IMPULSIVE CHOICE, ALCOHOL SELF-ADMINISTRATION, 
AND PRE-EXPOSURE TO REWARD DELAY

by

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ABSTRACT

Impulsive Choice, Alcohol Self-Administration, and Pre-Exposure to Reward Delay

by

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Impulsive choice (i.e., preference for smaller, sooner over larger, later rewards) is cross-sectionally and longitudinally associated with drug dependence in humans. Similarly, impulsive choice is associated with greater drug self-administration in rodents. These findings suggest that impulsive choice plays a causal role in drug use. However, little research has been designed to experimentally test this hypothesis or the boundary conditions under which it may operate.

The research reported in this document examined the relation between impulsive choice and alcohol consumption in rats. We developed and refined an experimental method, in which rats were pre-exposed to delayed rewards, to produce trait-like reductions in impulsive choice. We then examined the effects of this manipulation on subsequent alcohol consumption. If impulsive choice is causally related to alcohol consumption in this rodent model, then reduction of impulsive choice should be
accompanied by a reduction in alcohol consumption. However, in the experiment presented in Chapter 2, reductions in impulsive choice for food rewards were accompanied by unexpected *increases* in alcohol consumption. Accordingly, the goals of the experiments in Chapters 3 and 4 were to help determine the conditions that produced this unexpected finding.

Results reported in Chapters 3 and 4 suggest that the unexpected results reported in Chapter 2 were dependent on the way in which alcohol was introduced in that experiment and perhaps other variables specific to orally consumed alcohol (e.g., taste, slow onset of pharmacological effects). Moreover, in Chapter 3, examination of our own and newly reported data suggests that the naturally occurring relation between impulsive choice and alcohol consumption in rodents is not as robust as it is for other drugs of abuse (e.g., psychostimulants, such as cocaine or nicotine). Nonetheless, the work reported in these experiments developed a method of reducing impulsive choice which may be used in future research to examine its related effects on consumption of other drugs of abuse.

(139 pages)
PUBLIC ABSTRACT

Impulsive Choice, Alcohol Self-Administration, and Pre-Exposure to Reward Delay

Jeffrey S. Stein

Prior research indicates that drug dependence is associated with a tendency to discount the future. For instance, compared to control participants, drug-dependent participants more strongly prefer small, immediate rewards (e.g., $10 now) over larger, delayed rewards (e.g., $100 in 6 months). Similarly, in animal models of addiction, impulsive preference for small, immediate over larger, delayed food rewards in rats is associated with greater consumption of a number of drugs of abuse, including alcohol, cocaine, and nicotine. These and other findings suggest that this form of impulsive choice plays a causal role in addiction; however, this account has not been tested rigorously in an experimental context. Additional human and nonhuman research is needed to examine whether impulsive choice directly influences drug use. Findings from this research will improve basic understanding and perhaps aid in development of clinical treatments for addiction.

The research reported in this document developed and refined an experimental method (prolonged pre-exposure to delayed rewards) that produces long-lasting reductions in impulsive choice in rats and determined the effects of this method on subsequent alcohol consumption. If impulsive choice plays a direct, causal role in rodent alcohol consumption, then reductions in impulsive choice should be accompanied by reductions in alcohol consumption. However, in the experiment presented in Chapter 2,
reductions in impulsive choice for food rewards were accompanied by unexpected increases in alcohol consumption. Accordingly, the goals of the experiments in Chapters 3 and 4 were to help determine the conditions that produced this unexpected finding.

Generally, results of these and other experiments suggest that impulsive choice is not robustly associated with alcohol consumption in rodents, either following experimental manipulation of impulsive choice or under naturally occurring conditions. The work reported here, however, introduces an experimental method of reducing impulsive choice (developed in Chapter 2 and refined in Chapter 4) which may be used in future research to examine the relation between impulsive choice and other drugs of abuse (e.g., cocaine, nicotine).
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CHAPTER 1
INTRODUCTION

Impulsive Choice

Impulsivity is a multidimensional construct comprising an array of prematurely expressed, poorly planned or otherwise maladaptive behavioral forms (Bickel, Jarmolowicz, Mueller, Gatchalian, & McClure, 2012). Impulsive choice describes one such form and, across species, has been operationalized as pervasive preference for smaller, sooner rewards (SSRs) over larger, later rewards (LLRs; Ainslie, 1975; Rachlin & Green, 1972). The majority of theoretical and quantitative descriptions of impulsive choice assume an approximately hyperbolic decay of subjective reward value with increasing delay (for review, see Green & Myerson, 2004); however, other forms of the decay function have long been discussed in standard economic theory (e.g., exponential decay). For the purposes of the proposed research, the precise form of the value-decay function is largely irrelevant. Rather, one need only assume that: (a) decay in value occurs, and (b) there are individual differences in the rate of this decay--two criteria that have been extensively satisfied in prior studies of impulsive choice in all species investigated thus far.

The experimental tasks used to investigate impulsive choice vary within and between species (for review, see Stein & Madden, 2013). In the majority of human choice tasks, an experimenter arranges an LLR (most often, hypothetical) to be delivered in the future, while systematically titrating the amount of an SSR until the participant is indifferent between the two options (Du, Green, & Myerson, 2002; Rachlin, Raineri, &
Cross, 1991). The amount of the SSR at indifference indexes the degree to which delay has caused the LLR’s value to decay; repeating this titration procedure across a range of delays yields the full value-decay function. Other tasks feature fill-in-the-blank methods in which a participant is asked to state the amount of money he or she would accept immediately in lieu of an LLR (e.g., Rossow, 2008).

In the nonhuman literature, subjects are most often rats or pigeons and impulsive choice is assessed with tasks at least formally similar to the titration procedures used in humans. That is, an experimenter arranges repeated choices between small and large food rewards; across successive trials, the amount of the SSR (Richards, Mitchell, de Wit, & Seiden, 1997) or the delay to the LLR (Mazur, 1987) is titrated until the animal is indifferent between the options. In other tasks, the reward parameters remain relatively fixed and the measure of impulsive choice, rather than an indifference point, is the percentage of trials in which subjects choose the LLR (e.g., Evenden & Ryan, 1996; Rachlin & Green, 1972). In both sets of tasks (titrating and fixed), the delays examined are on the order of seconds (e.g., 0-60 s; Evenden & Ryan. 1996) rather than weeks, months, or years as in the human tasks, and subjects complete sessions until stable patterns of choice are observed.

**Impulsive Choice and Drug Abuse and Dependence**

Accumulating evidence from human cross-sectional studies (e.g., cigarette smokers vs. non-smokers) indicates that impulsive choice in laboratory tasks is strongly associated with alcohol and other drug use (for meta-analysis, see MacKillop et al.,
Additional data indicate that these relations are not solely due to the effects of drug toxicity on impulsive choice, but that impulsive choice precedes and predicts adoption of alcohol and other drug use. For example, in longitudinal studies, impulsive choice in varying screening tasks in childhood predicts the subsequent adoption of tobacco (Audrain-McGovern et al., 2009) and exacerbates the risk for adolescent alcohol use posed by deficits in working memory (Khurana et al., 2012).

These findings reported above appear to generalize across species, as impulsive choice in rats reliably predicts greater self-administration of many drugs of abuse, such as alcohol (e.g., Poulos, Le, and Parker, 1995), cocaine (e.g., Anker, Perry, Gliddon, & Carroll, 2009; Perry, Larson, German, Madden, & Carroll, 2005; Perry, Nelson, & Carroll, 2008), methylphenidate (e.g., Marusich & Bardo, 2009), and nicotine (e.g., Diergaarde et al., 2008). Concordant findings across species is useful in understanding the relation between human impulsive choice and drug dependence, as the ability to isolate and more readily manipulate relevant variables in the nonhuman context may facilitate discovery.

One possible account of the relations discussed above is that impulsive choice plays an etiological role in drug use (for reviews, see Perry & Carroll, 2008; Stein & Madden, 2013). However, such a direct role of impulsive choice in drug dependence has not been established because these two variables could co-vary with a third--and ultimately causal--variable. Stronger conclusions could be made if experimental reductions in impulsive choice were accompanied by reductions in drug dependence. Moreover, such findings would suggest novel behavioral treatments for drug dependence.
Purpose

The following experiments developed and refined an experimental method to reduce impulsive choice, in which rats were pre-exposed to delayed rewards from adolescence through early adulthood. Because prior work suggests that impulsive choice in rats is associated with greater alcohol consumption (e.g., Poulos et al., 1995), we sought to determine whether reductions in impulsive choice would be accompanied by concomitant reductions in alcohol consumption. Such a finding would support a direct, causal role of impulsive choice in alcohol consumption, and perhaps other drug use.

References


Stein, J. S., & Madden, G. J. (2013). Delay discounting and drug abuse: Empirical,
CHAPTER 2

IMPULSIVE CHOICE, ALCOHOL SELF-ADMINISTRATION, AND PRE-EXPOSURE TO REWARD DELAY: I. A PRELIMINARY INVESTIGATION

Abstract

Naturally occurring impulsive choice has been found to positively predict alcohol consumption in rats. However, the extent to which experimental manipulation of impulsive choice may modify alcohol consumption remains unclear. In the present study, we sought to: (a) train low levels of impulsive choice in rats using early, prolonged exposure to reward delay, and (b) determine the effects of this manipulation on subsequent alcohol consumption. During a prolonged training regimen, three groups of male, adolescent Long-Evans rats (21-22 days old at intake) responded on a single lever for food rewards delivered after either a progressively increasing delay, a fixed delay, or no delay. Post-tests of impulsive choice were conducted, as was an evaluation of alcohol consumption using a limited-access, two-bottle test. Following delay-exposure training, both groups of delay-exposed rats made significantly fewer impulsive choices than did rats in the no-delay group. In addition, fixed-delay rats consumed significantly more alcohol during daily, 30-min sessions than no-delay rats. Possible mechanisms of these effects are discussed, as is the significance of these findings to nonhuman models of addiction.
Introduction

Impulsivity comprises an array of potentially discrete behavioral forms, including motor disinhibition, inattention, excessive risk-taking, and deficits in intertemporal decision-making (Bickel, Jarmolowicz, Mueller, Gatchalian, & McClure, 2012). The latter form describes a preference for smaller, sooner over larger, later rewards. This form of impulsivity involves an explicit choice between reward alternatives and is often referred to as impulsive choice to distinguish it from other forms of impulsivity.

In humans, a growing research literature reveals that greater impulsive choice in laboratory tasks is strongly associated with drug abuse and dependence (for meta-analysis, see MacKillop et al., 2011). This relation remains robust across many drugs of abuse, including alcohol (e.g., Vuchinich & Simpson, 1998), opioid drugs (e.g., Madden, Petry, Badger, & Bickel, 1997), cocaine (e.g., Coffey, Gudleski, Saladin, & Brady, 2003), methamphetamine (e.g., Hoffman et al., 2006), and nicotine (e.g., Reynolds, Richards, Horn, & Karraker, 2004).

One possible account of this relation is that impulsivity plays an etiological role in drug abuse and dependence (for reviews, see Perry & Carroll, 2008; Stein & Madden, 2013). That is, individuals who disproportionately value reward immediacy over reward magnitude may be more motivated by immediate drug effects than the temporally distant (but objectively more valuable) benefits of abstinence (e.g., social, occupational, or financial rewards). Provisional support for this hypothesis comes from longitudinal studies in which impulsive choice in varying screening tasks in childhood precedes and
predicts the subsequent adoption of cigarette smoking (Audrain-McGovern et al., 2009) or cocaine use (Ayduk et al., 2000).

More evidence that impulsive choice precedes drug abuse and dependence comes from nonhuman laboratory models in which a sample of adult rats is screened on an impulsive-choice task, divided into sample-dependent quantiles, and subsequently assessed under various drug self-administration (SA) tasks (for review, see Stein & Madden, 2013). Most relevant to the present study, Poulos, Le, and Parker (1995) first reported that degree of impulsive choice in rats positively predicted consumption of a 12% (wt/vol) alcohol solution in a two-bottle test (Richter & Campbell, 1940). Greater impulsive choice in rats has also been shown to predict greater drug intake during many discrete SA phases, including acquisition and escalation of cocaine SA (Anker, Perry, Gliddon, & Carroll, 2009; Perry, Larson, German, Madden, & Carroll, 2005; Perry, Nelson, & Carroll, 2008) and cue- and drug-induced reinstatement of cocaine and nicotine SA (Broos, Diergaarde, Schoffelmeer, Pattij, & de Vries, 2012; Diergaarde et al., 2008; Perry et al., 2008).

Despite the apparent predictive validity of impulsive choice in these nonhuman models, a direct etiological role of impulsive choice in drug SA has not been established because these two variables could co-vary with a third--and ultimately causal--variable. Stronger statements might be made if impulsive choice could be experimentally manipulated before nonhumans were given drug SA opportunities. If experimental reduction of impulsive choice reduces drug SA relative to subjects not exposed to this manipulation, then a direct etiological role of impulsive choice in drug SA would be
further supported. By extension, this finding would suggest that therapies designed to reduce impulsivity might also reduce drug abuse and dependence in humans. However, if experimental reduction of impulsive choice does not result in reduced drug SA, then the predictive relation between these variables more likely owes to an as yet unknown third variable.

In this chapter, a behavioral method of training low levels of impulsive choice in rats was examined, as well as potential concomitant effects of this manipulation on alcohol SA. Two experimental groups of adolescent rats were first trained to respond (on a single lever) for delayed food pellets, and subsequently completed 120 training sessions (spanning into mid-adulthood) in which lever pressing initiated either an escalating delay or a fixed delay to food pellets. Following this training, impulsive choice and alcohol consumption in these two delay-exposed groups was compared to a group that responded for immediate pellets throughout training.

**Methods**

**Subjects**

Subjects were 44 experimentally naïve, male Long-Evans rats (Harlan Sprague-Dawley, Indianapolis, IN). Rats were of post-natal days 21-22 at intake and were housed individually in polycarbonate cages in a temperature- and humidity-controlled room on a 12-hr light/dark cycle (lights on at 7:00 am) throughout the experiment. Water was available continuously in the home cage. Following three days of ad-libitum food access, rats were weighed daily and food-restricted to the strain’s average, age-adjusted 85%
free-feeding weight (calculated from the vendor-supplied growth curve). Rats were randomly assigned to either no-delay (ND; \( n = 14 \)), fixed-delay (FD; \( n = 14 \)), or progressive-delay (PD; \( n = 16 \)) groups. More rats were intentionally assigned to the PD group to accommodate potential increased between-subject variability in this group’s dependent measures as a result of variability in terminal training delays. Food restriction continued throughout all experimental phases, with the exception of the alcohol SA test (see below) in which all rats received ad-libitum food access in their home cages. After alcohol SA, 85% free-feeding weights were recalculated for individual rats using mean body weight over the last three days of a post-alcohol period. In all phases described below, rats completed sessions 7 days per week between the hours of 7:00 am and 1:00 pm, with individual rats completing sessions at the same time each day (time of day counterbalanced across groups).

**Apparatus**

Twelve identical operant conditioning chambers were used (24.1 x 30.5 x 21 cm; Med Associates, St. Albans, VT). Each was equipped with a white-noise speaker and housed within a sound-attenuating cubicle. Centered on the rear wall and 6.5 cm above the grid floor was a retractable response lever. Identical left and right levers were positioned at the same height on the front wall; above each was a 28-V DC cue light. A pellet feeder (Coulbourn Instruments, Allentown, PA), equipped with an infrared pellet detector (Pinkston, Ratzlaff, Madden, & Fowler, 2008), delivered grain-based pellets (45 mg; Bio-Serv, Frenchtown, NJ) into a receptacle between the levers.
Twelve identical polycarbonate home cages were used in the alcohol SA test. Cages were equipped with two glass drinking tubes (Dyets, Inc., Bethlehem, PA), each affixed to the left and right walls on one end of the cage. Drinking tubes were positioned above small, glassware bowls (Pyrex; World Kitchen, LLC, Rosemont, IL) to contain potential leakage. The room was equipped with a white-noise speaker and illuminated by a 40W red light.

Procedures

Figure 2-1 depicts the order and duration of all experimental conditions described below.

