isles, A. R., Burgoyne, P. S., & Wilkinson, L. S. (2007). X-monosomy effects on visuospatial attention in mice: A candidate gene and implications for Turner Syndrome and Attention Deficit Hyperactivity Disorder. *Biological Psychiatry*, *61*(12), 1351–1360. https://doi.org/10.1016/j.biopsych.2006.08.011

de Wit, H., & Mitchell, S. H. (2015). Drug effects on delay discounting. In G. J. Madden & W. K. Bickel (Eds.), *Impulsivity: The behavioral and neurological science of discounting.* (pp. 213–241). American Psychological Association. https://doi.org/10.1037/12069-008

Dent, C. L., Humby, T., Lewis, K., Ward, A., Fischer-Colbrie, R., Wilkinson, L. S., Wilkins, J. F., & Isles, A. R. (n.d.). *Impulsive Choice in Mice Lacking Paternal Expression of Grb10 Suggests Intragenomic Conflict in Behavior*. 8.

Dixon, M. R., & Cummings, A. (2001). Self-control in children with autism: Response allocation during delays to reinforcement. *Journal of Applied Behavior Analysis*, *34*(4), 491–495. https://doi.org/10.1901/jaba.2001.34-491

Dixon, M. R., & Holcomb, S. (2000). Teaching self-control to small groups of dually diagnosed adults. *Journal of Applied Behavior Analysis*, *33*(4), 611–614. https://doi.org/10.1901/jaba.2000.33-611

Dixon, M. R., & Tibbetts, P. A. (2009). The effects of choice on self-control. *Journal of Applied Behavior Analysis*, *42*(2), 243–252. https://doi.org/10.1901/jaba.2009.42-243

Duckworth, A. L., & Kern, M. L. (2011). A meta-analysis of the convergent validity of self-control measures. *Journal of Research in Personality*, *45*(3), 259–268. https://doi.org/10.1016/j.jrp.2011.02.004

Eisenberger, R., & Masterson, F. A. (1987). Effects of prior learning and current motivation on self-control. In M. L. Commons, J. E. Mauzer, J. A. Nevin, & H. Rachlin (Eds.), *Quantitative analyses of behavior: Vol. V*.

Evenden, J. L., & Ryan, C. N. (1996). The pharmacology of impulsive behaviour in rats: The effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology*, *128*(2), 161–170. https://doi.org/10.1007/s002130050121

Fox, A. T., Hand, D. J., & Reilly, M. P. (2008). Impulsive choice in a rodent model of attention-deficit/hyperactivity disorder. *Behavioural Brain Research*, *187*(1), 146–152. https://doi.org/10.1016/j.bbr.2007.09.008

Helms, C. M., Reeves, J. M., & Mitchell, S. H. (2006). Impact of strain and d-amphetamine on impulsivity (delay discounting) in inbred mice. *Psychopharmacology*, *188*(2), 144–151. https://doi.org/10.1007/s00213-006-0478-0

Humby, T., Laird, F. M., Davies, W., & Wilkinson, L. S. (1999). Visuospatial attentional functioning in mice: Interactions between cholinergic manipulations and genotype: Attentional function in mice. *European Journal of Neuroscience*, *11*(8), 2813–2823. https://doi.org/10.1046/j.1460-9568.1999.00701.x

Hurst, J. L., & West, R. S. (2010). Taming anxiety in laboratory mice. *Nature Methods*, *7*(10), 825–826. https://doi.org/10.1038/nmeth.1500

Isles, A. R., Humby, T., Walters, E., & Wilkinson, L. S. (2004). Common genetic effects on variation in impulsivity and activity in mice. *Journal of Neuroscience*, *24*(30), 6733–6740. https://doi.org/10.1523/JNEUROSCI.1650-04.2004

Isles, A. R., Humby, T., & Wilkinson, L. S. (2003). Measuring impulsivity in mice using a novel operant delayed reinforcement task: Effects of behavioural manipulations and d- amphetamine. *Psychopharmacology*, *170*(4), 376–382. https://doi.org/10.1007/s00213-003-1551-6

Lambourne, S. L., Humby, T., Isles, A. R., Emson, P. C., Spillantini, M. G., & Wilkinson, L. S. (2007). Impairments in impulse control in mice transgenic for the human FTDP-17 tau V337M mutation are exacerbated by age. *Human Molecular Genetics*, *16*(14), 1708–1719. https://doi.org/10.1093/hmg/ddm119

Logue, A. W., & Mazur, J. E. (1981). Logue AW, Mazur JE. Maintenance of self-control acquired through a fading procedure: Follow-up on Mazur and Logue (1978). *Behaviour Analysis Letters*, *1*(3), 131–137.

Madden, & Bickel (Eds.). (2015). *Impulsivity: The Behavioral and Neurological Science of Discounting*. American Psychological Association. https://doi.org/10.1037/12069-000

Madden, G. J., & Johnson, P. S. (2015). A delay-discounting primer. In *Impulsivity: The behavioral and neurological science of discounting.* (pp. 11–37). American Psychological Association. https://doi.org/10.1037/12069-001

Marshall, A. T., Smith, A. P., & Kirkpatrick, K. (2014). Mechanisms of impulsive choice: I. Individual differences in interval timing and reward processing: Individual differences in choice and timing. *Journal of the Experimental Analysis of Behavior*, *102*(1), 86–101. https://doi.org/10.1002/jeab.88

Mazur, J. E., & Logue, A. W. (1978). Choice in a "self-control" paradigm: Effects of a fading procedure. *Journal of the Experimental Analysis of Behavior, 30*(1), 11–17.

McKerchar, T.L., & Renda, C.R. (2012). Delay and probability discounting in humans: An overview. *The Psychological Record, 62*(4), 817-834.

Mitchell, S. H. (2014). Assessing delay discounting in mice: Assessing delay discounting in mice. In C. R. Gerfen, A. Holmes, D. Sibley, P. Skolnick, & S. Wray (Eds.), *Current Protocols in Neuroscience* (pp. 8.30.1-8.30.12). John Wiley & Sons, Inc. https://doi.org/10.1002/0471142301.ns0830s66

Mitchell, S. H., Reeves, J. M., Li, N., & Phillips, T. J. (2006). Delay discounting predicts behavioral sensitization to ethanol in outbred WSC mice. *Alcoholism: Clinical and Experimental Research*, *30*(3), 429–437. https://doi.org/10.1111/j.1530-0277.2006.00047.x

Mori, M., Tsutsui-Kimura, I., Mimura, M. & Tanaka, K.F. (2018). 5-HT_3 antagonists decrease discounting rate without affecting sensitivity to reward magnitude in the delay discounting task in mice. Psychopharmacology, *253,* 2619-2629. https://doi.org/10.1007/s00213-018-4954-0

NIH. (2002). *Background on Mouse as a Model Organism*. https://www.genome.gov/10005834/background-on-mouse-as-a-model-organism/

NIH. (2015, August 27). *Knockout Mice Fact Sheet*. National Human Genome Research Institute. https://www.genome.gov/about-genomics/fact-sheets/Knockout-Mice-Fact-Sheet

Odum, A. L. (2011a). Delay discounting: Trait variable? *Behavioural Processes*, *87*(1), 1–9. https://doi.org/10.1016/j.beproc.2011.02.007

Odum, A.L. (2011b). Delay discounting: I'm a K, you're a K, *Journal of the Experimental Analysis of Behavior, 96,* 427-439.

Odum, A. L., & Baumann, A. A. L. (2015). Delay discounting: State and trait variable. In *Impulsivity: The behavioral and neurological science of discounting.* (pp. 39–65). American Psychological Association. https://doi.org/10.1037/12069-002

Olson, E. A., Hooper, C. J., Collins, P., & Luciana, M. (2007). Adolescents' performance on delay and probability discounting tasks: Contributions of age, intelligence, executive functioning, and self-reported externalizing behavior. *Personality and Individual Differences*, *43*(7), 1886–1897. https://doi.org/10.1016/j.paid.2007.06.016

Peters, C. M. & Hayes, L. J. (2020). Mice as subjects in collaborative research. Mexican Journal of Behavior Analysis, 46, 244-258.

Patel, S., Stolerman, I. P., Asherson, P., & Sluyter, F. (2006). Attentional performance of C57BL/6 and DBA/2 mice in the 5-choice serial reaction time task. *Behavioural Brain Research*, *170*(2), 197–203. https://doi.org/10.1016/j.bbr.2006.02.019

Peck, S., Rung, J. M., Hinnenkamp, J. E., & Madden, G. J. (2019). Reducing impulsive choice: VI. Delay-exposure training reduces aversion to delay-signaling stimuli. *Psychology of Addictive Behaviors*, No Pagination Specified-No Pagination Specified. https://doi.org/10.1037/adb0000495

Pinkston, J. W., & Lamb, R. J. (2011). Delay discounting in C57BL/6J and DBA/2J mice: Adolescent-limited and life-persistent patterns of impulsivity. *Behavioral Neuroscience*, *125*(2), 194–201. https://doi.org/10.1037/a0022919

Renda, C. R., & Madden, G. J. (2016). Impulsive choice and pre-exposure to delays: III. Four-month test-retest outcomes in male Wistar rats. *Behavioural Processes*, *126*, 108–112. https://doi.org/10.1016/j.beproc.2016.03.014

Renda, C. R., Rung, J. M., Hinnenkamp, J. E., Lenzini, S. N., & Madden, G. J. (2018). Impulsive choice and pre-exposure to delays: Iv. effects of delay- and immediacy-exposure training relative to maturational changes in impulsivity. *Journal of the Experimental Analysis of Behavior*, *109*(3), 587–599. https://doi.org/10.1002/jeab.432

Richards, J. B., Mitchell, S. H., de Wit, H., & Seiden, L. S. (1997). Determination of discount functions in rats with an adjusting-amount procedure. *Journal of the Experimental Analysis of Behavior*, *67*(3), 353–366. https://doi.org/10.1901/jeab.1997.67-353

Rosenthal, N., & Brown, S. (2007). The mouse ascending: Perspectives for human-disease models. *Nature Cell Biology*, *9*, 993–999.

Renda, R. C., Rung, J.M., Peck, S., & Madden, G. J. (2021). Reducing impulsive choice VII. effects of duration of delay-exposure training. *Animal Cognition, 24,* 11-21. https://doi.org/10.1007/s10071-020-01412-0

Rung, J. M., Buhusi, C. V., & Madden, G. J. (2018). Reducing impulsive choice: V. The role of timing in delay-exposure training. *Behavioural Processes*, *157*, 557–561. https://doi.org/10.1016/j.beproc.2018.04.018

Rung, J. M., & Madden, G. J. (2018). Experimental reductions of delay discounting and impulsive choice: A systematic review and meta-analysis. *Journal of Experimental Psychology. General*, *147*(9), 1349–1381. https://doi.org/10.1037/xge0000462

Schweitzer, J. B., & Sulzer-Azaroff, B. (1988). Teaching tolerance for delay in impulsive children. *Journal of the Experimental Analysis of Behavior*, *50*, 173–186.

Shamosh, N. A., & Gray, J. R. (2008). Delay discounting and intelligence: A meta-analysis. *Intelligence*, *36*(4), 289–305. https://doi.org/10.1016/j.intell.2007.09.004

Stein, J. S., Johnson, P. S., Renda, C. R., Smits, R. R., Liston, K. J., Shahan, T. A., & Madden, G. J. (2013). Early and prolonged exposure to reward delay: Effects on impulsive choice and alcohol self-administration in male rats. *Experimental and Clinical Psychopharmacology*, *21*(2), 172–180. https://doi.org/10.1037/a0031245

Stein, J. S., Renda, C. R., Hinnenkamp, J. E., & Madden, G. J. (2015). Impulsive choice, alcohol consumption, and pre-exposure to delayed rewards: II. Potential mechanisms. *Journal of the Experimental Analysis of Behavior, 103*(1), 33–49. https://doi.org/10.1002/jeab.116

Strickland, J.C., & Johnson, M.W. (2021). Rejecting impulsivity as a psychological construct: a theoretical, empirical, and sociocultural argument. *Psychological Review,* 128(2): 336-361.

Turturici, M., Ozga, J. E., & Anderson, K. G. (2018). Pair housing alters delay discounting in Lewis and Fischer 344 rats. *The Psychological Record*, *68*(1), 61–70. https://doi.org/10.1007/s40732-018-0268-1

Table 1

Summary of procedural differences between common discounting assessments

Variable	Independent Variable	Dependent Variable	Trial Arrangement
Adjusting Delay	Delay Length	Indifference points	No Blocks
Adjusting Amount	Delay Length	Indifference points	No blocks
Within-Sessions	Reward Amount	Percentage of choice	Blocks

Table 2

Summary of the training conditions for the three groups of subjects.

Group	Delays (s)	No. of days at each delay
A	S1: 0, 1, 2, 4, 8	10
	S2: 0, 1, 2, 4, 8	10
	S3: 0, 1, 2, 4, 8	10
B	S1: 0, 2, 4, 8, 12	10
	S2: 0, 2, 4, 8, 12	10
	S3: 0, 2, 4, 8, 12	10
C	S1: 0, .5, 1, 2, 4	10
	S2: 0, 1, 2, 4, 8	10
	S3: 0, 2, 4, 8, 12	10

Table 3

Summary of the training conditions for three groups of subjects during the reversal probe.

Group	Delays
1	8, 4, 2, 1, 0
2	12, 8, 4, 2, 0
3	12, 8, 4, 2, 0

Table 4

Summary of the comparison groups for statistical analysis.

Delay Progression	
Group	Delays (s)
1	S1: 0, 1, 2, 4, 8
	S2: 0, 1, 2, 4, 8
	S3: 0, 1, 2, 4, 8
2	S1: 0, 2, 4, 8, 12
	S2: 0, 2, 4, 8, 12
	S3: 0, 2, 4, 8, 12
3	S1: 0, .5, 1, 2, 4
	S2: 0, 1, 2, 4, 8
	S3: 0, 2, 4, 8, 12

Number of Trainings	
Group	Delays (s)
1	S1: 0, 1, 2, 4, 8
	S2: 0, 1, 2, 4, 8
	S3: 0, 1, 2, 4, 8
2	S1: 0, 2, 4, 8, 12
	S2: 0, 2, 4, 8, 12
	S3: 0, 2, 4, 8, 12
3	S1: 0, .5, 1, 2, 4
	S2: 0, 1, 2, 4, 8
	S3: 0, 2, 4, 8, 12

Delay Length	
Group	Delays (s)
1	S1: 0, 1, 2, 4, 8
	S2: 0, 1, 2, 4, 8
	S3: 0, 1, 2, 4, 8
2	S1: 0, 2, 4, 8, 12
	S2: 0, 2, 4, 8, 12
	S3: 0, 2, 4, 8, 12
3	S1: 0, .5, 1, 2, 4
	S2: 0, 1, 2, 4, 8
	S3: 0, 2, 4, 8, 12

Table 5

Mann-Whitney test statistics for G1 vs G3 over S3

	10 Session Average		
	Mdn		Mann-Whitney Test
	1	3	
Block 1	0.16	0.57	U = 7.00, z = -2.43, p= 0.014*, r = -0.63
Block 2	0.04	0.30	U = 8.50, z = -2.27, p= 0.021*, r = -0.59
Block 3	0.02	0.15	U = 8.00, z = -2.34, p= 0.021*, r = -0.60
Block 4	0.01	0.03	U = 15.50, z = -1.51, p= 0.152, r = -0.39
Block 5	0.00	0.02	U = 12.00, z = -2.03, p= 0.072*, r = -0.52