An autoshaping procedure was used to establish rear-lever pressing. For ND rats, the intertrial interval (ITI) was 55 s (during which all levers were retracted and cue lights extinguished), followed by 5 s of concurrent rear-lever insertion and rear cue-light illumination. Following this 5-s period, the cue light was extinguished, the lever was retracted, and two food pellets were delivered immediately to the receptacle; however, the rat could earn the reward at any time during this period with a single lever press.

These parameters provided a ratio of ITI to trial duration (I:T) of 11:1. In contrast, autoshaping with PD and FD rats involved delayed rewards. Following a 247.5-s ITI, the rear-lever and cue light were activated. After 5 s or a single lever press, the rear lever retracted but its cue light remained illuminated for 17.5 s prior to reward delivery (an I:T ratio of 11:1, equated across groups to increase the probability that lever training would be completed in a comparable number of sessions; Gibbon, Baldock, Locurto, Gold, &
Figure 2-1. Order and approximate duration (in postnatal days; PNDs) of experimental conditions.
Terrace, 1977). Training continued until individual rats pressed the lever to earn $\geq 90\%$ of the scheduled rewards for two consecutive sessions.

The next 120 sessions were composed of 80 trials, each 60 s in duration. Trials began when the rear lever was inserted into the chamber and its cue light was illuminated. A single lever press retracted the lever and initiated a delay to the delivery of two food pellets. The cue light remained on throughout the delay. If the lever was not pressed within 20 s of trial onset, the trial was terminated (lever retracted and cue light extinguished for the remainder of the trial) and was scored as an omission. Following pellet delivery (or omissions), no stimuli were presented until the beginning of the next trial.

For the rats assigned to the FD and ND groups, the delays to food were, respectively, 17.5 s and 0.01 s (henceforth referred to as 0 s). The delay for PD rats was initially 17.5 s and was gradually increased based on performance. Specifically, at every 4\textsuperscript{th} trial, the computer queried a moving window of the last 120 trials. If the mean response latency across these trials was less than 4 s and fewer than 12 omissions had occurred, the delay was increased by 0.057\%. This schedule of delay adjustments allowed for a maximum terminal delay of 68.36 s over the course of 120 sessions. Beginning with session 100, trial duration was increased to 80 s for all rats to accommodate the adjusted delays of the PD group.

In the next several sessions, in order to train responding on the side levers, the left or right levers on the front wall of the chamber were presented individually in random order with the constraint that each was presented 40 times per session. Pressing
the lever once led to two pellets delivered after the terminal delay from the delay-exposure training phase. Once consistent side-lever pressing was trained (≥ 90% of trials completed for two consecutive sessions), several choice-training sessions were conducted in which both levers were inserted at the beginning of each trial. The purpose of these sessions was to ensure sensitivity to differences in reward amount. Thus, pressing one lever led to one pellet and pressing the other led to three pellets (assignment counterbalanced within each group). The delays to both rewards were identical and were unchanged from the terminal delay-exposure training phase. These sessions continued until each rat chose the larger reward on ≥ 90% of the trials, and made no more than five omissions, for two consecutive sessions.

Next, impulsive choice was assessed in 20 sessions using a within-session, increasing-delay procedure (Evenden & Ryan, 1996). Sessions were composed of two 20-trial blocks with a 7-min inter-block blackout period. The first six trials in each block were forced-exposure trials in which only one lever and its associated cue light were presented (order determined randomly every two trials). The remaining 14 trials in a block were choice trials, in which both levers were presented. Trials began with the insertion of the rear lever and the illumination of its associated cue light. Following a single rear-lever response, the rear lever was retracted and its cue light was extinguished. One or both side levers (depending on trial type) on the opposite wall were then inserted into the chamber and their associated cue lights were illuminated. Retaining the lever assignments from choice-training sessions, pressing one lever led to one food pellet and the other led to three pellets. In the first trial block, the delay to both rewards was 0 s. In
the second trial block, the delay to the larger reward was increased to 15 s (cue light on
during the delay). An ITI ensured that trials began every 80 s regardless of the reward
chosen. As in previous phases, a 20-s omission criterion was used.

Two sessions in which the delay to both rewards remained at 0 s across both trial
blocks (Evenden & Ryan, 1996) were pseudorandomly interspersed among those of the
impulsive-choice test. These no-delay sessions were otherwise identical to those
described above, but were not programmed over the final six sessions analyzed.

Upon completion of the impulsive-choice test, rats were provided with ad-libitum
food and water access in the home cage for 7 days prior to, and throughout, the alcohol
SA test.

Alcohol SA procedures closely followed those used by Poulos et al. (1995). Rats
were weighed prior to each session and placed in prepared cages for 30 min. The cages
were equipped with glass drinking tubes, one containing deionized water and the other an
alcohol solution. The left or right position of the solution within the polycarbonate cage
alternated strictly across sessions. Following each session, the weights of the remaining
alcohol solution and water (plus leakage, if present) were recorded.

Four alcohol concentrations (3, 6, 12, and 24% wt/vol) were assessed in
ascending order: 8 days at the 3% concentration and 10 days each at the 6, 12, and 24%
concentrations.

After completion of the alcohol SA test, rats continued to receive ad-libitum food
and water access in their home cages for 11 days prior to the reinstatement of food
restriction.
When rats returned to 85% of post-alcohol free-feeding weights, the impulsive-choice retest was conducted using the same parameters as in the initial test of impulsive choice.

Statistical Analysis

All statistical tests were conducted using SPSS (version 19.0, SPSS Inc., Chicago, IL). In all analyses, an alpha level of .05 was considered statistically significant. All pairwise comparisons were examined using Bonferroni correction. Unless otherwise noted, data obtained in the last six sessions of each condition were analyzed.

Separate one-way ANOVAs were used to evaluate between-group differences in the following behavioral outcomes: (a) number of sessions required to acquire rear- and side-lever pressing, (b) the number of sessions required to demonstrate \( \geq 90\% \) choice of the larger number of pellets (in choice-training sessions), and (c) mean response latencies and omissions at the conclusion of delay-exposure training.

In the impulsive-choice test and retest, dependent measures were percent large-reward choice in the first and second trial blocks (0-s and 15-s delays, respectively). In the alcohol SA test, dependent measures were mean consumption of alcohol (g/kg) and water (mL/kg), as well as mean body weight (g), at each alcohol concentration. Dependent measures in the impulsive-choice and alcohol SA tests were non-normally distributed (positive skew) and were not amenable to transformation. Group differences in each of the measures above were therefore examined using separate generalized estimating equation (GEE) models, a generalized regression technique that allows
analysis of correlated repeated measures, but makes fewer parametric assumptions than
do traditional methods (for overview, see Ballinger, 2004).

In each GEE model, main effects of group and the relevant within-subjects
variable (e.g., test type in the impulsive-choice model, alcohol concentration in the
alcohol SA model) were included, as well as group x within-subject variable interactions.
GEE models were implemented using first-order auto-regressive working correlation
matrices. In the impulsive-choice GEE model, mean alcohol consumption (collapsed
across concentration) was included as a covariate to examine the possibility that alcohol
exposure influenced impulsive choice between test and retest. In the alcohol SA GEE
model, mean body weight at each concentration was included as a covariate to examine
the possibility that between-group differences in alcohol consumption were mediated by
differing metabolic or motivational processes (e.g., calorie seeking) between groups
unrelated to the value of drug reward.

Results

As shown in Table 2-1, acquisition of rear-lever pressing was undifferentiated
between groups, $F(2, 41) = 1.54, p > .05$. At the conclusion of 120 days of delay-
exposure training, the mean adjusted delay for the PD group was 44.82 s (±1.66; range:
34.13-57.60 s). A significant main effect of group, $F(2, 41) = 3.86, p < .05$, was detected
on rear-lever response latencies (see Table 2-2); however, pairwise comparisons revealed
no significant differences between groups ($p > .05$, in all cases). Table 2-2 also shows a
Table 2-1

*Mean number of sessions required to meet the acquisition criteria during rear-lever, side-lever, and choice training for all groups (± SEM).*

<table>
<thead>
<tr>
<th>Training Phase</th>
<th>Group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PD</td>
<td>FD</td>
<td>ND</td>
</tr>
<tr>
<td>Rear lever</td>
<td>4.19 (0.44)</td>
<td>5.14 (0.50)</td>
<td>5.29 (0.54)</td>
</tr>
<tr>
<td>Side levers</td>
<td>4.00 (0.40)Ω≡</td>
<td>2.57 (0.17)</td>
<td>2.29 (0.16)</td>
</tr>
<tr>
<td>Choice</td>
<td>5.69 (0.51)Ω≡</td>
<td>4.10 (0.46)</td>
<td>3.50 (0.40)</td>
</tr>
</tbody>
</table>

Ω and ≡ indicate PD/FD and PD/ND differences, respectively, in pairwise comparisons (p < .01).

Table 2-2

*Mean rear-lever response latencies and omissions per session over the last six delay-exposure training sessions for all groups (± SEM).*

<table>
<thead>
<tr>
<th>Dependent measure</th>
<th>Group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PD</td>
<td>FD</td>
<td>ND</td>
</tr>
<tr>
<td>Response latencies (s)</td>
<td>1.96 (0.24)</td>
<td>1.26 (0.19)</td>
<td>1.25 (0.19)</td>
</tr>
<tr>
<td>Omissions</td>
<td>3.28 (0.30)Ω≡</td>
<td>0.69 (0.14)</td>
<td>0.58 (0.12)</td>
</tr>
</tbody>
</table>

Ω and ≡ indicate PD/FD and PD/ND differences, respectively, in pairwise comparisons (p < .01).

significant main effect of group on response omissions, $F(2, 41) = 52.15, p < .0001$, with PD rats omitting more trials than FD or ND rats ($p < .001$, in both cases).

Prior to the impulsive-choice test, a main effect of group, $F(2, 41) > 6.08, p < .01$, was detected in the number of sessions required to acquire side-lever pressing (see Table 2-1), with PD rats requiring about 1.5 more sessions than FD or ND rats ($p < .01$). Likelihood, PD rats required more sessions than the FD or ND groups to demonstrate ≥ 90% preference for the larger reward just prior to the impulsive-choice test ($p < .01$; see Table 2-1).
**Impulsive-choice Test**

In the first trial block, when neither reward was delayed, there was no significant main effect of group on percent large-reward choice in either the test, Wald $\chi^2 = 3.05; p = .22$ (left panel of Figure 2-2), or retest, Wald $\chi^2 = 0.20; p = .63$ (right panel of Figure 2-2); thus, all groups were equally able to discriminate reward amounts (one vs. three pellets) in the absence of delay. For this reason, choice in the first trial block was excluded from all subsequent analyses.

The effects of delay-exposure training were evident in the second trial block (15-s delay) in the initial impulsive-choice test (left panel of Figure 2-2), with pairwise comparisons in the GEE model indicating that both PD and FD rats made fewer impulsive choices than ND rats ($p = .003$, in both cases).

![Figure 2-2](image.png)

*Figure 2-2.* Mean (± SEM) percent large-reward choice across trial blocks in the impulsive-choice test (left panel) and retest (right panel). ≡ and ‡ indicate, respectively, PD/ND and FD/ND differences ($p < .01$, in both cases).
Alcohol SA Test

The top, middle, and bottom panels of Figure 2-3 depict, respectively, mean alcohol consumption (g/kg), water consumption (mL/kg), and body weight (g) collapsed across all sessions at each concentration in the alcohol SA test. Significant main effects of group, Wald $\chi^2 = 8.43; p = .02$, and concentration, Wald $\chi^2 = 48.75; p < .001$, on alcohol consumption were detected, as was a group x concentration interaction, Wald $\chi^2 = 13.84; p = .03$. No effect of body weight on alcohol consumption was observed, Wald $\chi^2 = .01; p = .94$.

Collapsed across concentration, pairwise comparisons indicated that FD rats consumed more alcohol than ND rats ($p = .02$), but no other overall between-group differences were significant (PD/ND difference: $p = .09$). Pairwise comparisons at individual concentrations revealed greater alcohol consumption in FD rats at 12% wt/vol alcohol compared to ND rats ($p = .02$), but FD/ND differences at other concentrations were not significant ($p > .15$, in all cases). No other pairwise comparisons were statistically significant at any concentration (PD/ND difference at 12 and 24% wt/vol alcohol: $p = .14$ and .07, respectively).

No significant main effects of group were detected in either water consumption or body weight, Wald $\chi^2 < 2.51; p > .29$ in both cases, and the group x concentration interactions were not significant, Wald $\chi^2 < 8.05; p > .23$ in both cases. However, a main effect of concentration was detected on water consumption, Wald $\chi^2 = 9.15; p = .03$, and body weight, Wald $\chi^2 = 665.60; p = < .001$. No significant between-group differences were observed in pairwise comparisons of either dependent measure either when
Figure 2-3. Mean (± SEM) consumption of alcohol (top panel) and water (middle panel) at each alcohol concentration. Also depicted is mean body weight at each alcohol concentration (bottom panel). # indicates FD/ND difference \((p < .05)\), when data were collapsed across concentration. † indicates FD/ND difference at individual concentrations \((p < .05)\). Data points have been displaced slightly on the x-axes, for clarity.

collapsed across concentration \((p > .51, \text{ in all cases})\) or at individual concentrations \((p > .95, \text{ in all cases})\).
**Impulsive-choice Retest**

The right panel of Figure 2-2 depicts percent large-reward choice in the impulsive-choice retest. Pairwise comparisons in a GEE model revealed no between-group differences in large-reward choice in the retest at the second trial block \((p > .15, \text{ in all cases})\).

Across the test and retest of impulsive choice (second trial block only), a GEE model revealed significant main effects of group, Wald \(\chi^2 = 8.48; p = .02\), and test type, Wald \(\chi^2 = 4.97; p = .03\), on percent large-reward choice, and a group x test type interaction, Wald \(\chi^2 = 7.83; p = .02\). The latter reflects a significant decline in large-reward choice in the PD group \((p = .04)\) relative to the other groups \((p > .25, \text{ in both cases})\). Finally, although the analysis was not specific to PD rats (the only group in which a significant difference was observed in impulsive choice between test and retest), alcohol consumption in the intervening alcohol SA test was a trend-level predictor of change in percent large-reward choice, Wald \(\chi^2 = 3.63; p = .06\).

**Discussion**

The present study demonstrates that early and prolonged exposure to reward delay decreases impulsive choice in rats. In the initial test of impulsive choice, rats in both experimental groups (FD & PD) made significantly fewer impulsive choices than did ND rats. Thus, the present findings extend a relatively small literature on training variables known to impact impulsive choice in nonhuman animals (Logue, Rodriguez, Peña-Correal, & Mauro, 1984; Mazur & Logue, 1978). The present methodology is most
similar to that reported by Eisenberger, Masterson, and Lowman (1982), who exposed adult rats to progressively increasing intervals between response-independent food pellets (0-78 s) across 24 training sessions. In a subsequent test, rats exposed to these increasing inter-pellet intervals made significantly fewer impulsive choices than did rats exposed to shorter intervals (5 s). Although the effect observed by Eisenberger et al. in their impulsive-choice test (i.e., approximately 38 vs. 18% large-reward choice, across groups) was relatively smaller than that in the present study, any number of methodological differences (e.g., duration of the training regimen, age of rats during training, or the use of response-independent vs. dependent food delivery) prohibit direct, quantitative comparison between studies.

While the effects of delay-exposure training on impulsive-choice were largely expected, its effects on alcohol consumption were not. In humans, accumulating evidence demonstrates that greater impulsive choice is strongly associated with alcohol abuse and dependence (e.g., Petry, 2001; Vuchinich & Simpson, 1998). In rats, Poulos et al. (1995) reported that impulsive choice positively predicted subsequent alcohol consumption in a two-bottle test almost identical to the one used here. Likewise, selectively bred, alcohol-preferring rat and mouse lines make more impulsive choices non-alcohol-preferring comparison lines (Oberlin & Grahame, 2009; Wilhelm & Mitchell, 2008). Thus, in the absence of experimental manipulation, greater impulsive choice in rats appears strongly related to greater alcohol consumption. However, in the present study, when impulsive choice was experimentally manipulated, rats in the FD group consumed more alcohol
than did ND rats; the difference between PD and ND rats only approached significance ($p = .09$ overall, and .07 at the 24% concentration).