*$p < .05$

Table 6

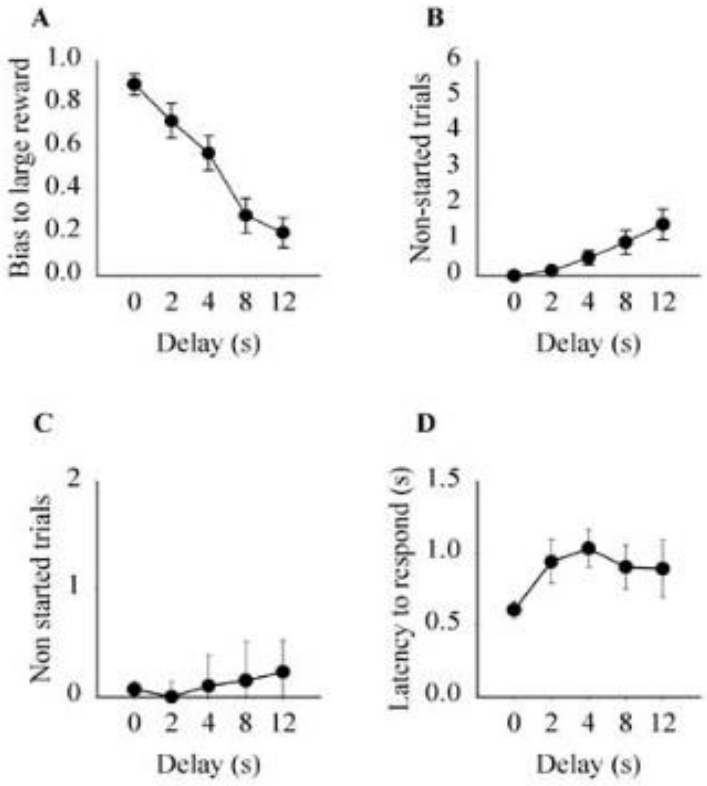
Friedman's Test statistics for G2

	N	Chi-Square	df	Sig.
Block 1	8	6.867	2	0.032
Block 2	8	10.516	2	0.005
Block 3	8	13.067	2	0.001
Block 4	8	9.071	2	0.011
Block 5	8	13.455	2	0.001

*$p < .05$

Figure 1

Changes in Choice Bias (reprinted from Isles et al., 2003)



Note. Graphs showing the changes in choice bias (**A**), non-started trials during the choice trials (of a possible six choice trials per block) (**B**), non-started trials during the forced trials (of a possible two trials per block) (**C**), and response latency (**D**) at baseline performance. Values shown are means±SEM

Figure 2

Choice bias across strains (reprinted from Isles et al., 2004)

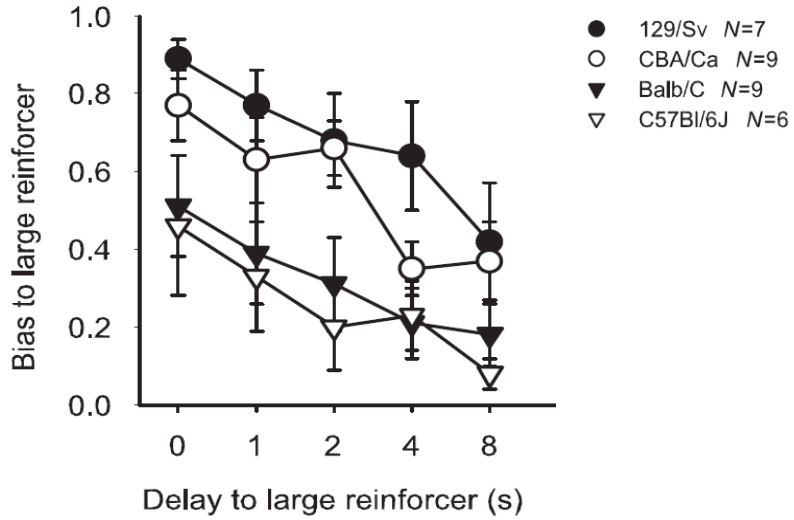


Figure 3

Choice bias (reprinted from Mori et al., 2018)

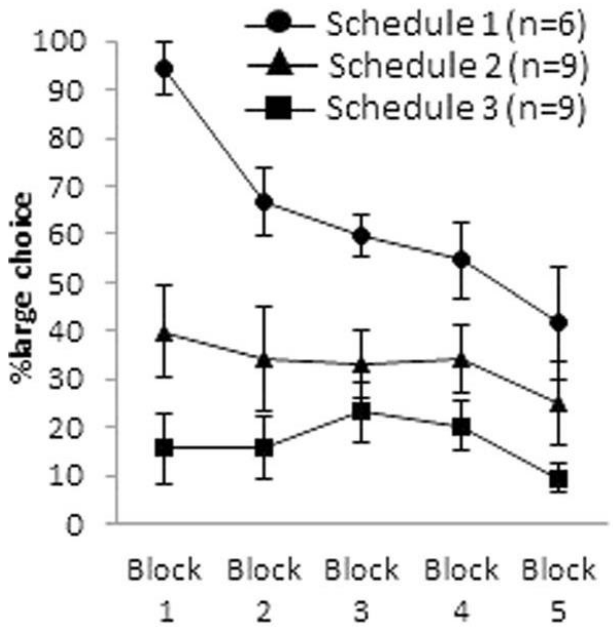


Figure 4

Correct nose-poke (count/session) (reprinted from Mori et al., 2018)

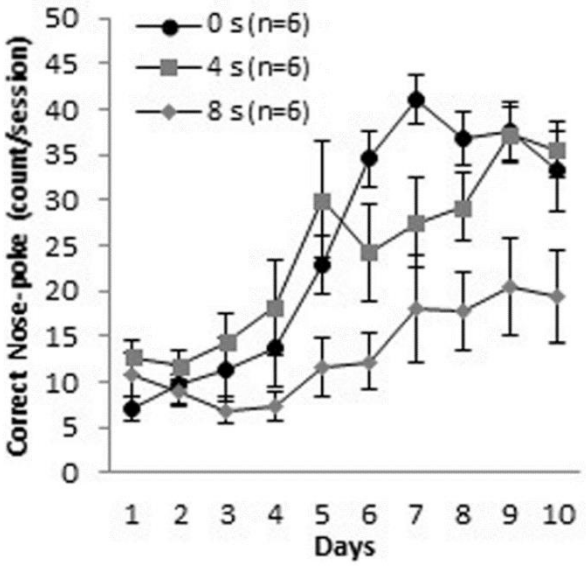


Figure 5

Percentage Accuracy (count/session) (reprinted from Mori et al., 2018)

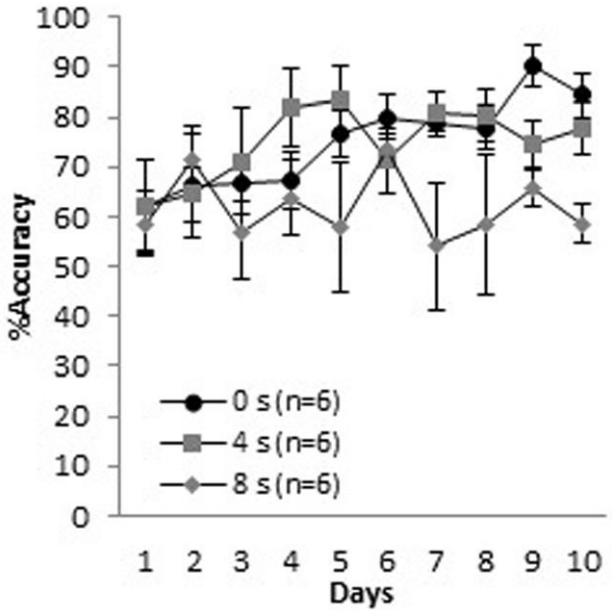


Figure 6

Correct nose-poke (count/session) (reprinted from Mori et al., 2018)

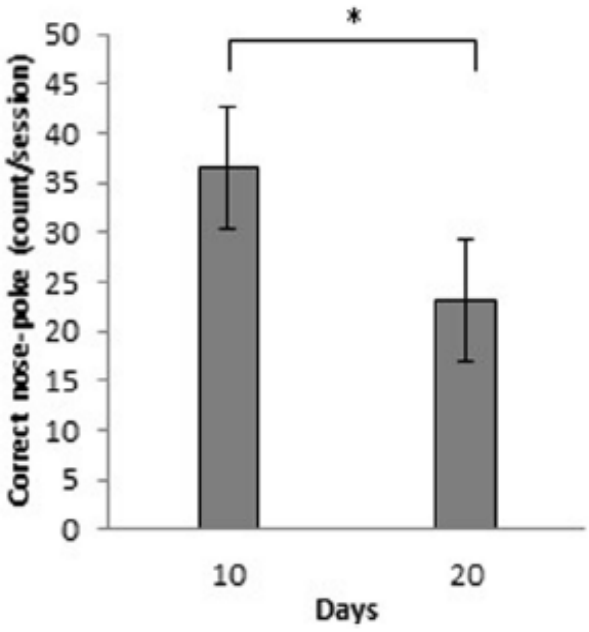


Figure 7

Percentage Accuracy (count/session) (reprinted from Mori et al., 2018)