The finding that PD rats (exposed to substantially longer delays than FD rats) were undifferentiated from ND rats in alcohol consumption, whereas FD rats were, is itself a matter of interest. Inclusion of PD training was intended as a parametric manipulation of delay exposure, and was thus hypothesized to produce behavioral effects greater than those observed in FD rats. However, that PD rats made significantly more response omissions during delay-exposure training and required significantly more sessions to acquire delayed side-lever pressing than FD rats suggests that the continuous challenge of progressively increasing delays may have obstructed the effects of delay-exposure training on alcohol consumption.

Although the finding that FD rats consumed significantly more alcohol than ND rats was unexpected, a recent study conducted by Broos et al. (2012; Experiment 2) reports a qualitatively consistent outcome. Broos et al. used an acute dose of methylphenidate (1.0 mg/kg ip) to significantly decrease impulsive choice in rats and reported a concomitant *increase* in cue-induced reinstatement of cocaine seeking. Conversely, increases in impulsive choice following acute doses of SCH-23390 (0.01 mg/kg sc) were accompanied by *decreases* in cue-induced cocaine reinstatement relative to saline. Broos et al.’s findings, however, should be interpreted with caution if one considers potential neuropharmacological interaction between experimenter- and self-administered drugs. For instance, in humans, both methylphenidate and cocaine produce similar physiological and subjective drug effects, and cannot be differentiated in a drug-
discrimination task (Rush & Baker, 2001). In rats, Schenk and Partridge (1999) reported that a priming dose of methylphenidate produced reinstatement of cocaine SA. Thus, the increases in cocaine reinstatement observed by Broos et al. may have simply been primed by methylphenidate—a possibility that would have nothing to do with impulsive choice. Likewise, the reduction of cocaine SA reinstatement following SCH-23390 may have been a product of this drug’s motor-suppressing effects at doses similar or equal to the one used by Broos et al. (e.g., 0.01 mg/kg, Hoffman & Beninger, 1985; 0.17 mg/kg, Morelli & Di Chiara, 1985).

A similar interpretational problem may be found in a study by Oberlin, Bristow, Heighton, and Grahame (2010), who reported that reduction of impulsive choice in high-alcohol-preferring (HAP) mice following an acute dose of amphetamine (1.2 mg/kg) was not accompanied by concomitant changes in alcohol consumption in a two-bottle test. However, in light of the present study’s counterintuitive effects of experimentally-reduced impulsive choice on alcohol consumption, an alternative explanation for Oberlin et al.’s null finding exists. Namely, acute amphetamine has been shown to produce a dose-related decrease in two-bottle alcohol consumption in rats (Linseman, 1990). Thus, the impact of competing mechanisms that simultaneously increase and decrease alcohol consumption may have washed out any significant effects of amphetamine on alcohol consumption. In light of the considerations above, manipulation of impulsive choice via training variables (as in the present study) may be preferred over pharmacological methods.
Future research may be designed to address the precise determinants of the effects of delay-exposure training on alcohol consumption. Such an analysis would bear directly on the validity of training-related SA behavior as a measure of drug seeking in nonhuman models, as opposed to an otherwise unrelated or more general behavioral process. No between-group differences were apparent in water consumption in the present study, suggesting that our observed effect was not mediated, in general, by differential consummatory behavior between groups. Further, potential differences in calorie seeking were controlled statistically in the present study by assuming body weight as a relevant proxy measure. We also varied the feeding regimen between the impulsive-choice and alcohol SA tests (restriction vs. ad-libitum access) to minimize the potential that consumption across groups would be differentially motivated by alcohol’s caloric properties—a practice common among studies that have shown a relation between impulsive choice and alcohol SA (Oberlin & Grahame, 2009; Poulos et al., 1995; Wilhelm & Mitchell, 2008), and even when the drug examined has no caloric properties (Diergaard et al., 2008; Marusich & Bardo, 2009; Yates, Marusich, Gipson, Beckmann, & Bardo, 2012; cf. Anker et al., 2009; Koffarnus & Woods, 2011; Perry et al., 2005, 2008). Nonetheless, a more stringent experimental control may be employed in future studies. For example, investigating consumption of an isocaloric sucrose solution in separate cohorts of delay-exposed and delay-naïve rats would allow examination of caloric or taste variables as alternative explanations for our findings.

As a second alternative explanation for our findings, training-related increases in alcohol consumption may have been mediated by stress exposure. Prior to the alcohol-SA
test, experimental rats had been exposed to reward delay from early adolescence through middle adulthood (PNDs 25-150). If reward delay is a stressor, then the results of the present study may be placed in the context of a larger experimental literature on the effects of acute and chronic stress on drug SA (for reviews, see Koob, 2008; Piazza & Le Moal, 1998; Sinha, Shaham, & Heilig, 2011). For example, stressors such as restraint, foot shock, and social isolation have increased SA of multiple drugs of abuse in rats, including alcohol (Bozarth, Murray, & Wise, 1989; Goeders & Guerin, 1994; Erb, Shaham, & Stewart, 1996; Shaham, 1993; Shaham & Stewart, 1995). This effect has been linked to several neurochemical and neuroendocrine systems, including limbic dopamine and hypothalamic-pituitary-adrenal axis function (e.g., Schulkin, McEwen, & Gold, 1994; Shepard, Barron, & Myers, 2000). Future studies in this line may be designed to examine behavioral and neurobiological indicators of stress in delay-exposed rats, such as exploratory behavior in an open-field maze or the stress-related steroid corticosterone. However, no work has identified delay as an explicit source of stress in rats. Further, why delay-exposed rats wouldn’t have actively avoided this putative stressor throughout impulsive-choice testing, by choosing the immediate reward, remains a paradox.

As a final alternative explanation for our results, training-related increases in alcohol consumption may have been mediated by the relatively slow pharmacokinetic profile of oral alcohol. Onset of drug action varies directly as a function of route of administration (Fowler et al., 2008; Parasarmpuria et al., 2007; Volkow et al., 2000). Absorption of orally ingested alcohol in rats sufficient to produce pharmacologically active blood alcohol concentrations requires significant delays (e.g., Livy, Parnell, &
West, 2003; Spirduso, Mayfield, Grant, & Schallert, 1989). Thus, prior experience in
detecting and exploiting contingent relations between responding and delayed rewards
may have better prepared rats to detect and exploit the contingent relation between
alcohol consumption and its delayed pharmacological effects.

A few potential limitations of the present study deserve comment. First, impulsive
choice was assessed at only one non-zero delay. The purpose of this was to minimize
testing-related exposure to delay--our putative independent variable--in ND rats.
However, the use of only one non-zero delay may have limited our ability to detect
parametric differences in impulsive choice between PD and FD rats--differences that may
have emerged had longer delays to the larger reward been explored.

Second, only male rats were examined, thus preventing identification of potential
sex differences in our dependent variables. Examinations of the relation between
naturally occurring impulsive choice and nonhuman drug SA have predominantly been
conducted with male rats (e.g., Broos et al., 2012; Diergaard et al., 2008; Koffarnus &
Woods, 2013; Marusich & Bardo, 2009; Poulos et al., 1995), presumably to avoid any
uncontrolled effect of estrous cycle. While there are known sex differences in absolute
levels of drug SA, the relation between impulsive choice and drug SA appears the same
as that observed in males when female rats have been examined (Anker et al., 2009;
Oberlin & Grahame, 2009; Perry et al., 2005, 2008). Nonetheless, the literature would
benefit from systematic investigation of potential sex differences as they may uniquely
pertain to the effects of delay-exposure training.
Third, in confounding the passage of time with alcohol exposure, the generality of the effects of delay-exposure on impulsive choice across time are difficult to interpret. In the impulsive-choice retest (approximately 65 days following the initial test), no significant differences between delay-exposed and ND rats were observed. In addition, PD rats made significantly more impulsive choices in the retest compared to the initial test. However, whether these findings were due to the passage of time, or to differential alcohol exposure between groups, is unanswerable from the experimental design used. Thus, firm conclusions regarding the effects of alcohol on impulsive choice (e.g., Evenden & Ryan, 1999; Olmstead, Hellemans, & Paine, 2006; Poulos, Parker, & Le, 1998), or the effects of delay exposure on impulsive choice across time should be deferred to studies designed explicitly to test such relations.

Fourth, prior literature documents anxiogenic effects and neurobiological deficits in adolescent and adult rats exposed to chronic and severe food restriction (e.g., 50-60% ad-libitum food intake; Gur, Newman, Avraham, Dremencov, & Berry, 2003; Huether, Zhou, Schmidt, Wiltfang, & Rüther, 1997; Jahng et al., 2007). Rats in the present experiment were subjected to food restriction from early adolescence to middle adulthood (PNDs 25-175) in order to encourage operant responding. However, our use of food restriction is unlikely to have substantially impacted our findings, as the level of restriction in the present study was much milder (approximately 85% of ad-libitum food intake) than has been widely found to produce behavioral and neurochemical abnormalities in the studies cited above. Relatively little research has been designed to examine such neurobehavioral effects as a result of the mild food restriction employed in
the study of operant food responding (cf. Carr, Tsimberg, Berman, & Yamamoto, 2003). Further, the use of adolescent food restriction in the present study was a variable held constant across all groups, and thus did not likely pose a threat to internal validity.

As a final limitation, delay exposure in the present study was a composite variable consisting of both delayed-reward autoshaping and a prolonged, 120-day training regimen. In addition, training began during adolescence (a period of highly plastic responsiveness to experimental variables; Chapillon, Patin, Roy, Vincent, & Caston, 2002) to increase the likelihood that delay exposure would produce stable, trait-like patterns of behavior in adulthood. The primary goal of this multi-faceted approach was to create distinct groups of varying levels of impulsivity to explore related group differences in alcohol SA. The extent to which any variable in the delay-exposure regimen weighed independently on our observed effects cannot be resolved from the experimental design used. However, future studies may be designed to isolate these variables, or parametrically manipulate the duration of the training regimen, to determine their effects on impulsive choice and alcohol consumption.

In conclusion, the present data suggest that the relation between impulsive choice and alcohol SA is not a straightforward one--experimentally reducing impulsive choice did not decrease alcohol consumption in rats; to the contrary, it appears to have increased it. Thus, the present data do not accord with previous findings suggesting that impulsive choice precedes and predicts drug SA in rats (e.g., Diergaarde et al., 2008; Perry et al., 2005, 2008; Koffarnus & Woods, 2013; Poulos et al., 1995). Nonetheless, further investigation will be required to determine the generality of the present findings across
other nonhuman drug SA models (e.g., iv cocaine SA), in which many of the variables reviewed above (e.g., oral alcohol’s slow pharmacokinetic profile or caloric properties) would not play a role. Whether these future investigations yield findings similar, or opposite, to those of the present study might yield further evidence for, or against, respectively, a direct causal relation between impulsive choice and drug SA.

References


control of serotonergic transmission in the hippocampus and hypothalamus of rats. *Nutritional Neuroscience, 6*, 169-175.


Livy, D. J., Parnell, S. E., & West, J. R. (2003). Blood ethanol concentration profiles: A
comparison between rats and mice. Alcohol, 29, 165-171.


Oberlin, B. G., & Grahame, N. J. (2009). High-alcohol preferring mice are more
impulsive than low-alcohol preferring mice as measured in the delay discounting task. Alcoholism: Clinical and Experimental Research, 33, 1294-1303.


CHAPTER 3
NATURALLY OCCURRING IMPULSIVE CHOICE AND
ALCOHOL SELF-ADMINISTRATION

Abstract

Prior human research indicates robust, positive relations between impulsive choice (i.e., preference for smaller, immediate over larger, delayed rewards) and alcohol use disorders. However, varied findings in the nonhuman literature reveal a relatively ambiguous relation between impulsive choice and alcohol consumption in rodents. In addition, few rodent studies have investigated potential relations between impulsive choice and common covariates of alcohol consumption (e.g., avidity for sweet substances or anxiety-like behavior). Ninety-two male Long-Evans rats completed an impulsive-choice task. From this larger sample, extreme high- and low-impulsive groups (n = 30 each) were retained for further testing. In separate tests, subsequent open-field behavior and consumption of oral alcohol (12% w/v) and isocaloric sucrose were examined. Impulsive choice was then retested to examine whether behavior remained stable over the course of the experiment. No significant relations emerged between impulsive choice and either alcohol or sucrose consumption. However, impulsive choice predicted greater anxiety-like behavior (avoidance of the center field, defecation) in the open-field test. In turn, greater anxiety predicted lower alcohol and sucrose consumption. Finally, choice remained generally stable across the experiment, although high-impulsive rats tended toward less impulsive choice in the retest. Although impulsive choice and alcohol
consumption appear to share some variance with anxiety-like behavior, the present data offer no support for a relation between impulsive choice and alcohol consumption in Long-Evans rats. Together with mixed rodent data from prior reports, these findings attenuate cross-species comparisons to human relations between impulsive choice and alcohol use disorders.

**Introduction**

Impulsivity is a multi-dimensional construct comprising an array of prematurely expressed, poorly planned, or otherwise maladaptive behavioral forms (Bickel, Jarmolowicz, Mueller, Gatchalian, & McClure, 2012). Impulsive choice describes one such form and, across species, has been operationalized as preference for smaller, relatively immediate rewards over larger, more delayed rewards. In humans, accumulating evidence indicates that impulsive choice in laboratory tasks is strongly associated with substance-use disorders (for meta-analysis, see MacKillop et al., 2011).

This association is not solely due to the effects of drug toxicity on impulsive choice, as impulsive choice has been shown to precede and predict adoption of drug use. For example, in human longitudinal studies, impulsive choice in varying screening tasks in childhood predicts the subsequent adoption of tobacco or cocaine use (Audrain-McGovern et al., 2009; Ayduk et al., 2000) and exacerbates the risk for adolescent alcohol abuse posed by working-memory deficits (Khurana et al., 2013). Likewise, in rats, impulsive choice reliably predicts greater self-administration of psychostimulant drugs, such as cocaine (e.g., Perry, Larson, German, Madden, & Carroll, 2005; Perry,
Nelson, & Carroll, 2008), methylphenidate (Marusich & Bardo, 2009), and nicotine (Diergaarde et al., 2008).

When combining data across species, impulsive choice appears to play a substantive role (primary or mediational) in vulnerability to drugs of abuse. However, despite a clear association between impulsive choice and human alcohol-use disorders (see MacKillop et al., 2011), the literature on impulsive choice and rodent alcohol consumption is relatively mixed. Poulos, Le, and Parker (1995) first reported that impulsive choice in outbred rats predicted greater alcohol consumption in a limited-access, two-bottle test. Likewise, rat and mouse lines bred for differential alcohol consumption or preference have shown directional differences in impulsive choice similar to that reported by Poulos et al. (1995; e.g., Oberlin and Grahame, 2009; Wilhelm and Mitchell, 2008).

Further review of the literature, however, indicates mixed relations between impulsive choice and rodent alcohol consumption. For example, inbred C57BL/6J mice consume more alcohol (e.g., Belknap, Crabbe, & Young, 1993; Risinger, Brown, Doan, & Oakes, 1998) but are less impulsive (Helms, Reeves, & Mitchell, 2006) than DBA/2J mice, indicating that impulsive choice and alcohol consumption do not perfectly co-vary. Likewise, lower levels of impulsive choice have been reported in a short-term selected mouse line bred for high alcohol consumption, compared to a line bred for low consumption (Wilhelm, Reeves, Phillips, & Mitchell, 2007). However, this effect was specific to short delays (2-4 s) and was not observed in global measures of impulsive choice.
Two studies of outbred rats raise additional uncertainty regarding the relation between impulsive choice and rodent alcohol consumption. First, impulsive choice has failed to predict acquisition of instrumental alcohol self-administration, as well as economic demand for self-administered alcohol (i.e., the degree to which rats will defend consumption against increasing response requirements; Diergaard, van Mourik, Y., Pattij, Schoffelme, & De Vries, 2012). Second, training low levels of impulsive choice (via pre-exposure to reward delay) was shown to increase, not decrease, alcohol consumption (the opposite of what might be predicted from an otherwise positive relation between these variables). Thus, to the extent that impulsive choice has been found to relate to alcohol consumption (e.g., Poulos et al., 1995), this relation appears dissociable when impulsive choice is experimentally manipulated.

Attempts to reconcile the mixed data reviewed above may reveal that impulsive choice and alcohol consumption interact dynamically with other variables that modulate the strength and direction of observed relations. A preliminary step in investigating such interaction is to examine how impulsive choice relates to common covariates of alcohol consumption. Two candidate variables for investigation are: (a) avidity for sweet substances (e.g., sucrose or saccharin), and (b) anxiety. For example, sucrose or saccharin consumption has been shown to correlate positively with alcohol consumption within outbred strains and across selectively bred lines (e.g., Belknap et al., 1993; Gosnell & Krahn, 1992; for review, see Kampov-Polevoy, Garbutt, & Janowsky, 1999). In contrast, anxiety-like behavior in open-field and elevated-plus mazes (e.g., diminished exploration, greater defecation) has been shown to correlate both positively (e.g., Roman & Colombo,
2009; Spanagel et al., 1995) and negatively (e.g., Henniger, Spanagel, Wigger, Landgraf, & Hölter, 2002; Izídio & Ramos, 2007; Möller, Wiklund, Thorsell, Hyytiá, & Heilig, 1997) with alcohol consumption. The direction of these findings may depend on how anxiety interacts with the genetic background of rats used to test the phenotypic correlation (for review, see Sharko, Fidel, & Wilson, 2013). Nonetheless, both avidity for sweet substances and anxiety have long been associated with alcohol consumption, although little is known about how these two factors relate to impulsive choice.

In consideration of the uncertainties outlined above, the present research was designed to revisit and expand upon the relations between impulsive choice, alcohol consumption, avidity for sweet substances, and anxiety. A sample of outbred Long-Evans rats was screened on an impulsive-choice task. Extreme high- and low-impulsive groups (HiI and LoI, respectively; \( n = 30 \) each) were briefly tested for anxiety-like behavior in an open field (e.g., avoidance of the center field, defecation). Subsequently, rats were given access to 12\% wt/vol alcohol in a two-bottle test to investigate potential between-group differences in alcohol consumption. In a separate, but identical, two-bottle test, rats were also given access to isocaloric sucrose.

**Methods**

**Subjects**

Subjects were 92 experimentally naïve, male Long-Evans rats (Harlan Sprague-Dawley, Indianapolis, IN). Rats completed the experiment in two consecutive cohorts (\( n = 46 \) each; Cohorts 1 and 2). Rats were approximately 90 days old at intake and were
housed individually in polycarbonate cages in a humidity- and temperature-controlled room on a 12-hr light/dark cycle (lights on at 7:00 a.m.). Water was available continuously in the home cage. The use of ad-libitum feeding or food restriction varied by experimental condition (described further below). Unless otherwise noted, rats completed sessions seven days per week between 7:00 and 11:00 a.m. All animals were maintained under the standards of the Institutional Animal Care and Use Committee of Utah State University.

**Apparatus**

Thirty identical operant conditioning chambers were used (Med Associates, St. Albans, VT; ENV-008). Each chamber was equipped with a white-noise speaker (ENV-225SM) and was housed within a sound-attenuating cubicle (ENV-022V). In the center of the rear wall and on opposing sides of the front wall (6.5 cm above the grid floor) were retractable response levers (ENV-112CM). A cue light (ENV-221M) was positioned above all levers. A pellet feeder equipped with a photocell beam to verify reward delivery (ENV-200R2MA) dispensed grain-based pellets (45 mg; Bio-Serv, Frenchtown, NJ) into a food receptacle between the levers.

One open-field arena was used to test anxiety-like behavior. The arena (41 cm x 41 cm x 41 cm) consisted of four black acrylic walls and a white acrylic floor. The room was equipped with a white-noise speaker and was illuminated by ambient light of approximately 60 lux intensity at the level of the arena floor. Sessions were recorded using a digital video camera (Logitech, Inc., Newark, CA); behavior was analyzed using a combination of video tracking software (Smart, version 3.0, Coulbourn Instruments,
Fifteen identical polycarbonate cages were used to examine alcohol and sucrose consumption. Cages were equipped with two glass drinking tubes (Dyets, Inc., Bethlehem, PA) located above glassware bowls to contain potential leakage. The experimental room was equipped with a white-noise speaker and was illuminated by a 40W red light.

**Procedures**

Figure 3.1 depicts the order and approximate duration of all conditions.

Rats were trained to respond on rear and side levers using the autoshaping procedure described in Chapter 2. Sessions consisted of 100 trials each. Lever pressing was trained on each lever until rats earned ≥ 90% of available rewards on that lever for two consecutive sessions.

Next, rats completed a variable number of choice-training sessions, the purpose of which was to minimize variance in sensitivity to differences in reward magnitude. At the beginning of every trial, the rear lever was inserted into the chamber and its cue light was illuminated. A rear-lever press retracted that lever and produced insertion of one or both side levers; when pressed, side levers retracted and produced immediate delivery of either 1 or 3 food pellets (depending on lever; assignment of reward magnitude was counterbalanced across rats). Trials were terminated and counted as an omission if more than 20 s elapsed without a response on active levers. Following pellet delivery (or omissions), no stimuli were presented until the beginning of the next trial. A variable-

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1 The autoshaping procedure used was that described for ND rats in Chapter 2.
Figure 3-1. Order and approximate duration (in days) of experimental conditions. White space indicates periods in which experimental sessions were not completed.
length ITI ensured that trials began every 60 s.

Sessions consisted of 60 trials, divided into three, 20-trial blocks. A 7-min blackout period separated each block. The first six trials in each block were forced-exposure trials in which only one choice lever and its associated cue light were presented (order determined randomly every two trials). Choice-training sessions continued until individual rats chose the larger reward on ≥ 90% of the trials, and made no more than five omissions, for two consecutive sessions.

Following choice training, impulsive choice was assessed using the within-session, increasing-delay task (Evenden & Ryan, 1996). Trial and session structure was identical to that described for choice-training sessions, with the following exceptions. In the first trial block, the delay to both rewards (1 and 3 pellets) was 0 s. In the second and third trial blocks, the large-reward delay increased, respectively, to 15 s and 30 s. The chosen reward’s associated cue light remained illuminated during these delays.

In order to ensure continued sensitivity to differences in reward magnitude, two 0-s probe sessions (identical to choice-training sessions, described above) were pseudorandomly interspersed among the delay session described above. Rats completed 20 sessions in the impulsive-choice test, with no 0-s probe sessions programmed over the terminal six sessions.

Upon completion of the impulsive-choice test, percent large-reward choice across delays (last six sessions) was used to calculate the area under each rat’s impulsive-choice curve (AUC; Myerson, Green, & Warusawitharana, 2001), a summary measure of impulsive choice expressed as a proportion of the maximum possible area. Rats with
AUC values in the approximate upper and lower tertiles of the distribution (LoI and HiI, respectively; $n = 30$ each) were retained for further testing.

Upon completion of the impulsive-choice test, rats were provided with ad-libitum food access in their home cage for 14 days prior to examination of alcohol and sucrose consumption. On the 13th day of ad-libitum food access, the open-field test was conducted by placing individual rats in the middle of the arena. Ten minutes later, rats were returned to their home cage. Testing took place between 7:00 and 9:00 a.m.

Alcohol and distilled water were mixed to a 12% wt/vol solution every 1-2 days. Sucrose and distilled water were mixed daily to a 21% wt/vol solution (isocaloric to alcohol). The 12% solution was chosen because this was the concentration at which prior studies (Poulos et al., 1995; Stein et al., 2013 in Chapter 2) observed significant relations between impulsive choice and alcohol consumption. To determine completion order of alcohol and sucrose tests, rats were matched into pairs based on AUC; from each pair, rats were randomly assigned to one of two order groupings: alcohol first, followed by sucrose ($Alc-Suc$) or sucrose first, followed by alcohol ($Suc-Alc$).

On the day following the open-field test, alcohol and sucrose testing began. Each test consisted of 20 daily, 30-min sessions of access to the active test solution and water in separate drinking tubes. Alcohol and sucrose tests were separated by four days in which no sessions were conducted. The placement side for the test solution and water alternated daily. Following the session, pre-post differences in weights (0.01 g resolution) of drinking tubes were recorded. Leakage, if present, was subtracted from consumption measures.
After completion of alcohol and sucrose tests, rats continued to receive ad-libitum food access in their home cages for 14 days prior to the reinstatement of food restriction. The impulsive-choice retest was then conducted exactly as described for the initial test, excluding choice-training sessions.

**Data Analysis**

All analyses were conducted in SPSS (version 21, SPSS Inc., Chicago, IL), using a significant alpha level of .05.

During training, dependent measures were days to meet the lever-training and choice-training criteria. In the impulsive-choice test and retest, AUC was the dependent measure. In the open-field test, anxiety-related dependent measures were entries into the center field (defined via software as an inner square comprising 25% of the total area), defecation count (number of fecal boluses) and latency to defecate (min). Instances in which rats did not defecate during the session were coded as a 10-min latency (the length of the session). Locomotor activity (distance traveled) was also measured. For all measures described above, data were examined using *t* tests or, where data were non-normally distributed and not amenable to transformation, nonparametric Mann-Whitney *U* or Wilcoxon ranked-sign tests.

In the alcohol and sucrose tests, the 20 test sessions were subdivided into four, 5-session blocks. Dependent measures included mean alcohol or sucrose consumption (g/kg), as well as water consumption (mL/kg), at each session block. Alcohol consumption was non-normally distributed; thus, prior to analysis, data were natural log-transformed. Measures of sucrose and water consumption required no transformation.
Alcohol and sucrose consumption were analyzed using repeated-measures MANOVA, including group (LoI/HiI) and order (Alc-Suc/Suc-Alc) as between-subjects factors, session block (1-5) as a within-subjects factor, and all possible factorial two- and three-way interactions. Water consumption during alcohol and sucrose testing was examined using a separate, but identical, MANOVA. Follow-up univariate ANOVA was used where significant effects were observed in MANOVA. Greenhouse-Geisser adjusted degrees of freedom were used where data violated assumptions of sphericity.

Finally, group analyses described above were supplemented by calculating correlations between days to criterion during autoshaping, AUC, open-field measures, and alcohol and sucrose consumption. The autoshaping measure was included because previous authors report negative relations between cue-oriented behavior and impulsive choice (Flagel et al., 2010; Lovic, Saunders, Yager, & Robinson, 2011). Spearman rho coefficients were used because residuals in many regression analyses were non-normally distributed and heteroscedastic. Kaiser-Mayer-Olsen and Bartlett’s tests were used to examine inter-correlation between consumption across session block (both alcohol and sucrose) and Fisher’s z transformation was used to compare the magnitude of observed correlations by order (Alc-Suc/Suc-Alc).

**Results**

In the test and retest of impulsive choice, no differences emerged between Cohorts 1 and 2 in either LoI or HiI rats (in all cases, $U > 83$, NS); thus, data in all subsequent analyses were collapsed across cohorts.
Lever and Choice Training

Median number of sessions to complete autoshaping was 5 (IQR: 4.00-6.00) in LoI rats and 6 (IQR: 4.75-8.00) in HiI rats. Median number of sessions to complete choice training was 5 in both LoI (IQR: 4.00-6.25) and HiI (IQR: 4.00-7.00) rats. Neither of these measures differed by group.

Impulsive Choice

Figure 3-2 depicts mean (± SEM) percent large-reward choice across delays in the initial impulsive-choice test. Choice was stable across the terminal six sessions, as no main effect of session ($F(5, 885) = 0.41$, NS) or Session x Delay interaction ($F(10, 885) = 0.73$, NS) was observed. The tertile-based selection criterion yielded a highly

![Figure 3-2. Median percent large-reward choice across delays for LoI and HiI rats (n = 30 each) in the initial impulsive-choice test (left panel). Error bars represent interquartile range. Corresponding AUC values are also depicted in box-and-whisker plots (right panel). Horizontal lines within each box indicate group medians. Lower and upper box edges indicate observed 25th and 75th percentiles, respectively. Whiskers indicate minimum and maximum observed values. ****Significantly different than LoI rats (p < .0001)
significant difference in AUC between groups (median LoI AUC: .85, IQR: .63-.99; median HiI AUC: .26, IQR: .25-.26; \( U = 0, p < .001 \)).

**Open-field Behavior**

Videos for one LoI and one HiI rat were incomplete (experimenter error), so software-derived open-field measures (center entries and distance traveled) for these rats were excluded from analysis. Defecation data in these rats were unaffected, as defecation count was scored in vivo (post-session) and defecation latency could be derived from incomplete video.

Figure 3-3 depicts results of the open-field test. Significantly longer defecation latencies (\( U = 283.5, p < .01 \)) and more center entries (\( t(58) = 2.57, p < .05 \)) were observed in LoI compared to HiI rats. No group differences in defecation count (\( U = 343, \) NS) or distance traveled (\( t(56) = 1.06, \) NS) were observed.

*Figure 3-3.* Box-and-whisker plots depicting behavioral measures from the open-field test in LoI and HiI rats. Horizontal lines within each box indicate group medians. Lower and upper box edges indicate observed 25\textsuperscript{th} and 75\textsuperscript{th} percentiles, respectively. Whiskers indicate minimum and maximum observed values. *Significantly different than LoI rats (\( p < .05 \)). **Significantly different than LoI rats (\( p < .01 \)).
Alcohol and Sucrose Consumption

Data for four sessions (three alcohol and one sucrose, affecting separate rats) were lost due to equipment failure (loss of vacuum seal in drinking tube, causing unmeasurable leakage). Missing data were imputed using linear interpolation.

Figure 3-4 depicts mean alcohol and sucrose consumed (g/kg) per session block in the alcohol and sucrose tests. Results of MANOVA revealed no significant main effect of group on alcohol and sucrose consumption ($F(2, 55) = 1.50$, NS, $\eta^2 = .05$), nor significant interactions between group and any other factor (in all cases, $F < 1.32$, NS, $\eta^2 < .18$). Likewise, no significant main effect of order was observed ($F(2, 55) = 2.37$, NS, $\eta^2 = .08$). However, there was a significant main effect of session block ($F(8, 49) = 14.03$, $p < .001$, $\eta^2 = .70$) and an Order x Session Block interaction ($F(8, 49) = 4.82$, $p < .001$, $\eta^2 = .44$), indicating the effects of session block depended on order. Follow-up univariate tests revealed significant Order x Session Block interactions for both alcohol ($F(3.01$,

![Figure 3-4](image_url)

*Figure 3-4. Mean (± SEM) alcohol consumed (g/kg) in LoI and HiI rats across session blocks. Insets depict mean (± SEM) sucrose consumed (g/kg). Depicted are rats from the ALC-SUC and SUC-ALC orders (left and right panels, respectively; $n = 15$ each group, each panel).*
168.79) = 9.28, \( p < .001, \eta^2 = .14 \) and sucrose \( (F(2.96, 165.95) = 7.56, \ p < .001, \eta^2 = .12) \). However, no three-way Order x Session Block x Group interaction was observed for either alcohol or sucrose (in both cases, \( F < 1.77, \ NS, \eta^2 < .04 \)).

A significant main effect of session block on water consumption was observed \( (F(8, 49) = 9.17, \ p < .001, \eta^2 = .60) \), but no effects of order or group (in both cases, \( F(2, 55) < 0.41, \ NS, \eta^2 < .02 \)) or interactions between any factor \( (F(8, 49) < 1.10, \ NS, \eta^2 < .16) \). Follow-up univariate tests indicated a significant effect of session block (consumption declining over blocks) when alcohol was concurrently available \( (F(3.15, 176.46) = 12.56, \ p < .001, \eta^2 = .18) \), but only a marginally significant effect of block when sucrose was available \( (F(3.63, 203.20) = 2.25, \ p = .07, \eta^2 = .04) \).

**Correlational Analyses**

Table 3-1 provides correlation coefficients between days to criterion during autoshaping, AUC, alcohol and sucrose consumption, and open-field measures. Consumption measures were highly inter-correlated across session blocks (in both cases, KMO > .80; Bartlett’s \( \chi^2 > 246.65, \ p < .001 \)) and comparisons of coefficients revealed that correlation magnitude differed significantly by order in only one instance (noted below); thus, sucrose and alcohol consumption were collapsed across order and session block.

Days to criterion during autoshaping was significantly correlated with both consumption measures and both defecation measures, indicating that delayed operant learning predicted lower alcohol and sucrose consumption and higher anxiety. However, the correlation between days to criterion and sucrose consumption varied significantly by
Table 3-1

Spearman rho correlation coefficients between behavioral measures.