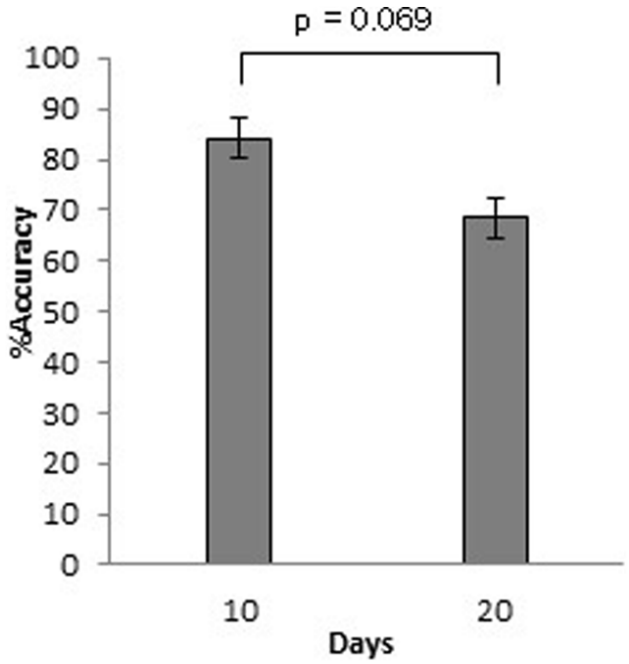


Figure 8

Within-sessions procedural layout for proposed experiment.

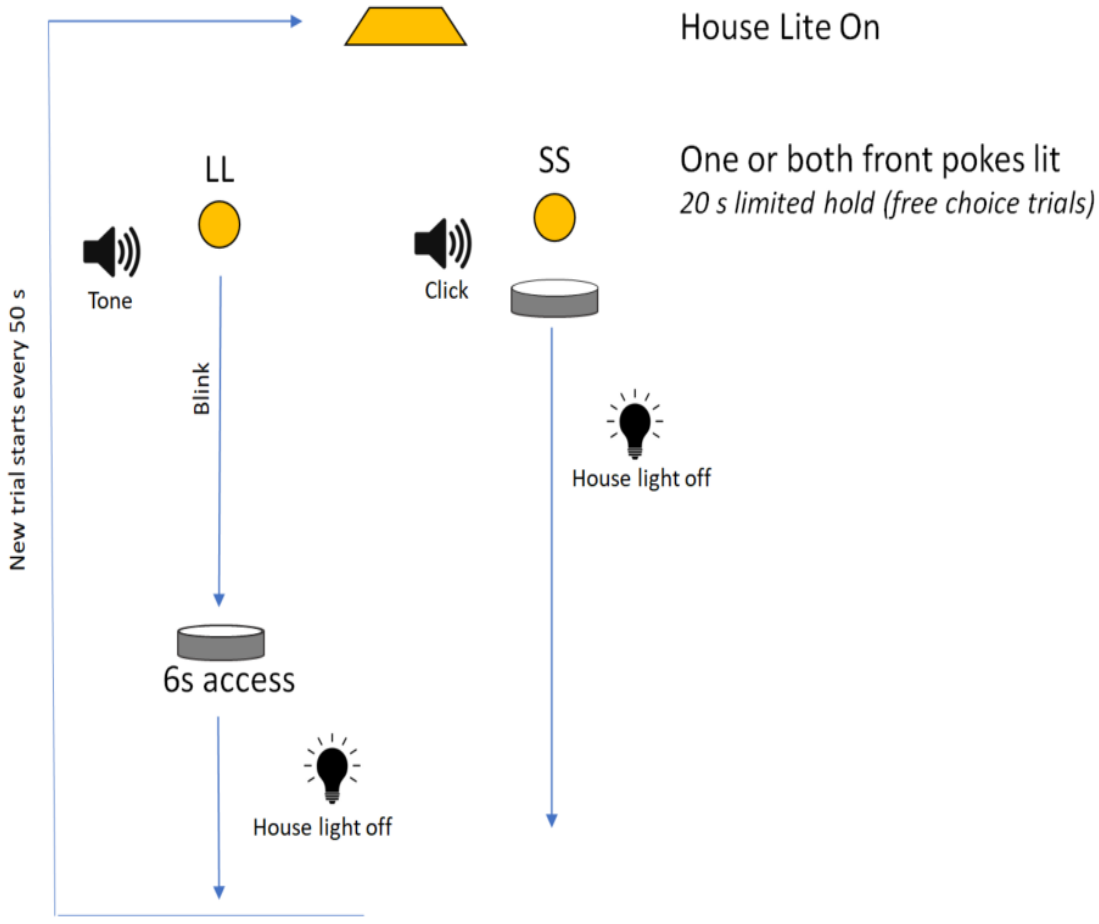
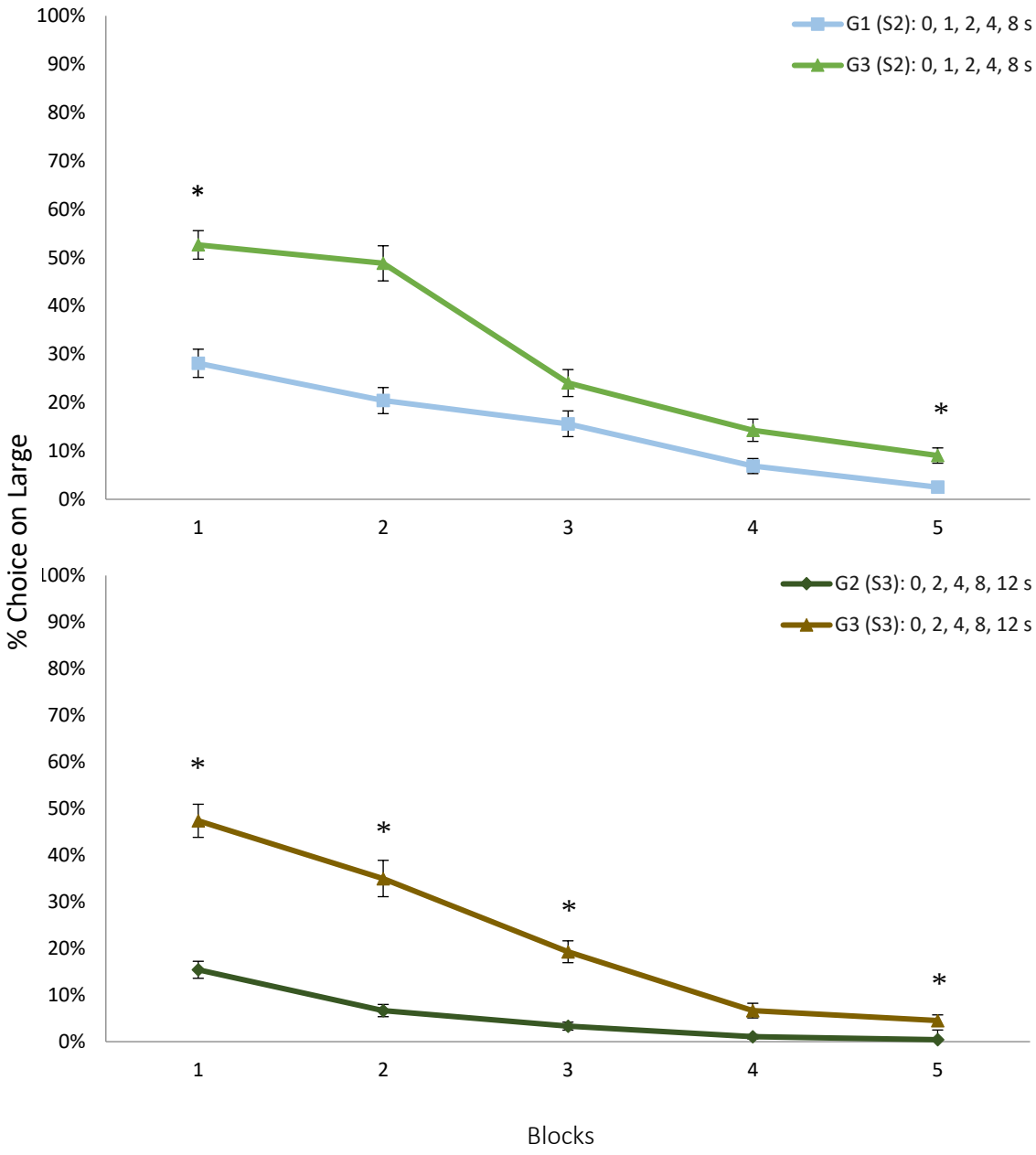