<table>
<thead>
<tr>
<th></th>
<th>Autoshaping</th>
<th>AUC</th>
<th>Alcohol</th>
<th>Sucrose</th>
<th>Distance Traveled</th>
<th>Center Entries</th>
<th>Defecation Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>-.41**</td>
<td>.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>-.35*</td>
<td>.19</td>
<td></td>
<td></td>
<td></td>
<td>-.01</td>
<td>.59***</td>
</tr>
<tr>
<td>Distance</td>
<td>-.14</td>
<td>.19</td>
<td>.11</td>
<td></td>
<td>.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Center Entries</td>
<td>.07</td>
<td>.20</td>
<td>-.01</td>
<td>-.01</td>
<td>.59***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defecation Count</td>
<td>.43**</td>
<td>-.30*</td>
<td>-.18</td>
<td>-.50***</td>
<td>-.25</td>
<td>-.10</td>
<td></td>
</tr>
<tr>
<td>Defecation Latency</td>
<td>-.36*</td>
<td>.39*</td>
<td>.29*</td>
<td>.57***</td>
<td>.36*</td>
<td>.20</td>
<td>-.89***</td>
</tr>
</tbody>
</table>

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

order (z = 2.33, p < .05).

Consistent with group analyses, AUC was not significantly correlated with alcohol or sucrose consumption. In contrast, AUC was significantly correlated with both defecation measures, indicating that lower levels of impulsive choice were associated with lower anxiety. However, unlike group analyses, AUC did not significantly correlate with center entries.

Defecation measures were significantly correlated with both alcohol and sucrose consumption, although these relations were more consistent for defecation latency than defecation count. Such relations indicate that higher anxiety was associated with lower alcohol and sucrose consumption. Finally, distance traveled was significantly correlated
with defecation latency and center entries, but not with any other behavioral measure.

**Impulsive-choice Retest**

Group differences in AUC remained significant at the retest ($U = 1, p < .001$) and AUC was highly correlated across tests ($r = .92, p < .001$). No significant test-retest change in AUC was observed in LoI rats ($W = -145, \text{NS}$); however, in HiI rats, significantly higher AUC was observed in the retest ($W = 127, p < .05$).

**Discussion**

Results of the present study provide no support for a relation between impulsive choice and either alcohol or sucrose consumption in male Long-Evans rats. Alcohol and sucrose consumption were positively related to each other, but neither measure was predicted by impulsive choice. Instead, results revealed that anxiety-like behavior significantly correlated with both impulsive choice and alcohol and sucrose consumption, but in opposing directions (Figure 3-4 and Table 3-1). That is, anxiety (more defecation, shorter defecation latencies, and fewer center entries) was associated with greater impulsive choice and, in turn, anxiety (defecation, but not center entries) was also associated with *lower* alcohol and sucrose consumption. Finally, individual differences in impulsive choice remained generally stable across the course of the experiment. Although impulsive choice in HiI rats significantly decreased from test to retest, this change was modest (median AUC difference: .01) and restricted variability in initial AUC values (IQR: .25-.26) likely facilitated statistical significance.
Impulsive Choice

The absence of a relation between impulsive choice and alcohol consumption contrasts with some prior reports of positive relations between these variables in rats (Oberlin & Grahame, 2009; Poulos et al., 1995; Wilhelm & Mitchell, 2008). Although alcohol consumption differed visually between LoI and HiI groups in the present study (Figure 3-4), this difference was not significant and tended in a direction opposite to what would be observed if greater impulsive choice predisposed organisms toward alcohol reward (e.g., Poulos et al., 1995). Together with other reports (Diergaarde et al., 2012; Helms et al., 2006; Oberlin, Bristow, Heighton, & Grahame, 2010; Stein et al., 2013; Wilhelm et al., 2007), the present data indicate that positive covariance between impulsive choice and rodent alcohol consumption is not a generalized phenomenon.

By contrast, the relation between human impulsive choice and alcohol-use disorders is relatively robust. In a recent meta-analysis, MacKillop et al. (2011) reviewed 17 studies comparing impulsive choice in alcohol abusing populations and controls. These authors reported greater impulsive choice in alcohol abusers in 65% of the studies examined. The remaining studies reported no relation between these variables. Of particular interest, group differences in impulsive choice were significantly more robust for studies examining populations meeting clinical criteria for alcohol dependence than those examining subclinical populations (Cohen’s $d$ effect sizes = 0.50 and 0.26, respectively). Given this finding, examination of outbred rats in this and prior studies (Diergaarde et al., 2012; Poulos et al., 1995; Stein et al., 2013) may be of limited utility in understanding the phenomenology of human impulsive choice and alcohol
dependence. However, examinations of alcohol-preferring inbred or selectively bred rodents (putative models of alcohol dependence) have yielded mixed results similar to those observed in outbred rats (Helms et al., 2006; Oberlin & Grahame, 2009; Wilhelm & Mitchell, 2008; Wilhelm et al., 2007;). Thus, in consideration of the concerns outlined above, caution is warranted when interpreting rodent data on impulsive choice and alcohol consumption.

Sucrose Consumption

In the present study, no relation between impulsive choice and sucrose consumption was observed (for a similar finding, see Koffarnus & Woods, 2013). However, sucrose and alcohol consumption were positively related to each other, (e.g., Gosnell & Krahn, 1992). Importantly, such covariance is not unique to the nonhuman literature, as greater avidity for sweet substances has been associated with alcohol dependence in humans (e.g., Kampov-Polevoy et al., 1999).

Across species, avidity for alcohol and sweet substances is thought to be mediated by similar biological mechanisms, including taste and feeding-related neuropeptides, as well as dopamine and opioid systems (for reviews, see Fortuna, 2010; Leggio et al., 2011). Thus, because avidity for alcohol and sucrose share similar sources of control, the failure of impulsive choice to predict alcohol consumption in the present study is consistent with its failure to predict sucrose consumption. Nonetheless, impulsive choice and avidity for sweet substances have previously been shown to co-vary with selective breeding. Specifically, Perry, Nelson, Anderson, Morgan, & Carroll (2007) reported higher levels of impulsive choice in rats bred for high saccharin consumption (HiS)
compared to rats bred for low saccharin consumption (LoS). Interestingly, more-impulsive HiS rats have been shown elsewhere to consume more alcohol than LoS rats (Dess, Badia-Elder, Thiele, Kiefer, & Blizard, 1998); however, scarcity of additional data prevents further interpretation.

**Anxiety**

When examining open-field measures in the present study, impulsive choice predicted greater anxiety-like behavior (i.e., more defecation, shorter defecation latencies, and more center entries). This finding represents a relatively novel contribution to the nonhuman literature, although the underlying mechanism remains unclear. One possibility is that pre-existing anxiety interfered with learning of response-reward contingencies (evidenced by significant correlations between defecation measures and days to criterion during autoshaping; Table 3-1). Such interference would likely be more pronounced when imposing response-reward delays during impulsive-choice testing, producing preference for immediate over delayed food in anxious rats. Whatever the mechanism, a relation between anxiety and impulsive choice would not be unique to rodents, as self-report measures of anxiety (Rounds, Beck, & Grant, 2007; Salters-Pedneault & Diller, 2013) and elevated cortisol levels (Takahashi, 2004) have been associated with impulsive choice in humans. However, acute or repeated administration of anxiolytic drugs has thus far not decreased impulsive choice in humans (Acheson, Reynolds, Richards, & de Wit, 2006; Reynolds, Richards, Dassinger, & de Wit., 2004) and has had mixed effects in rats and pigeons (Cardinal, Robbins, & Everitt, 2000; Eppolito France, & Gerak, 2011; Evenden & Ryan, 1996; Huskinson & Anderson, 2012;
Wolff & Leander, 2002). Further investigation is necessary. Thus, whether anxiety plays a causal role in impulsive choice remains to be determined.

Whereas impulsive choice predicted greater anxiety-like behavior in the present study, anxiety-like behavior predicted lower alcohol and sucrose consumption. This negative relation between anxiety and consumption reproduces some prior findings (e.g., Alsiö et al., 2009; Henniger et al., 2002; Izídio & Ramos, 2007; Dess & Minor, 1996) and is consistent with pharmacological data in which anxiety reduction, via pre-session administration of anxiolytic drugs, increases alcohol consumption in rodents (e.g., Hedlund and Wahlstrom, 1997; Schmitt, Waldhofer, Weigelt, & Heimke, 2002). However, our findings conflict with additional data in which anxiety is instead associated with higher consumption of alcohol in both rodents and humans (e.g., Roman & Colombo, 2009; Spanagel et al., 1995; for review, see Sharko et al., 2013). Given these conflicting reports, the role of anxiety in alcohol consumption is complex and requires further study. Nonetheless, because anxiety in the present study related to impulsive choice and alcohol consumption in opposing directions, anxiety may have obstructed the otherwise positive relation between these variables reported previously (e.g., Poulos et al., 1995). Future studies may be designed to explore this putative modulatory influence by administering anxiolytic medication prior to alcohol sessions.

Conclusions

Despite relatively robust relations between impulsive choice and alcohol-use and other addictive disorders in humans (for review, see MacKillop et al., 2011) and psychostimulant self-administration in rodents (for review, see Stein & Madden, 2013),
the present and prior studies have revealed no consistent relations between impulsive choice and alcohol consumption in rodents. This inconsistency indicates that the relation between impulsive choice and rodent alcohol consumption may be dynamically modulated by a number of other variables. This preliminary investigation identified one possible variable--anxiety--which, if nothing else, should be measured in future studies of impulsive choice and alcohol consumption. We caution, however, that the apparent complexity of the relations between these variables likely constrains their translational utility.

References


Poor impulse control predicts inelastic demand for nicotine but not alcohol in rats. *Addiction Biology, 17*(3), 576-587.


Oberlin, B. G., & Grahame, N. J. (2009). High-alcohol preferring mice are more impulsive than low-alcohol preferring mice as measured in the delay discounting task. *Alcoholism: Clinical and Experimental Research, 33*(7), 1294-1303.


Stein, J. S., & Madden, G. J. (2013). Delay discounting and drug abuse: Empirical,


CHAPTER 4

IMPULSIVE CHOICE, ALCOHOL SELF-ADMINISTRATION, AND
PRE-EXPOSURE TO REWARD DELAY: II. FOLLOW-UP INVESTIGATION

Abstract

In a prior study (Stein et al., 2013 [Chapter 2]), we reported that rats pre-exposed
to delayed rewards made fewer impulsive choices, but consumed more alcohol (12% wt/vol),
than rats pre-exposed to immediate rewards. To understand the mechanisms that
produced these findings, we again pre-exposed rats to either delayed (17.5 s; n = 32) or
immediate (n = 30) rewards. In posttests, delay-exposed rats made significantly fewer
impulsive choices at 15- and 30-s delays to a larger, later food reward than the
immediacy-exposed comparison group. Behavior in an open-field test provided little
evidence of differential stress exposure between groups. Further, consumption of either
12% alcohol or isocaloric sucrose in subsequent tests did not differ between groups.
Because Stein et al. introduced alcohol concentration gradually (3-12%), we speculate
that their group differences in 12% alcohol consumption were not determined by
alcohol’s pharmacological effects, but by another variable (e.g., taste) that was preserved
as an artifact from lower concentrations. We conclude that pre-exposure to delayed
rewards generalizes beyond the pre-exposure delay; however, this same experimental
variable does not robustly influence alcohol consumption.
Introduction

Across species, *impulsive choice* has been operationalized as preference for smaller, relatively immediate rewards over larger, more delayed rewards (e.g., Ainslie, 1975). In human cross-sectional studies (e.g., comparisons of alcoholics vs. controls), impulsive choice in laboratory tasks is strongly associated with substance-use disorders (for meta-analysis, see MacKillop et al., 2011). One account of this association is that impulsive choice plays an etiological role in the development of such disorders, as a generalized tendency to over-value immediate outcomes (or devalue delayed outcomes) might be expected to produce persistent preference for immediate drug effects over the delayed benefits of abstinence (e.g., long-term good health; for review, see Perry & Carroll, 2008; Stein & Madden, 2013). Some evidence supports this interpretation, as impulsive choice in longitudinal studies has been shown to precede and predict subsequent adoption of tobacco, cocaine, and alcohol use (e.g., Audrain-McGovern et al., 2009; Kim-Spoon, McCullough, Bickel, Farley, & Longo, 2014; Khurana et al., 2013), indicating that the relation between impulsive choice and substance-use disorders cannot be solely explained as a consequence of prior drug exposure.

Rodent studies have yielded relations between impulsive choice and drug self-administration at least formally consistent with the human longitudinal data reviewed above. That is, impulsive choice in screening tasks has been shown to precede and predict greater self-administration of a range of drugs, including alcohol, cocaine, nicotine, and
methylphenidate (e.g., Diergaarde et al. 2008; Koffarnus & Woods, 2013; Marusich & Bardo, 2009; Perry, Larson, German, Madden, & Carroll, 2005; Poulos, Le, & Parker, 1995; for review, see Stein & Madden, 2013). Despite these naturally occurring relations, few rodent studies have been designed to determine whether experimental changes in impulsive choice yield predictable changes in drug self-administration. If experimental reductions in impulsive choice produce concomitant reductions in drug self-administration, a direct causal relation between these variables would be strengthened. In translation, this finding would suggest that treating impulsivity in human populations would yield therapeutic effects on substance-use disorders. If, however, experimental reductions in impulsive choice do not reliably reduce drug self-administration, then the naturally occurring relation between these variables may owe to the mutual influence of a third, unexamined variable (biological or behavioral).

Adopting the experimental logic above, Stein et al. (2013) reduced impulsive choice in rats (via prolonged pre-exposure to delayed rewards) in order to examine potential concomitant reductions in alcohol consumption. These authors pre-exposed two experimental groups to sessions in which food was available from a single lever following either fixed (17.5 s; n = 14) or escalating (17.5-44 s, on average; n = 16) delays. In subsequent testing, both experimental groups more frequently preferred a larger, later reward (three pellets, delayed by 15 s) over a smaller, sooner reward (one pellet, delivered immediately) than a comparison group pre-exposed to immediate rewards (n = 14). However, when these authors examined alcohol consumption across
ascending concentrations (3-24%), the group pre-exposed to fixed delays, although less impulsive, consumed significantly more alcohol (12%) than the comparison group exposed to immediate reward—a effect that counters the naturally occurring relation between these variables (e.g., Poulos et al., 1995). In this chapter, two variables that may have been responsible for this finding were addressed.

First, a large experimental literature documents that chronic stress, particularly during adolescence, increases alcohol consumption in rodents (for review, see Becker, Lopez, & Doremus-Fitzwater, 2011). If one assumes that delay to reward is a stressor, then chronic pre-exposure to this putative stressor might be expected (independent of its effects of on impulsive choice) to increase subsequent alcohol consumption. Few studies, however, have examined delay as a possible source of stress in rodents. In at least one study, acute exposure to a prolonged (15 min), non-operant delay to highly palatable food was shown to increase the stress hormone, corticosterone, in rats (Cifani, Polidoro, Melotto, Ciccocioppo, & Massi, 2009). However, the specific methodology used in this study (acute delay exposure in a model of “yo-yo” dieting) may limit the generality of this finding and its relevance to the data reported by Stein et al. (2013). Further study is required to estimate the potential role of stress in the effects of delay pre-exposure on alcohol consumption.

Second, Stein et al. (2013) examined alcohol consumption under ascending alcohol concentrations, a preparation designed to match prior studies in the literature (e.g., Poulos et al., 1995). A problem here is the degree to which this choice may have

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2 Only a trend-level difference was observed ($p = .07$) between the group pre-exposed to escalating delays and the comparison group.
introduced sources of variance (e.g., taste) that were unrelated to alcohol’s pharmacological effects. Indeed, such concern is warranted, as reanalysis of Stein et al.’s data indicates that likely subpharmacological consumption at 3% alcohol (approximately 0.1-0.2 g/kg/30 minutes, on average) significantly predicted consumption at the 12% concentration at which group differences emerged ($r = .59, p < .001; N = 44$). Thus, it appears the variable(s) that determined consumption of 12% alcohol were preserved as an artifact from the 3% concentration. As a result, the degree to which delay pre-exposure increases rats’ preference for alcohol’s pharmacological effects, independent of the influence of extra-pharmacological properties experienced at lower concentrations, has yet to be determined. To this end, examining consumption of 12% alcohol in isolation is necessary. Moreover, examining consumption of a sucrose solution in a separate test is necessary to estimate the influence of delay pre-exposure on preference for sweet substances in the absence of pharmacological effects.