Figure 9

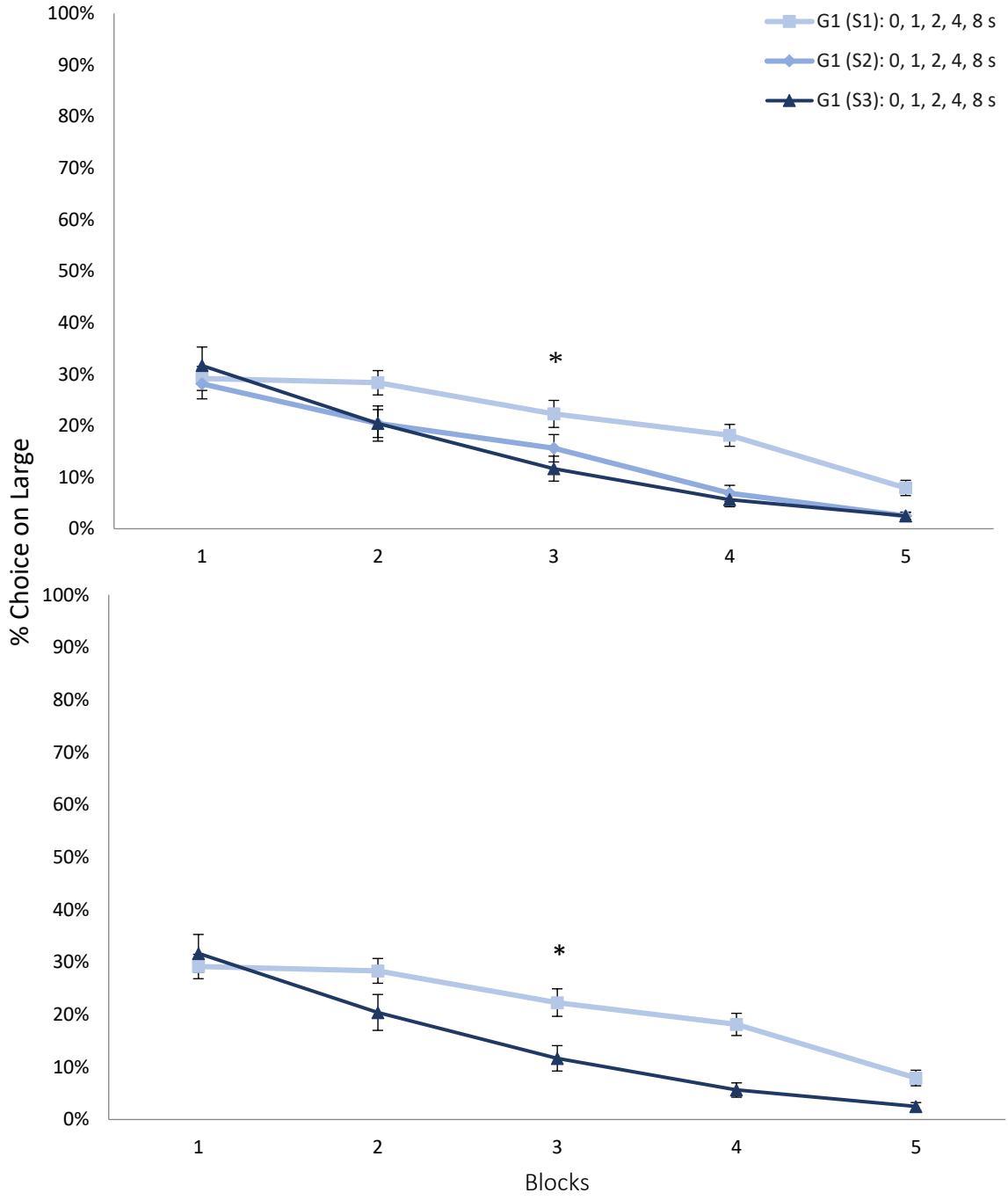
Delay Progression Comparisons



Note. Average percentage of choice on the large alternative, for G1 and G3 over S2 (upper panel). Average percentage of choice on the large alternative, for G2 and G3 over S3 (lower panel). Values displayed are means ± SEM. Asterisks indicate a statistically significant difference.

Figure 10

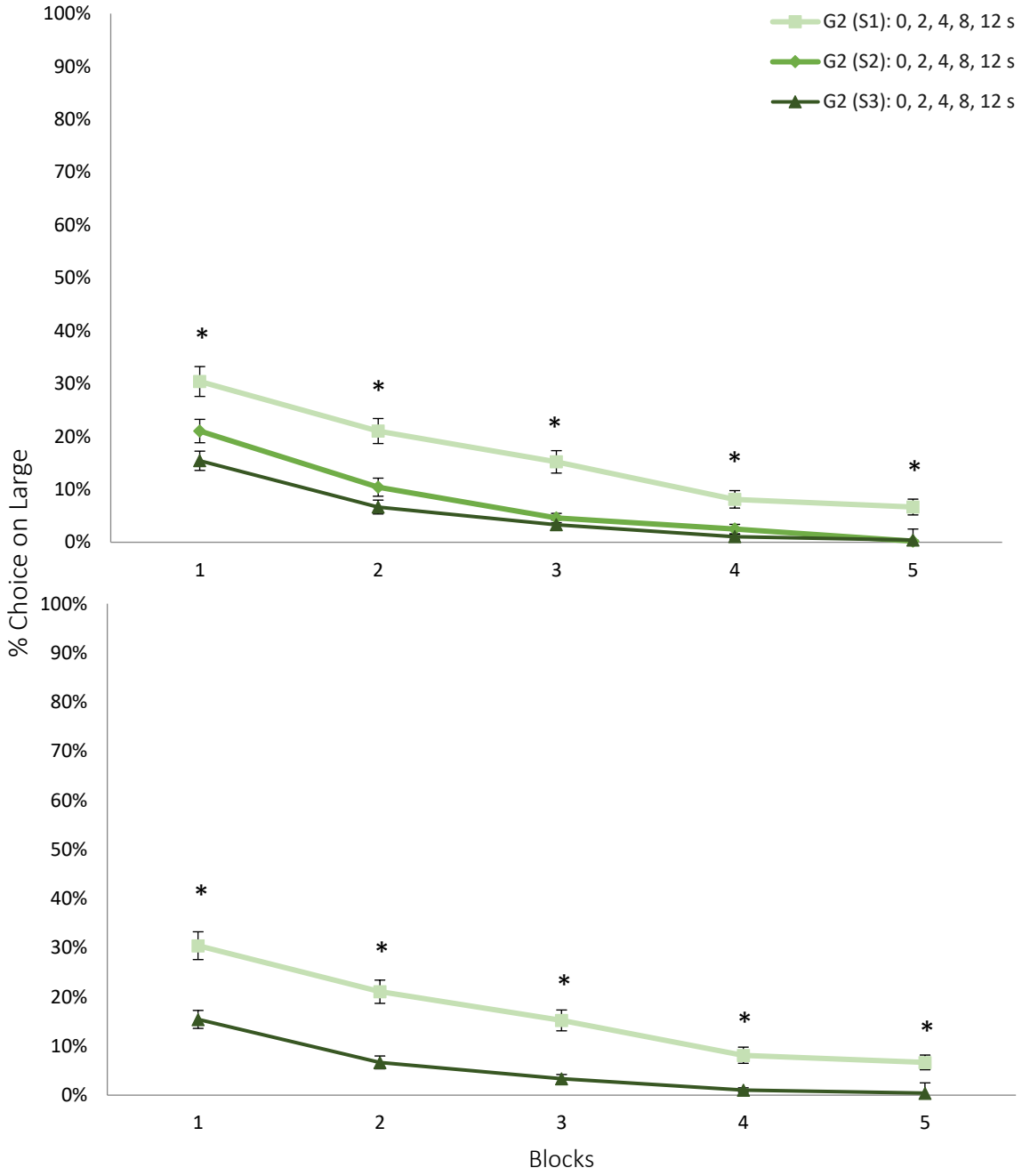
Number of Trainings Comparison 1



Note: Average percentage of choice on the large alternative, for G1 over S1, 2 and 3 (upper panel). Average percentage of choice on the large alternative, for G1 over S1 and 3 (lower panel). Values displayed are means ± SEM. Asterisks indicate a statistically significant difference.

Figure 11

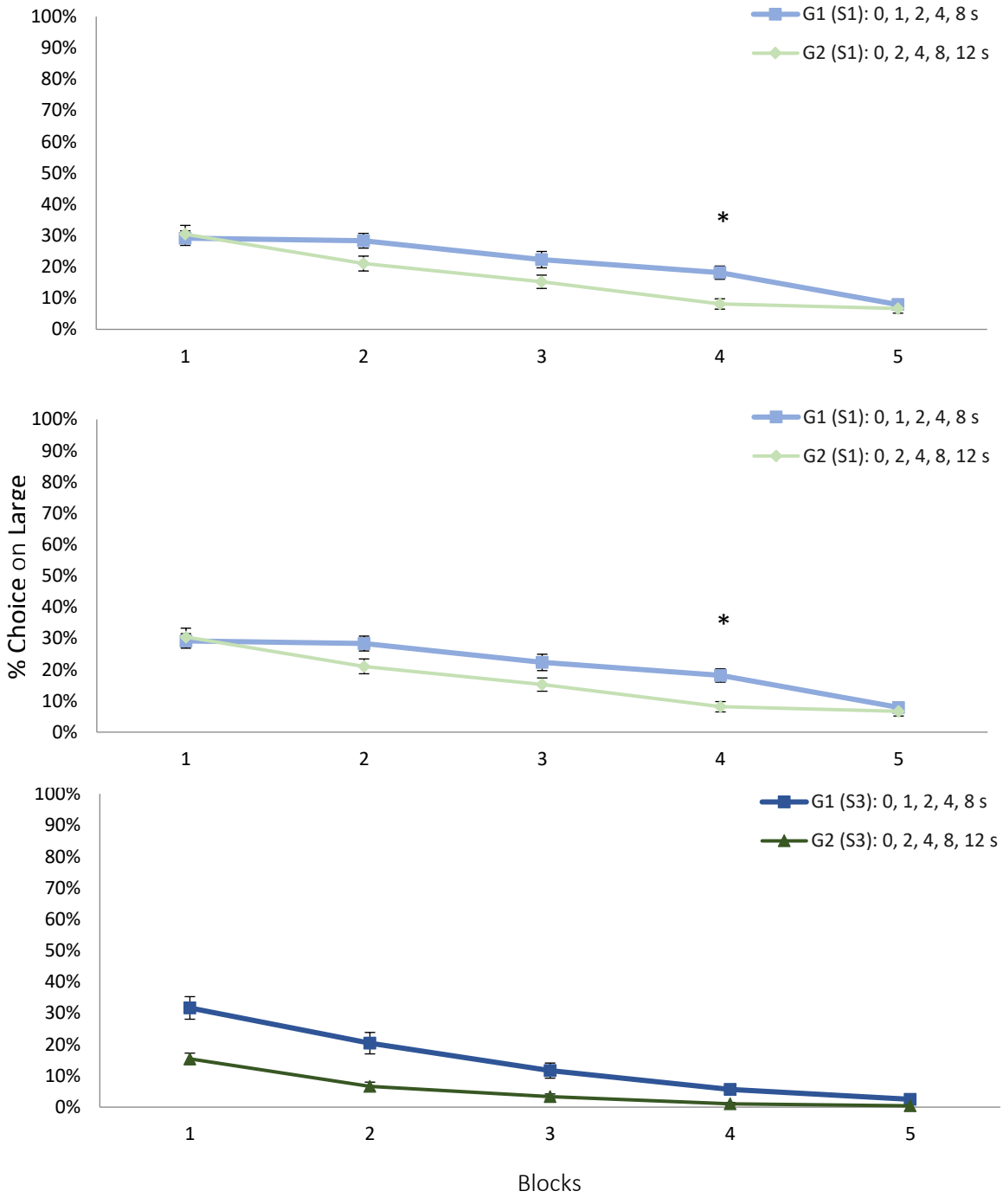
Number of Trainings Comparison 2



Note. Average percentage of choice on the large alternative, for G2 over S1, 2 and 3 (upper panel). Average percentage of choice on the large alternative, for G2 over S1 and 3 (lower panel). Values displayed are means ± SEM

Figure 12

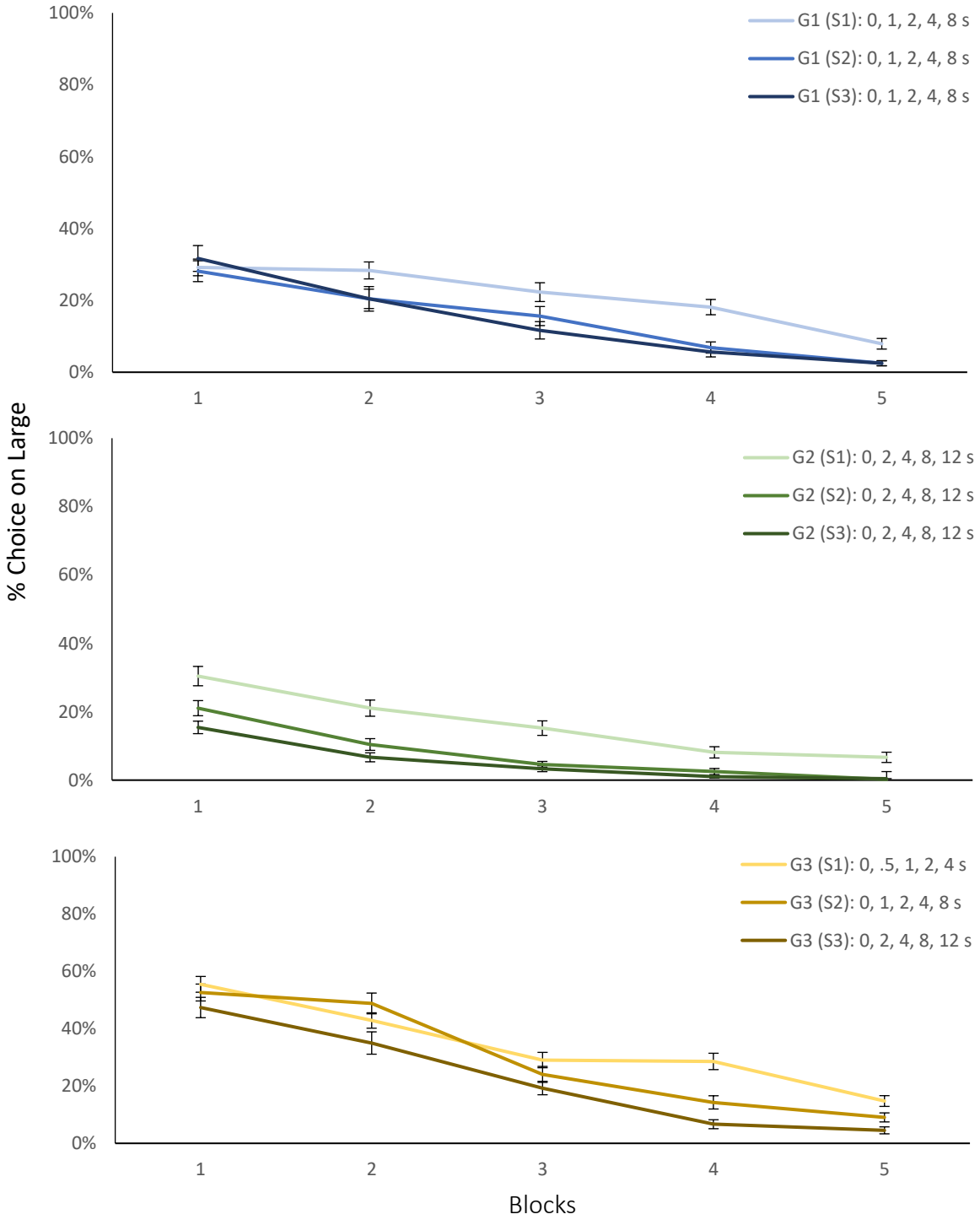
Delay Length Comparisons



Note. Average percentage of choice on the large alternative, for G1 and 2 over S1 (upper panel), S2 (middle panel) and S3 (lower panel). Values displayed are means ± SEM. Asterisks indicate a statistically significant difference.