Aside from the specific concerns outlined above regarding Stein et al.’s (2013) data, variables that impact impulsive choice are of broad interest to those studying impulsive choice and its relation to addiction and other pathologies (e.g., ADHD; Scheres, Tontsch, Thoeny, & Kaczkurkin, 2010). To this end, the present chapter seeks to provide a thorough examination of the effects of delay pre-exposure on impulsive choice. Because these authors tested impulsive choice under only a single non-zero large-reward delay (15 s), the degree to which the effects of pre-exposure may generalize to longer delays is unknown. In addition, little is known about the mechanism(s) by which delay pre-exposure exerts its effects on impulsive choice. To this end, examination of
secondary behavioral measures (e.g., response latency, time spent visiting the food receptacle during delays), while determining how such measures relate to impulsive choice, may provide important clues. For example, if delay pre-exposure reduces impulsive choice by altering timing processes, then this may be evident in the temporal precision of food receptacle visits during delays (i.e., scalloped patterns typical of timing under fixed-interval schedules). In turn, such secondary analyses are likely to inform future research designed explicitly to investigate functional mechanisms underlying the effects of delay pre-exposure on impulsive choice.

In light of the considerations outlined above, the present chapter sought to systematically reproduce and extend the effects of delay pre-exposure on impulsive choice and alcohol consumption reported by Stein et al. (2013). Two groups of Long-Evans rats were pre-exposed to either a fixed delay to food rewards (17.5 s; n = 32) or immediate food rewards (n = 30). In these delay-exposed (DE) and immediacy-exposed (IE) rats, a variety of behavioral measures were examined: (1) behavioral indicators of prior stress exposure in an open-field test (e.g., exploration of the open field, motor activity, defecation; Colorado, Shumake, Conejo, Gonzalez-Pardo, & Gonzalez-Lima, 2006; Katz, Roth, & Carroll, 1981); (2) impulsive choice under both 15-s and 30-s LLR delays, including secondary behavioral measures of response latency (a potential measure of motivation), time spent in the food receptacle during delays (a possible measure of mediating behavior), and temporal precision of intra-delay receptacle visits (a potential measure of timing); (3) consumption of 12% wt/vol alcohol and isocaloric sucrose (in

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3 The terms DE and IE are interchangeable with the terms FD and ND, respectively, from Chapter 2.
separate tests); and (4) impulsive choice in a retest, more than two months following the initial test.

Methods

Subjects

All animals were maintained under the standards of the Institutional Animal Care and Use Committee of Utah State University. Subjects were 63 experimentally naïve, male Long-Evans rats (Harlan Sprague-Dawley, Indianapolis, IN), received at the facility at postnatal day 21. Rats were individually housed in polycarbonate cages in a temperature- and humidity-controlled colony room. Lights in the colony operated on a 12-hr light/dark cycle (lights on at 7:00 a.m.). Water was freely available in all home cages.

Rats were randomly assigned to either DE \((n = 32)\) or IE \((n = 31)\) groups. One IE rat died of natural causes early in training, leaving only 30 rats in the IE group. Following three days of ad-libitum food access, rats were weighed daily and restricted to 85% of the vendor-supplied, age-adjusted free-feeding weight. Food restriction continued until 14 days prior to open-field, alcohol and sucrose tests, at which point rats were provided with ad-libitum food access in their home cages. Following alcohol and sucrose testing, food restriction was reinstated using rats’ 85% free-feeding weights (recalculated from free-feeding weights 12-14 days following the final alcohol or sucrose session).

Unless otherwise specified below, experimental sessions were conducted seven days per week between the hours of 7:00 a.m. and 1:00 p.m. Sessions were conducted at
approximately the same time each day, with DE and IE rats counterbalanced across time of day.

**Apparatus**

Thirty identical operant conditioning chambers were used (24.1 x 30.5 x 21 cm; Med Associates, St. Albans, VT). Each chamber was housed within a sound-attenuating box outfitted with a white-noise speaker. Centered on the rear wall of the chamber and 6.5 cm above the chamber’s grid floor was a retractable response lever. Two identical retractable levers were located at the same height on the front wall, one to the left and one to the right of the rear lever. Above each lever was a 28-V DC cue light. A pellet feeder delivered grained based pellets (45 mg; Bio-Serv, Frenchtown, NJ) to a receptacle centered below the two front wall levers.

Sixteen polycarbonate cages were used in the alcohol and sucrose tests. Each cage was equipped with two drinking tubes (Dyets, Inc., Bethlehem, PA), located above a small glassware bowl (Pyrex; World Kitchen, LLC, Rosemount, IL) used to collect leakage. Alcohol and sucrose tests occurred in a room equipped with a white-noise speaker and illuminated by a 40W red light.

An open-field arena was used to measure exploratory behavior. The arena (41 cm x 41 cm x 41 cm) consisted of four black acrylic walls and a white acrylic floor. Testing occurred in a room with a white-noise speaker and illuminated with approximately 60 lux of light at the level of the arena floor. All sessions were recorded using a digital video camera (Logitech, Inc., Newark, CA) mounted 81 cm above the arena floor. Smart (version 3.0) video tracking software and manual scoring were used to analyze behavior.
Procedures

Figure 4-1 illustrates the order and duration of all experimental phases, as well as the approximate age of rats during these phases. An autoshaping procedure was used to establish rear lever pressing. To produce comparable rates of acquisition across DE and IE groups, a constant ratio of intertrial interval to trial length of 11:1 was used (Gibbon, Baldock, Locurto, Gold, & Terrance, 1977). For DE and IE rats, the ITI was 247.5 and 55 s, respectively. During the ITI, no programmed stimuli were presented. Following the ITI, the rear-lever/light complex was activated (i.e., lever inserted and light illuminated) for 5 s. For DE rats, either a single response or termination of this 5-s period (whichever occurred first) deactivated the lever; the cue light remained illuminated for 17.5 s prior to delivery of two food pellets. For IE rats, deactivation of the lever and light occurred simultaneously and was contiguous with pellet delivery. Sessions consisted of 25 trials and continued until rats earned \( \geq 90\% \) of the scheduled rewards across two consecutive sessions.

All rats completed 90 sessions of delay pre-exposure; sessions were composed of 125 trials. Each trial began with the activation of the rear-lever/light complex. A single lever press deactivated the rear lever and initiated a delay to the delivery of two food pellets. This delay was 17.5 s for DE rats and 0.01 s [henceforth 0 s] for IE rats. In this and all subsequent phases, the cue light above the lever remained illuminated during the delay. If the rear lever was not pressed within 20 s of trial onset, the rear-lever/light complex was deactivated and the trial was scored as an omission. A variable-length ITI ensured that trials started every 50 s, regardless of rear-lever response latency or
Figure 4-1. Order and approximate duration (in days) of experimental conditions. Depicted durations of delay pre-exposure and the initial impulsive-choice test include side-lever and choice-training, respectively. Some durations varied slightly depending on sessions required to meet training criteria (see text; Table 4-1). White space indicates periods in which sessions were not completed.
omissions.

Immediately following the 90 sessions of responding on the rear lever, all rats were briefly trained to respond on the two front-wall levers. Session structure and stimuli were identical to those used during delay pre-exposure, except that the rear lever was not used. Instead, either the left or right (determined randomly without replacement every two trials) front-lever/light complex was activated at the beginning of every trial. Front-lever training continued until rats completed ≥ 90% of programmed trials for two consecutive sessions.

Prior to the impulsive-choice test, all rats completed choice training in order to ensure sensitivity to reward amount (e.g., Evenden & Ryan, 1996). Choice-training sessions consisted of three blocks of 20 trials, with each block separated by a 7-min blackout period. Each block consisted of six forced-choice trials (only one front lever available; left-right order determined randomly, as described above) and 14 free-choice trials (both front levers available). At the beginning of all trials, the rear-lever/light complex was activated. A single response deactivated the complex and activated one or both front-lever/light complexes, depending on trial type (forced- or free-choice). A single response on a front-wall lever deactivated both levers and initiated a delay to the delivery of food; the cue light above the selected lever remained on during the delay, while the unselected cue light was turned off. One lever produced a 1-pellet reward whereas the other delivered 3 pellets; sides counterbalanced within and between groups. Within each group, the delay to both rewards was the same; however, these delays differed between groups (as before, 17.5 s in DE rats and 0 s in IE rats). Failures to
respond within 20 s of lever insertion terminated the trial and were counted as omissions. Sessions continued until rats chose the larger reward on $\geq 90\%$ of the trials and made no more than five omissions during two consecutive sessions.

Following choice training, impulsive choice was assessed for all rats using a within-session, increasing-delay procedure (Evenden & Ryan, 1996). Sessions were identical to those described for choice training with the following exceptions: (a) the 1-pellet reward was delivered immediately; (b) the delay to the 3-pellet reward increased across the three trial blocks (0, 15, and 30 s); and (c) to ensure continued sensitivity to differences in reward amount, two probe sessions (0-s delays across trial blocks) were pseudo-randomly interspersed among those of the impulsive-choice test. Rats completed 20 total sessions in the impulsive-choice test, with no probe sessions programmed over the final six sessions.

Upon completion of the impulsive-choice test, rats were provided with ad-libitum food in the home cage for 14 days. On the 13th day of ad-libitum food, the rat was placed in the middle of the open-field arena and its movements were tracked for 10 minutes (e.g., Colorado et al., 2006). Testing took place between 7:00 and 9:00 a.m.

All rats were matched into pairs based on a summary measure of impulsive choice (area under the curve; Myerson, Green, & Warusawitharana, 2001). From each pair, one rat was randomly assigned to complete alcohol sessions; the other rat was assigned to complete sucrose sessions. The sample size used in this study was chosen so that the alcohol and sucrose conditions would contain numbers of rats ($n = 16$ and 15 DE and IE rats, respectively) similar to those used by Stein et al. (2013; $n = 14$, each group).
Alcohol and distilled water were mixed to a 12% wt/vol solution every 1-2 days. Sucrose and distilled water were mixed daily to a 21% wt/vol solution (isocaloric to alcohol). Alcohol and sucrose tests consisted of 20 daily, 30-min sessions. Each subject’s assigned test solution and water were poured into separate drinking tubes, with the placement side of the solution and water alternating daily. Following each session, the pre-post difference in weights (0.01-g resolution) of each drinking tube was recorded. Leakage, if present, was weighed and subtracted from consumption measures.

Following completion of the alcohol or sucrose tests, rats continued to receive ad-libitum food in their home cages for 14 days prior to the reinstatement of food restriction. Following this period, the impulsive-choice retest was conducted as described for the initial test, except that no choice-training sessions were conducted.

**Data Analysis**

All analyses were conducted in SPSS (version 21, SPSS Inc., Chicago, IL). For autoshaping, front-lever training, and choice training, the dependent measures were days to meet the training criteria. For delay pre-exposure, the 90 sessions were divided into 15, six-session blocks. Dependent measures at each session block included: rear-lever latency (s), percent trials omitted, and total time spent visiting the pellet receptacle during delays (measured by continuous 0.01-s beam breaks). An index of curvature was also examined in order to describe temporal precision of receptacle visits during delays. To calculate this index, the 17.5-s delay was divided into 35 half-second intervals. Cumulative time spent in the receptacle across intervals was then applied to the following equation (Fry, Kelleher, & Cook, 1960; e.g., Ward & Odum, 2005):
\[
C = \frac{(n-1)T_n - 2 \sum_{i=1}^{n-1} T_i}{nT_n},
\]

in which \(C\) represents curvature and \(T\) represents cumulative time in the receptacle through interval \(i\) or through the final interval \(n\). This measure \((C)\) is bounded symmetrically around 0 by discrete negative and positive values, but these bounds vary as a function of the number of intervals \((n)\) used in Equation 4-1. Thus, in order to make comparisons across delays of different durations, observed values were converted to a normalized measure \((C_n)\) by dividing observed \(C\) by the fraction: \((n - 1)/n\). Calculated using this method, \(C_n\) describes a continuum in which, at the extremes, time is spent in the receptacle only in the first interval \((C_n = -1)\) or only in the last interval \((C_n = 1)\). When \(C_n = 0\), time in the receptacle is evenly distributed across the intervals.

In the impulsive-choice test and retest, data were taken from the final six sessions. Dependent measures at each delay were percent LLR choice, rear-lever latency, and percent trials omitted. Front-lever latencies were not examined because many rats displayed exclusive or near-exclusive LLR or SSR preference in free-choice trials at one or more delays, thus restricting the data available for group comparisons. Finally, time spent in the receptacle and \(C_n\) (as described previously) were examined during 15- and 30-s LLR forced-choice trials (to which all rats were exposed, regardless of free-choice preference).

In the open-field test, the 10-min session was divided into 5, two-min blocks. Dependent measures at each block included entries into the center field (defined via software as an inner square comprising 25\% of the total area) and distance traveled (m).
Additional global measures included defecation count (number of fecal boluses) and defecation latency (min). Instances in which rats did not defecate during the session were coded as a 10-min latency (the length of the session).

In the alcohol and sucrose tests, the 20 sessions were divided into 5, four-session blocks. Dependent measures at each block included consumption of the test solution (alcohol or sucrose; g/kg), a preference score for the test solution (g solution/mL water consumed), and body weight.

Many of the measures described above were non-normally distributed (bimodality or skew) and not amenable to transformation. Where skew was evident in dependent measures, group medians (± interquartile range) are presented in figures; in all other cases, group means (± SEM) are presented. Due to violations of normality, all non-repeated measures within individual tests (days to criterion during autoshaping, front-lever and choice training, defecation data) were analyzed using nonparametric Mann-Whitney U tests. All repeated measures (those within delay pre-exposure, as well as impulsive-choice, open-field, and alcohol or sucrose tests) were analyzed using separate generalized estimating equations (GEEs; one for each measure, unless otherwise specified). Use of GEE allows for analysis of correlated, repeated measures; but unlike ANOVA, use of GEE makes no distributional assumptions (for overview, see Ballinger, 2004).

Unless otherwise specified, GEE models included group as the between-subjects factor and one within-subjects factor relevant to the test (e.g., session block during delay pre-exposure and alcohol or sucrose tests; delay during impulsive-choice tests). Because
time in the receptacle and $C_n$ could not be derived for IE rats during delay pre-exposure, only the within-subjects factor (session block) was examined. Likewise, because time in the receptacle and $C_n$ could not be derived in either group at the 0-s delay in the impulsive-choice test, only the 15- and 30-s delays were examined when analyzing these measures. In a GEE model examining test-retest changes in LLR choice, group was again the between-subjects factors (as in previous models); however, two within-subjects factors (delay and test) and a covariate (interim completion of either alcohol or sucrose testing) were included in the model. Where significant main effects or interactions were observed in all GEE models, post-hoc comparisons were examined at individual repeated-measures time points using sequential Bonferroni correction to control Type I error rate.

Finally, Spearman rho correlations were used to examine relations between LLR choice and the following measures in DE and IE rats: rear-lever latency, percent trials omitted, time in the receptacle, and $C_n$ from the impulsive-choice tests; and center entries, distance traveled, and defecation count and latency from the open-field tests Spearman rho correlations were also used elsewhere, as reported below, to supplement group analyses.

**Results**

**Autoshaping**

Both groups required a comparable number of sessions to meet the rear-lever autoshaping criterion (see Table 4-1; $U = 410$; NS).
Delay Pre-Exposure

The upper two panels of Figure 4-2A depict rear-lever latency and percent trials omitted across session blocks during delay pre-exposure. For latency, main effects of session block (Wald’s $\chi^2 = 118.38; p < .001$) and group (Wald’s $\chi^2 = 67.36; p < .001$) were observed, as well as a Group x Session Block interaction (Wald’s $\chi^2 = 14.52; p < .001$). Post-hoc comparisons at individual session blocks revealed significantly longer latencies in DE rats from session blocks 1-12 ($p < .05$ or lower; see Figure 4-2A) and no between-group differences from session blocks 13-15.