Figure 13

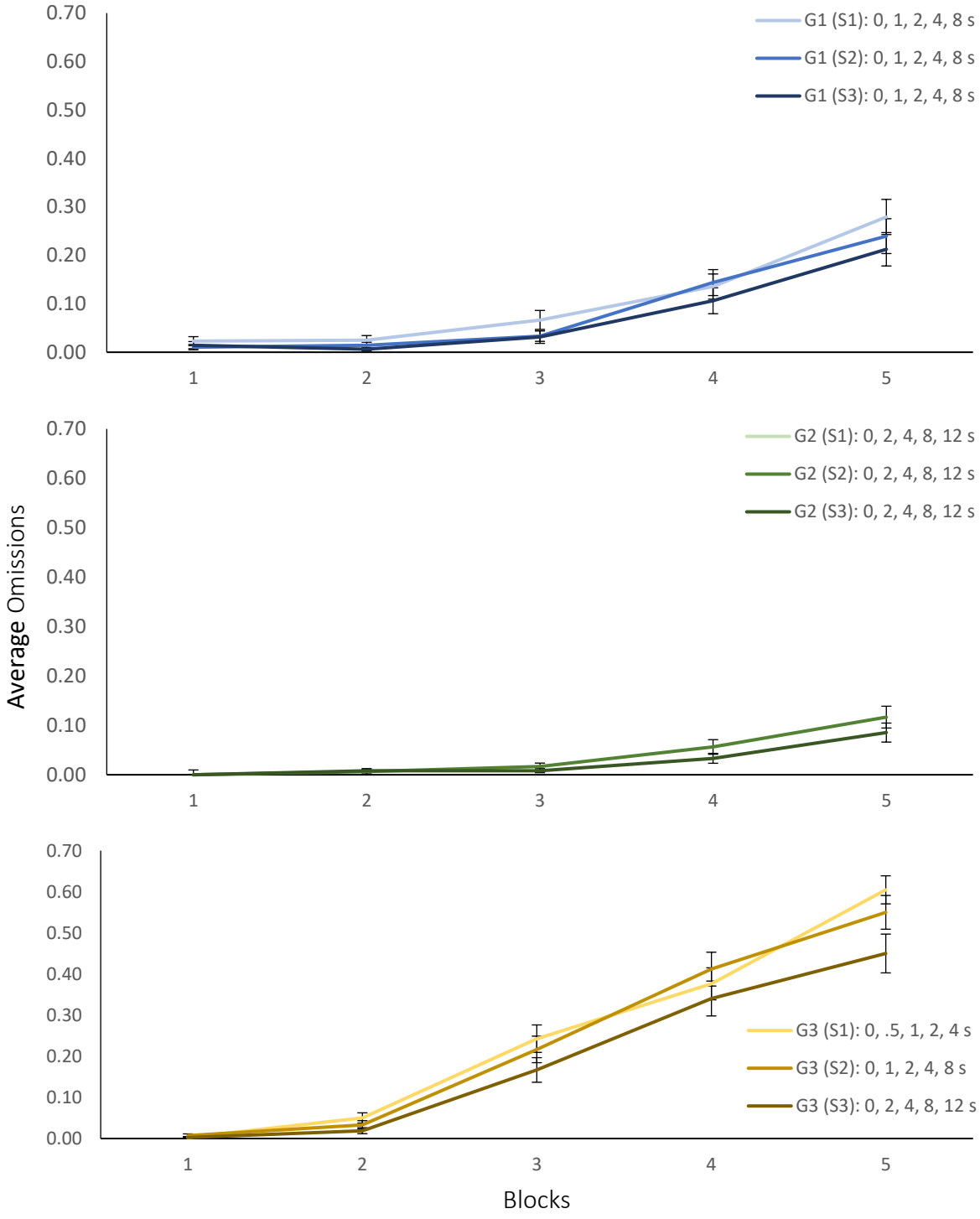
Percentage of choice on the LL for all groups across all series



Note. Average percentage of choice on the large alternative, for G1 (top panel), 2 (middle panel) and 3 (lower panel) over S1, 2 and 3. Values displayed are means ± SEM.

Figure 14

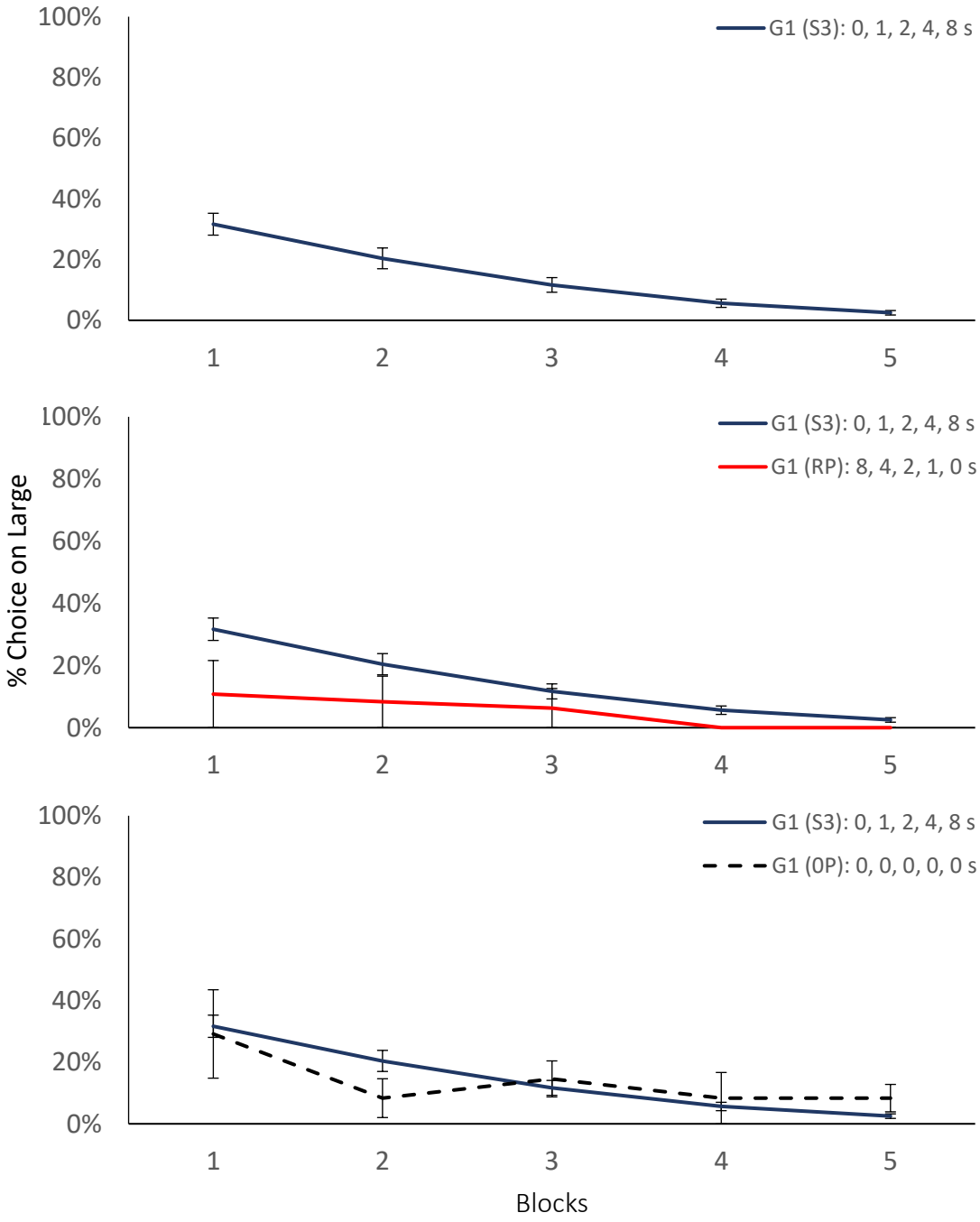
Average Omissions for all Groups across all Series



Note. Average number of omissions, for G1 (top panel), 2 (middle panel) and 3 (lower panel) over S1, 2 and 3. Values displayed are means ± SEM.