When percent trials omitted was examined, main effects of session block (Wald’s $\chi^2 = 342.3; p < .001$) and group (Wald’s $\chi^2 = 69.76; p < .001$) were observed, as well as a Group x Session Block interaction (Wald’s $\chi^2 = 39.27; p < .001$). Post-hoc comparisons at individual session blocks revealed longer latencies in DE rats from session blocks 1-9 ($p < .05$ or lower; see Figure 4-2A) and no between-group differences from session blocks 10-15.

The lower two panels of Figure 4-2A depict time in the receptacle and $C_n$ during delay pre-exposure. For DE rats, a main effect of session block was observed on time in

Table 4-1

*Median sessions to meet the training criterion (± interquartile range) during autoshaping, side-lever, and choice training in DE and IE rats*

<table>
<thead>
<tr>
<th>Group</th>
<th>Training Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Autoshaping</td>
</tr>
<tr>
<td>DE</td>
<td>7.00 (4.25-8.75)</td>
</tr>
<tr>
<td>IE</td>
<td>7.00 (6.00-9.00)</td>
</tr>
</tbody>
</table>

Significantly different than IE rats: *$p < .05$, **$p < .01$.**
Figure 4-2. Rear-lever latency and percent trials omitted per session, and time in the receptacle and \( C_n \) per trial, across session blocks during delay pre-exposure (Panel A); percent large-reward choice across delays in the impulsive-choice test (Panel B); and rear-lever latency and percent trials omitted per session, and time in the receptacle and \( C_n \) per trial, across delays in the impulsive-choice test (Panel C). Where specified, data reflect median (± interquartile range) observed values; all remaining data reflect mean (± SEM) values. Data points have been slightly displaced on the x-axis, for clarity. Significantly different than IE rats: *\( p < .05 \), **\( p < .01 \), ***\( p < .001 \).
the receptacle (Wald’s $\chi^2 = 90.38; p < .001$), with this measure rising to peak values by
the 10th session block and remaining approximately stable through the final session block.
Likewise, a main effect of session block was observed on $C_n$ (Wald’s $\chi^2 = 264.16; p < .001$); however, the relation between these variables was bitonic, with $C_n$ rising to peak values by the fourth session block and declining to baseline levels by the tenth session block.

**Front-Lever and Choice Training**

Rats in the DE group required significantly more sessions than IE rats to meet the
front-lever and choice-training criteria (see Table 4-1; in both cases, $U < 324; p < .05$).

**Impulsive-Choice Test**

Figure 4-2B depicts percent large-reward choice across delays in the impulsive-
choice test. Choice was stable across the final six sessions analyzed, as no significant
main effect of session on large-reward choice, or Delay x Session interaction, was
observed in any group (in all cases, Wald’s $\chi^2 < 0.90$; NS). Results of GEE revealed main
effects of group (Wald’s $\chi^2 = 34.24; p < .001$) and delay (Wald’s $\chi^2 = 593.26; p < .001$) on
large-reward choice, as well as a Group x Delay interaction (Wald’s $\chi^2 = 34.11; p < .001$).
Post-hoc comparisons revealed significantly greater large-reward choice (collapsed
across delay) in DE, compared to IE rats ($p < .001$). Comparisons at individual delays
revealed greater large-reward choice in DE, compared to IE rats, at both the 15- and 30-s
large-reward delays (in both cases, $p < .001$).

The upper two panels of Figure 4-2C depict rear-lever response latency (s) and
percent trials omitted across large-reward delays. Results of GEE revealed significant main effects of delay on both latency and omissions (in both cases, Wald’s $\chi^2 > 28.26; p < .001$), but no main effect of group (Wald’s $\chi^2 < 0.39; NS$). Results further revealed a significant Group x Delay interaction for omissions (Wald’s $\chi^2 = 12.41; p < .05$), but not latency (Wald’s $\chi^2 = 2.44; NS$). Post-hoc comparisons revealed significantly fewer omissions in DE, than IE rats at the 15-s large-reward delay ($p < .05$). Finally, large-reward choice did not significantly correlate with measures of rear-lever latency or percent trials omitted at either the 15- or 30-s delay.

The lower two panels of Figure 4-2C depict time in the receptacle and $C_n$ across delays in the impulsive-choice test. For time in the receptacle, results of GEE revealed significant main effects of group (Wald’s $\chi^2 = 4.49; p < .05$) and delay (Wald’s $\chi^2 = 137.09; p < .001$), as well as a Group x Delay interaction (Wald’s $\chi^2 = 10.90; p < .01$). Post-hoc comparisons revealed that DE rats spent significantly more time in the receptacle than IE rats at the 30-s delay ($p < .01$), but not at the 15-s delay.

When $C_n$ was examined, results of GEE revealed a significant main effect of delay (Wald’s $\chi^2 = 9.98; p < .01$), no main effect of group (Wald’s $\chi^2 = 0.79; NS$), but a significant Group x Delay interaction (Wald’s $\chi^2 = 4.89; p < .05$). Post-hoc comparisons, however, revealed no between-group differences at either delay.

Table 4-2 provides correlations between large-reward choice and measures of time in the receptacle and $C_n$ in the impulsive-choice test. Significant positive correlations at both delays were observed between large-reward choice in the first test of impulsive choice and the time that DE rats spent in the food receptacle. That is, DE rats that spent
Table 4-2

*Spearman rho correlations in DE and IE rats between large-reward choice (15- and 30-s delays) and simultaneously collected measures of time in the receptacle and C_n in the impulsive-choice test and retest*

<table>
<thead>
<tr>
<th>Test</th>
<th>Large-Reward Delay</th>
<th>Time in Receptacle</th>
<th>C_n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DE</td>
<td>IE</td>
</tr>
<tr>
<td>Test</td>
<td>15</td>
<td>.59***</td>
<td>.30</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>.71***</td>
<td>.32</td>
</tr>
<tr>
<td>Retest</td>
<td>15</td>
<td>.42</td>
<td>.14</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>.66***</td>
<td>.15</td>
</tr>
</tbody>
</table>

*p < .05, **p < .01, ***p < .001.

traveled was otherwise undifferentiated. Finally, LLR choices made at the 15- and 30-s more time in the receptacle during delays (regardless of when it occurred) tended to make more large-reward choices than those that spent less time in the receptacle. These correlations were not significant in IE rats. For C_n, a significant negative correlation was observed at the 15-s and 30-s delays in DE rats. That is, DE rats that evenly distributed their time in the feeder during the delay tended to make more large-reward choices than those that spent more time in the feeder at the end of the delay. In IE rats, this negative correlation was significant only at the 30-s delay.

**Open-Field Test**

The left panels of Figure 4-3A depict center entries and distance traveled in the open-field test. A main effect of time was observed on center entries (Wald’s $\chi^2 = 21.61$; $p < .001$), but there was no main effect of group (Wald’s $\chi^2 = 1.84$; NS) or Group x Time interaction (Wald’s $\chi^2 = 1.25$; NS). In contrast, main effects of group (Wald’s $\chi^2 = 7.09$; $p < .01$) and time (Wald’s $\chi^2 = 52.10$; $p < .001$) were observed on distance traveled, as well
as a Group x Time interaction (Wald’s $\chi^2 = 11.96; p < .05$). Post-hoc comparisons revealed that DE rats were less active than IE rats in the second two-minute block ($p < .05$) but distance traveled was otherwise undifferentiated. Finally, LLR choices made at the 15- and 30-s delays (test or retest) did not significantly correlate with either of these open-field measures in DE or IE rats.

The right panels of Figure 4-3A depict defecation count and defecation latency in the open-field test. No differences between groups were observed in either of these measures ($U > 424; NS$). As before, 15- or 30-s LLR choice (test or retest) did not significantly correlate with either of these measures in DE or IE rats.

**Figure 4-3.** Center entries and distance traveled across two-minute blocks in the open-field test, as well as defecation count and latency (Panel A); and consumption and body weight during the alcohol and sucrose tests (Panel B and C). Where specified, panels reflect median (± interquartile range) observed values; all other panels reflect mean (± SEM) observed values. Data points have been slightly displaced on the x-axis, for clarity. Significantly different than DE rats: $^*p < .05$. 
**Alcohol and Sucrose Tests**

Figure 4-3B depicts alcohol consumption (g/kg) and corresponding body weight (g) across session blocks among rats assigned to this test. A main effect of session block was observed on consumption (Wald’s $\chi^2 = 9.51; p < .05$), but no main effect of group (Wald’s $\chi^2 = 0.14; \text{NS}$) or Group x Session Block interaction (Wald’s $\chi^2 = 3.63; \text{NS}$).

Median alcohol preference (i.e., g solution/mL water consumed) across session blocks 1-5 (not pictured) ranged from 1.49 (IQR: 1.21-1.95) to 2.40 (IQR: 0.36-6.07) in DE rats and 1.32 (IQR: 1.15-1.75) to 2.26 (IQR: 0.14-1.72) in IE rats. Results of GEE revealed no main effect of session block (Wald’s $\chi^2 = 1.83; \text{NS}$) or group (Wald’s $\chi^2 = 0.95; \text{NS}$) on alcohol preference, and no significant Group x Session Block interaction (Wald’s $\chi^2 = 0.99; \text{NS}$).

Body weight significantly increased across session blocks (Wald’s $\chi^2 = 124.39; p < .001$), but weight was undifferentiated across groups (Wald’s $\chi^2 = 3.27; \text{NS}$) and there was no Group x Session Block interaction (Wald’s $\chi^2 = 2.66; \text{NS}$).

Figure 4-3C depicts sucrose consumption (g/kg) and corresponding body weight (g) for rats assigned to the test of sucrose consumption. No main effect of group was observed on consumption (Wald’s $\chi^2 = 2.25; p = \text{NS}$); however, a main effect of session block was observed (Wald’s $\chi^2 = 80.80; p < .001$), as well as a Group x Session Block interaction (Wald’s $\chi^2 = 17.82; p < .01$). Post-hoc comparisons revealed no significant group differences in consumption at individual session blocks.

Median sucrose preference across session blocks 1-5 (not pictured) ranged from 9.88 (IQR: 6.40-15.70) to 50.88 (IQR: 31.90-112.60) in DE rats and 10.89 (IQR: 7.60-
19.30) to 18.92 (IQR: 12.6-88.80) in IE rats. Results of GEE revealed a main effect of session block on sucrose preference (Wald’s $\chi^2 = 34.83; p < .001$), but no main effect of group (Wald’s $\chi^2 = 0.70; \text{NS}$) or Group x Session Block interaction (Wald’s $\chi^2 = 2.59; \text{NS}$).

Again, a main effect of session block was observed on body weight (Wald’s $\chi^2 = 122.10; p < .001$), but no main effect of group (Wald’s $\chi^2 = 0.07; \text{NS}$) or Group x Session Block interaction (Wald’s $\chi^2 = 3.94; \text{NS}$).

**Impulsive-Choice Retest**

Figure 4-4A depicts percent LLR choice across delays in the impulsive-choice retest. As in the initial test, choice was stable across the final six sessions, as no main effect of session or Delay x Session interaction was observed in either group (in all cases, Wald’s $\chi^2 < 0.76; \text{NS}$). Results of GEE revealed main effects of group (Wald’s $\chi^2 = 15.31; p < .001$) and delay (Wald’s $\chi^2 = 368.41; p < .001$), as well as a Group x Delay interaction (Wald’s $\chi^2 = 15.67; p < .001$). Post-hoc comparisons revealed greater LLR choice in DE, compared to IE, rats at both the 15- ($p < .001$) and 30-s ($p < .05$) delays.

Choice in DE rats varied substantially, with interquartile ranges of 5.16-100% and 0.51-98.46% LLR choice at 15- and 30-s LLR delays, respectively. In contrast, choice in IE rats varied little; interquartile ranges were 0-6.12% and 0-1.65% LLR choice at 15- and 30-s LLR delays, respectively.

The upper two panels of Figure 4-4B depict rear-lever response latency (s) and percent trials omitted across LLR delays. For both measures, results revealed a main effect of delay (in both cases, Wald’s $\chi^2 > 15.15; p < .001$), but no main effect of group (in
Figure 4-4. Percent LLR choice across delays in the impulsive-choice retest (Panel A); rear-lever latency and percent trials omitted per session, and time in the food receptacle and $C_n$ per trial, across LLR delays in the impulsive-choice retest (Panel B); and percent LLR choice at the 15- and 30-s delays in the impulsive-choice test and retest (Panel C). Gray lines indicate data for individual subjects. Where specified, panels reflect median (± interquartile range) observed values; all other panels reflect mean (± SEM) observed values. Data points have been slightly displaced on the x-axis, for clarity. Significantly different than IE rats: *$p < .05$, ***$p < .001$. 
both cases, Wald’s $\chi^2 < 0.88$; NS) or Group x Delay interaction (in both cases, Wald’s $\chi^2 < 1.64$; NS). As in the initial impulsive-choice test, LLR choice did not significantly correlate with measures of rear-lever latency or percent trials omitted at either the 15- or 30-s delay.

The lower two panels of Figure 4-4B depicts time in the receptacle and $C_n$ across LLR delays. For time in the receptacle, results of GEE revealed main effects of group (Wald’s $\chi^2 = 5.04; p < .05$) and delay (Wald’s $\chi^2 = 140.25; p < .001$), as well as a Group x Delay interaction (Wald’s $\chi^2 = 7.14; p < .01$). Post-hoc comparisons revealed that DE rats spent more time in the receptacle than IE at the 30-s delay ($p < .05$), but not at the 15-s delay.

When $C_n$ was examined, results of GEE revealed a main effect of group (Wald’s $\chi^2 = 11.32; p < .01$). While there was no main effect of delay (Wald’s $\chi^2 = .31$; NS), a Group x Delay interaction was observed (Wald’s $\chi^2 = 5.99; p < .05$). Post-hoc comparisons revealed larger $C_n$ values in DE rats at the 15-s delay ($p < .01$), but not at the 30-s delay.

Table 4-2 provides correlations between LLR choice and measures of time in the receptacle and $C_n$ in the impulsive-choice retest. For time in the receptacle, a significant positive correlation was observed at the 30-, but not 15-s, delay in DE rats. In contrast, no significant correlations were observed in IE rats. For $C_n$, a significant negative correlation was observed at the 30-, but not 15-s, delay in DE rats. Again, neither of these correlations was significant in IE rats.

Figure 4-4C depicts group and individual-subject comparisons between LLR
choice in the impulsive-choice test and retest (for space, 0-s delays are not depicted).

When test and retest were compared, there was no main effect of test (Wald’s $\chi^2 = 1.33$; NS); however, a Group x Test interaction was observed (Wald’s $\chi^2 = 5.24; p < .05$); specifically, post-hoc comparisons revealed a reduction in LLR choice (collapsed across delay) at the retest in DE rats ($p < .05$), but not in IE rats. No other two- or three-way interactions were observed (in both cases, Wald’s $\chi^2 < 4.47$; NS) and interim completion of either alcohol or sucrose testing was not a significant covariate (Wald’s $\chi^2 > 0.19$; NS).

Despite individual-subject changes in choice depicted in Figure 4-4C, LLR choice was strongly correlated across tests at both the 15-s delay (DE: $rho = .85$; IE: $rho = .69$; $ps < .001$) and 30-s delay (DE: $rho = .93$; IE: $rho = .80$; $ps < .001$).

**Discussion**

The results of the present study reproduce and extend previously reported effects of delay pre-exposure on impulsive choice (Chapter 2), demonstrating greater LLR choice in DE than IE rats at a 15-s LLR delay and generalization of this effect to a longer, 30-s LLR delay. Effects of delay pre-exposure were generally robust across time and intervening experience, as differences in choice between DE and IE rats were also observed in the impulsive-choice retest (more than two months following the initial test); however, a significant decline in LLR choice (collapsed across delays) was observed in DE rats between tests.

We observed no evidence of differential early or chronic stress exposure in DE compared to IE rats in the majority of measures in the open-field test (e.g., increased defecation, reduced center exploration; Colorado et al., 2006; Katz et al., 1981). Although
DE rats were less active than IE rats, motor activity was unrelated in correlational analyses to either impulsive choice or alcohol and sucrose consumption in either DE or IE rats. Thus, between-group differences again appear to be an effect of delay pre-exposure unrelated to stress. These data provide little evidence of a role of stress in the data reported in Chapter 2.