Figure 15

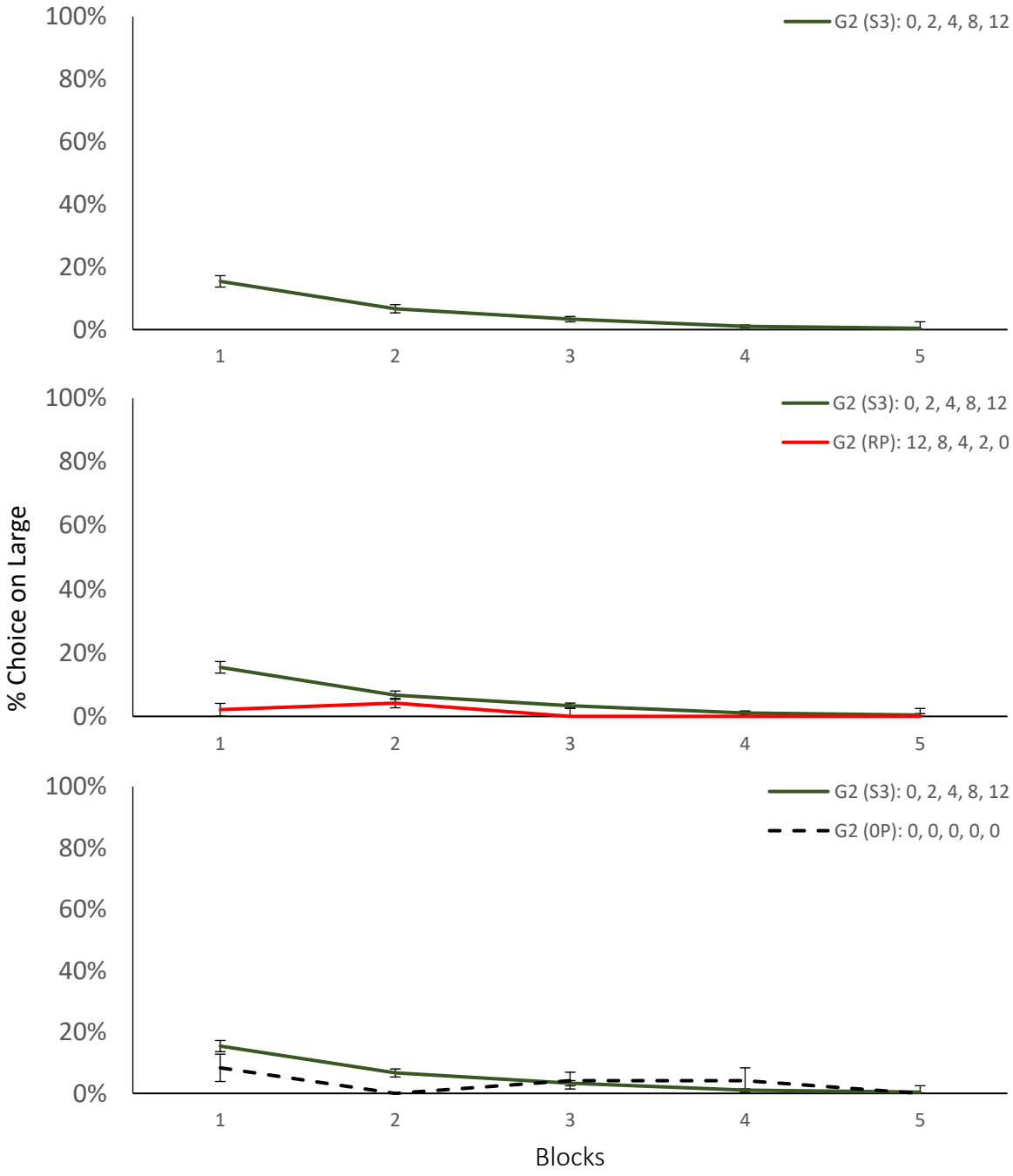
Group 1 Probe Data



Note. Average percentage of choice on the large alternative, for G1 over S3 (top panel), reverse delay probe (middle panel) and the 0 s delay probe (bottom panel). Values displayed are means ± SEM.

Figure 16

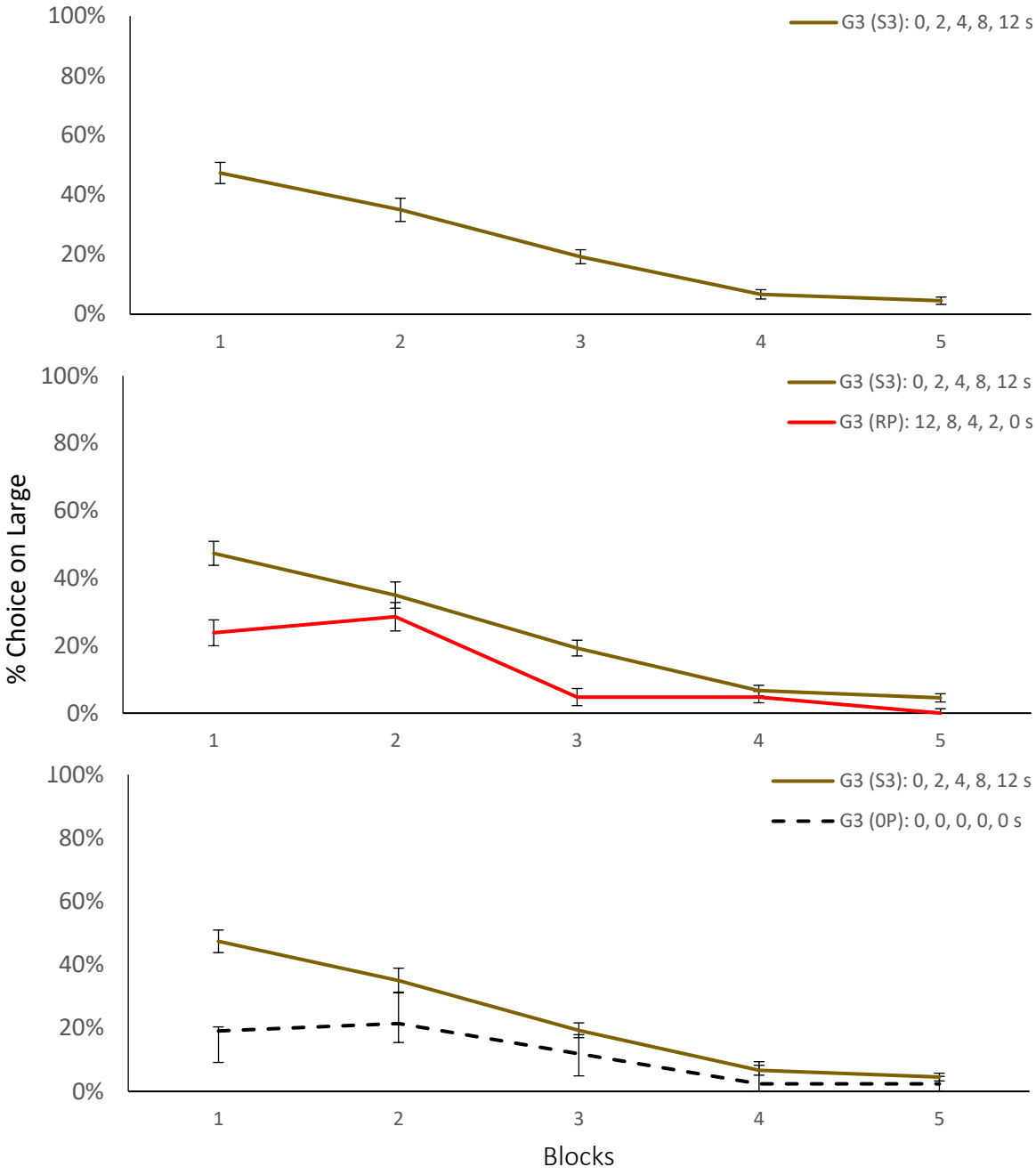
Group 2 Probe Data



Note. Average percentage of choice on the large alternative, for G2 over S3 (top panel), reverse delay probe (middle panel) and the 0 s delay probe (bottom panel). Values displayed are means ± SEM.

Figure 17

G3 Probe Data



Note. Average percentage of choice on the large alternative, for G3 over S3 (top panel), reverse delay probe (middle panel) and the 0 s delay probe (bottom panel). Values displayed are means ± SEM.