Rats in the DE and IE groups consumed comparable amounts of both alcohol and sucrose. However, a significant Group x Session Block interaction was observed on sucrose consumption, the source of which appears to be greater initial acceptance of sucrose in DE compared to IE rats in the first two session blocks, with consumption converging between groups by the three latter session blocks.

**Delay Pre-Exposure and Impulsive Choice**

Observed effects of delay pre-exposure on impulsive choice were heterogeneous despite significant group differences, as choice in a substantial proportion of DE rats failed to respond to treatment in the impulsive-choice test (approximately one third) and retest (one half). Regarding this heterogeneity, no clear predictors of treatment response were evident in open-field behavior (center entries, motor activity, and defecation) or response latency and trial omissions in the impulsive-choice tests. Total time spent in the food receptacle and the temporal precision of intra-delay receptacle visits ($C_n$) in the initial impulsive-choice tests were positively and negatively correlated, respectively, with LLR choice in DE rats. When group levels of these intra-delay behaviors were considered, DE rats spent significantly more time in the receptacle than IE rats during the 30-s (but not 15-s) LLR delay in both impulsive-choice tests. Rats in the DE group also
showed greater temporal precision of intra-delay receptacle visits at the 15-s (but not 30-s) LLR delay in the impulsive-choice retest, despite an otherwise inverse relation between $C_n$ and LLR choice within individual groups. Generally, imperfect covariance between group differences in impulsive choice and intra-delay behavior (across delays and tests) indicates that, while these measures may be related, they are to some extent dissociable. Thus, while delay pre-exposure increased time spent in the receptacle and $C_n$ (possible measures of mediating and timing behavior, respectively), neither of these measures likely reflects a primary mechanism underlying observed effects on impulsive choice; rather, such changes appear to be ancillary effects of delay pre-exposure.

Despite reproducing and extending the effects of delay pre-exposure on impulsive choice reported in Chapter 2, the present chapter yielded little indication of potential mechanism(s) underlying these effects. Because the relation between impulsive choice and LLR delay in psychophysical titration tasks conforms well to hyperbola-like equations in both humans and nonhumans (Mazur, 1987; Rachlin, 1989), it is thought that common behavioral processes underlie choice across species. However, some have recently challenged this notion, suggesting species-specific sources of behavioral control (Blanchard, Pearson, & Hayden, 2013; Killeen, 2011). As such, an understanding of the mechanism(s) by which delay pre-exposure exerts its effects should prove useful in elucidating one or more component processes involved in nonhuman impulsive choice. In this section, a number of candidate mechanisms (including testable predictions) will be considered.

As Killeen (2011) suggested, nonhuman impulsive choice may be a product of
differential associability across LLRs and SSRs. That is, intervening delay may weaken memory of the response that produced the LLR, thereby poorly establishing the response-reward association and producing relative SSR preference. In this way, the considerable number of trials during delay pre-exposure (over 11,000 programmed in the present study) may asymptotically strengthen the association between a response and delayed reward, thus increasing LLR choice. Future research may be designed to explore this potential role of associability in delay pre-exposure by examining whether DE rats show faster response acquisition than IE rats during trace conditioning (in which CS and US are temporally distant; e.g., Raybuck & Lattal, 2011).

Delay pre-exposure may also exert its effects on impulsive choice by altering timing processes. Although causal relations have yet to be established, prior human and nonhuman data demonstrate an association between impulsive choice and poor performance in timing tasks (e.g., Baumann & Odum, 2012; Marshall, Smith, & Kirkpatrick, 2014; but also see Galtress, Garcia, & Kirkpatrick, 2012). In the abstract, if delay pre-exposure produces changes in the subjective estimation of delay length, then such changes may alter the relative value of LLRs. However, only limited evidence of group differences in our measure of timing (i.e., greater $C_n$ values in DE, compared to IE, rats at the 15-s LLR delay only in the impulsive-choice retest), was observed. Moreover, $C_n$ values were negatively related to LLR choice in DE rats—the opposite of what one would predict if timing improvements were a mechanism underlying effects of delay pre-exposure on impulsive choice. We note, however, the degree to which our $C_n$ measure corresponds to validated timing measures is unclear. Future research may be designed to
examine the effects of delay pre-exposure on timing speed or precision in peak-interval or temporal bisection tasks (Catania, 1970).

Finally, in contrast with the accounts described above, a seemingly simple explanation for the present study’s effects on impulsive choice may involve habituation to the aversive properties of delay during pre-exposure sessions. Despite this account’s apparent parsimony, however, it is the most ambiguous. Reward delay in operant paradigms can be considered aversive from a behaviorally functional view (Perone, 2003), as delay is a response-contingent stimulus (albeit temporally diffuse) that serves to suppress behavior (e.g., response rate; Jarmolowicz & Lattal, 2013). However, appealing solely to this behaviorally functional definition is inadequate, as a putative role of habituation in delay pre-exposure yields predictions identical to those from all other accounts described above—for example, gradual reductions in response latency during, and decreased impulsive choice following, delay pre-exposure. A unique prediction for future research, however, is that a longitudinal assay of the stress hormone, corticosterone, should reveal declining evidence of stress over delay pre-exposure sessions.

The considerations above highlight some of the potential complexities in nonhuman impulsive choice. Identification of functional mechanisms underlying delay pre-exposure’s effects may provide important clues regarding component processes in impulsive choice. In this way, experiential variables like delay pre-exposure may be used as an experimental tool similar to pre-session drug administration in behavioral pharmacology (e.g., use of anxiolytic drugs to examine the role of anxiety in
experimental paradigms). We note, however, that the varied behavioral effects of pharmacological treatment (e.g., on the reinforcing efficacy of food, discrimination of reinforcer contingencies, or motor behavior; Chu et al., 2014; Johnson, Stein, Smits, & Madden, 2013; Hoffman & Benninger, 1985) often make it difficult to attribute changes in behavior to precise mechanisms. In the abstract, delay pre-exposure may recruit fewer of these nuisance variables and would therefore be better suited for use in experimentation. However, additional study is required.

Observed heterogeneity in DE rats’ LLR choice in the present study, while suboptimal from a therapeutic view, provides an interesting experimental advantage. Identification of one or more variables (behavioral or biological) that distinguish treatment responders from non-responders in future research may prove important in identifying the mechanism(s) by which delay pre-exposure exerts its effects. With this in mind, one limitation of the present study is that baseline levels of impulsive choice were not established prior to delay pre-exposure. In a recent paper, Bickel, Landes, Kurth-Nelson, & Redish (2014) reviewed five prior human data sets, finding evidence for rate dependence in treatment effects on impulsive choice. That is, the degree to which impulsive choice responded to treatment variables depended on baseline impulsive choice, with particularly impulsive participants responding the most to treatment and less impulsive participants responding very little or not at all. Given this finding, knowledge of baseline impulsive choice may prove useful in future studies on delay pre-exposure, as a similar (or inverse) form of rate dependence may have been operating in the present data. If the present methodology is to inform human research on impulsive choice and
substance-use disorders (e.g., by modeling treatment or causal relations), its 
phenomenology should resemble that observed in humans.

Finally, one limitation of the present study may be the absence of a true control 
group, as pre-exposure to immediate rewards may have served as an independent variable 
in its own right (e.g., increasing impulsive choice). We note, however, that impulsive 
choice in experimentally naïve rats in Chapter 2 (N = 92) differs minimally from that 
observed in IE rats in the present study. Thus, pre-exposure to delayed (as opposed to 
immediate) rewards appears to produce the largest proportion of observed effects on 
impulsive choice.

Future research should also be designed to examine the effects of delay pre-
exposure across a variety of impulsive-choice tasks, such as the adjusting-delay task (in 
which choice titrates LLR delay; Mazur, 1987) or adjusting-amount task (in which choice 
titrates SSR amount; Richards, Mitchell, de Wit, & Seiden, 1997). Use of these 
alternative tasks would determine whether delay pre-exposure produces a generalized, 
trait-like reduction in impulsive choice or whether our effects were specific to the 
parameters of the increasing-delay task used here. With this in mind, a recent study in our 
lab demonstrated within-subject correspondence in naïve rats between impulsive choice 
in the adjusting- and increasing-delay tasks (rho = .71; Craig, Maxfield, Stein, Renda, & 
Madden, 2014); however, whether these tasks yield comparable measures following 
experimental manipulation of impulsive choice has yet to be determined. Generality of 
findings across task type would increase the degree to which delay pre-exposure informs 
our understanding of impulsive choice.
**Delay Pre-Exposure and Alcohol Consumption**

Despite significantly reducing impulsive choice in the present study, delay pre-exposure produced no effect on consumption of 12% alcohol. This contrasts with prior data from Chapter 2, in which delay pre-exposure produced significantly greater consumption of 12% alcohol following initial exposure to 3% and 6% alcohol. Because the 12% concentration was introduced alone in the present study, it is possible that the effect of pre-exposure on alcohol consumption reported in Chapter 2 depended on gradual introduction of alcohol. Across species, self-report and electrophysiological data demonstrate that alcohol possesses both sweet and bitter taste components (e.g., Hoopman, Birch, Serghat, Portmann, & Mathlouthi, 1993; Lanier, Hayes, & Duffy, 2005; Lemon, Brasser, & Smith, 2004; Settle, 1979). Particularly relevant to Chapter 2’s data, alcohol’s sweet component and subjective ratings of pleasantness are more pronounced at low compared to high concentrations, whereas the opposite is true for alcohol’s bitter component (e.g., Hoopman et al., 1993; Scinska et al., 2000). Moreover, sucrose or saccharin preference correlates positively with alcohol consumption across species (e.g., Gosnell & Krahn, 1992; Kampov-Polevoy, Overstreet, Rezvani, & Janowsky, 1995; for review, see Kampov-Polevoy, Garbutt, & Janowsky, 1999), suggesting common sources of control. That said, no overall group differences in sucrose consumption were observed in the present study. However, the significant Group x Session Block interaction observed on sucrose consumption indicates at least some effect of delay pre-exposure on preference for sweet substances. Whereas a relatively high (and perhaps asymptotically rewarding) 21% sucrose concentration was used in the present study, future research may
employ lower concentrations to increase sensitivity to group differences in consumption. Although DE rats were more active in the open-field test than IE rats, motor activity was unrelated to alcohol or sucrose consumption in correlational analyses. Together with the absence of group differences in other open-field measures (center entries, defecation), these data suggest a minimal role of stress in Chapter 2’s report of greater alcohol consumption in DE compared to IE rats. However, this conclusion relies on open-field behavior as a proxy measure for prior stress exposure. Future studies may avoid this potential limitation through, as mentioned previously, use of direct measurement of stress hormone, corticosterone.

Beyond the data reported here, recent findings and a closer examination of the literature call into question the robustness of the baseline relation between naturally occurring impulsive choice and alcohol consumption in rodents (i.e., in the absence of delay pre-exposure). The primary evidence for a relation between these variables comes from Poulos et al. (1995), who reported that impulsive choice in outbred rats predicted greater consumption of 12% alcohol. However, subsequent studies failed to find similar predictive relations (Diergaarde, van Mourik, Pattij, Schoffelmeer, & de Vries, 2012; Chapter 2, this document). While additional work demonstrates that impulsive choice co-variates with selective breeding for alcohol consumption or seeking (i.e., in a direction similar to that reported by Poulos et al.; Beckwith & Czachowski, 2014; Oberlin & Grahame, 2009; Wilhelm & Mitchell, 2008), some demonstrates no relation (Beckwith & Czachowski, 2014), or the opposite relation (Wilhelm, Reeves, Phillips, & Mitchell, 2007) between these variables. These inconsistencies diminish the utility of examining
alcohol consumption in rodent models when attempting to understand the relation between experimental manipulation of impulsive choice and subsequent drug self-administration. In contrast, the relations between impulsive choice and self-administration of psychostimulant drugs such as cocaine (e.g., Anker, Perry, Gliddon, & Carroll, 2009; Koffarnus & Woods, 2013; Perry et al., 2005; Perry, Nelson, & Carroll, 2008), nicotine (e.g., Diergaarde et al., 2008), or methylphenidate (Marusich & Bardo, 2009), appear to be more consistent than those reported for alcohol. With this in mind, future research may be designed to examine the effects of delay pre-exposure on self-administration of these psychostimulant drugs across a range of experimental phases (e.g., acquisition, maintenance, extinction, and reinstatement).

Conclusions

Together with data from Chapter 2, the present data indicates that delay pre-exposure reliably reduces impulsive choice and that this effect generalizes to a delay longer than the one used during pre-exposure. In contrast, delay pre-exposure does not reliably affect alcohol consumption. Future research should be designed to investigate the mechanism(s) by which delay pre-exposure reduces impulsive choice, as well as the conditions under which delay pre-exposure impacts drug self-administration (e.g., drug type, behavioral measure).

References


vulnerability to distinct stages of nicotine seeking in rats. *Biological Psychiatry*, 63, 301-308.


Solute-solvent interactions and the sweet taste of small carbohydrates. Part II:

Jarmolowicz, D. P., & Lattal, K. A. (2013). Delayed reinforcement and fixed-ratio


Saccharin-induced increase in daily fluid intake as a predictor of voluntary

preference for sweets and excessive alcohol intake: A review of animal and

Katz, R. J., Roth, K. A., & Carroll, B. J. (1981). Acute and chronic stress effects on open

(2013). Working memory ability predicts trajectories of early alcohol use in

Killeen, P. R. (2011). Models of trace decay, eligibility for reinforcement, and delay of
reinforcement gradients, from exponential to hyperboloid. *Behavioural Processes, 87*, 57-63.


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Settle, R. G. (1979). The alcoholic's taste perception of alcohol: Preliminary
findings. *Currents in Alcoholism, 5*, 257-267.


CHAPTER 5
GENERAL DISCUSSION

Summary and Conclusions

In Chapter 2, prolonged pre-exposure to delayed rewards reduced impulsive choice in male Long-Evans rats. However, this reduction in impulsive choice was accompanied by increased consumption of 12% wt/vol alcohol, counter to the naturally occurring relation between these variables sometimes reported elsewhere (e.g., Poulos, Le, and Parker, 1995). However, this finding may have been dependent on gradual introduction of alcohol concentration and related recruitment of non-pharmacological motivational variables (e.g., taste). To aid in interpretation of data from Chapter 2, in Chapters 3 and 4 we examined naturally occurring and experimentally induced relations between impulsive choice and consumption of a single alcohol concentration (12%). In both cases, no relation between impulsive choice and alcohol consumption was observed.

Combined with previous findings (e.g., Beckwith & Czachowski, 2014; Diergaarde, van Mourik, Y., Pattij, Schoffelmeer, & De Vries, 2012; Wilhelm, Reeves, Phillips, & Mitchell, 2007), the findings reported here suggest the relation between impulsive choice and rodent alcohol consumption is not as robust as once thought. Inconsistent relations between these variables suggest the study of rodent models of alcohol self-administration are of limited utility in understanding the otherwise robust relation between impulsive choice and human alcohol dependence (see MacKillop et al., 2011).
Despite these limited effects, however, pre-exposure to delayed rewards in Chapters 2 and 4 produced reductions in impulsive choice that were robust against both time and intervening experience (Chapters 2 and 4). These findings extend previous work on similar variables known to reduce nonhuman impulsive choice (Eisenberger, Masterson, & Lowman, 1982; Mazur & Logue, 1978) and provide a methodology to be used in future work. Specifically, the relation between impulsive choice and self-administration of psychostimulant drugs, such as cocaine, nicotine, or methylphenidate appears reliable across varying rat strains and experimental paradigms (e.g., acquisition and escalation of self-administration, as well as behavioral-economic demand; e.g., Koffarnus & Woods, 2013; Perry, Larson, German, Madden, & Carroll, 2005; Perry, Nelson, & Carroll, 2008; for review, see Stein & Madden, 2013). Thus, against this clearer naturally occurring baseline, pre-exposure to delayed rewards may be used to examine potentially causal relations between impulsive choice and self-administration of psychostimulants. Such investigations may facilitate basic understanding and development of behavioral treatments for human drug dependence.

References


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*Invited Talks

Poster presentations


13. Johnson, P. J., Brewer, A. T., Stein, J. S., Francisco, M. T., & Madden, G. J. (May,
2009). Effects of pramipexole on choice for differential rewards using a within-session increasing delay procedure (May, 2009). Poster presented at the annual meeting of the Association for Behavior Analysis, Phoenix, AZ.


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REFERENCES

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