A NOVEL RISKY DECISION-MAKING TASK IN HIGH AND LOW ALCOHOL PREFERRING MICE

by

Claire R. Carron

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THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Nicholas J. Grahame, Chair
Department of Psychological Sciences
Dr. Cristine L. Czachowski
Department of Psychological Sciences
Dr. Christopher C. Lapish
Department of Psychological Sciences

Approved by:

Dr. Nicholas J. Grahame
Head of the Graduate Program

To all my family, friends, and dogs who show me unconditional love and support.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Author: Carron, Claire, R. MS
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Committee Chair: Nicholas J. Grahame

Deficits in impulse control and decision-making have been implicated in the development and maintenance of alcohol use disorders (AUDs). Individuals with AUD often make disadvantageous choices under conditions of probabilistic risk. The Iowa Gambling Task (IGT) is often used to measure risky decision-making, in which impaired individuals tend to favor large, infrequent rewards even when punished for these choices, rather than smaller, safer, and more advantageous rewards. It remains poorly understood if these deficits are behaviors under genetic control and if ethanol intoxication may alter decision-making. High and Low Alcohol Preferring (HAP3 and LAP3, respectively) mice were trained on a novel gambling task to investigate these possible influences. In Experiment 1, HAP3s and LAP3s responded for a 0.1% saccharin solution, choosing between a risky and a safe option. Importantly, choosing the risky option was meant to be ultimately disadvantageous. In Experiment 2, these same HAP3 mice responded for saccharin or saccharin plus 10% ethanol. Contrary to hypothesis, LAP3s preferred the risky option more than HAP3s. Alcohol increased preference for the risky lever, but only in male mice. HAP3 preference for the safe lever may be explained by higher motivation to obtain sweet rewards, or higher overall avidity for responding. Ethanol-induced changes in male risk behavior may be explained by higher androgen levels, but further investigation is required. Similarly, continued research is necessary to optimize a risky decision-making task for both lines, and thus investigate possible genetic differences in risk acceptance that correlate with differences in alcohol intake.

INTRODUCTION

Alcohol Use Disorder and Decision-Making Deficits

Alcohol abuse is a pervasive mental health issue, placing third in leading preventable causes of death in the United States, and costing the country an estimated $249 billion in 2010 (Mokdad et al., 2004; Sacks et al, 2015). One of the hallmarks of alcohol use disorder (AUD) is consumption of alcohol despite negative consequences (American Psychiatric Association, 2013). Individuals suffering from AUD make choices involving drinking that may initially be viewed as appealing, but are detrimental long-term, resulting in financial, social, and physical problems. Likely underlying these maladaptive behaviors are dysfunctional cognitive processes, including impaired decision-making and deficits in impulse control (Verdejo-García et al., 2006). There is evidence supporting these impaired cognitive processes as both trait- and state-dependent, and investigating these variables in a clearly defined manner is essential to improving our understanding of AUD risk (De Wit, 2009).

There are a variety of laboratory tasks used to investigate possible decision-making deficits in alcoholic populations. For example, delay discounting is commonly used to measure a dysfunctional preference for smaller, immediate rewards, such as alcohol intoxication, instead of larger delayed rewards. While delay discounting is a useful measure of decision-making with regards to waiting for more adaptive rewards, and people with substance use issues do consistently show deficits in this task (for one review see Reynolds, 2006), it does not include elements of uncertainty or punishment that are present in many real-life decisions. Probability discounting introduces an element of uncertainty to decision-making. In probability discounting tasks, instead of choosing between smaller/sooner and larger/later rewards, subjects decide between a small, but certain reward and a larger reward that occurs with varying probabilities. Many utilize probability discounting tasks to investigate decision-making with regards to risk, as they include these differing levels of certainty. However, a key piece of real-life decision-making that is still missing in these tasks is punishment. When a reward is not given in probability discounting tasks, the next trial begins and no punishment occurs. However, there may be a key difference in cognitive processing between decisions simply resulting in non-reward, and decisions resulting in actual

punishment. The Iowa Gambling Task (IGT) was developed by Bechara et al. (1994) to more closely model real-world decision-making by incorporating elements of loss and uncertainty.

The Iowa Gambling Task and AUD

In the IGT, individuals are instructed to choose from four different decks of cards, identical in appearance. Each choice results in a monetary gain or loss, and the ratio of gains to losses differs between decks. Two of the decks initially look more appealing due to larger rewards. However, continued selection of these "bad" decks results in monetary loss because they have larger, more frequent punishments. The other two decks, the "good" decks, give smaller monetary rewards on win trials, but are overall advantageous. Subjects are unaware of the contingencies of each deck, and thus must rely on intuitive decision-making processes to choose the most advantageous option. Impairments in the IGT are generally defined by inability to learn to avoid the "bad" decks over repeated trials. The task was designed to simulate decision-making situations that occur in real life, with elements of reward, punishment, and uncertainty. While the IGT was originally developed as a neuropsychological task to screen for damage to the ventromedial prefrontal cortex, impairments have been observed in a range of subjects, including those with AUD (see Kovács et al, 2017 for review and meta-analysis).

Bechara et al. (1994) hypothesized that impaired performance on the IGT is due to defective somatic marker mechanisms – emotional signals of the prospective consequences of an action (Damasio, 1994). The amygdala is critical for triggering somatic states in response to pleasurable or aversive stimuli. Once evoked, signals from these states lead to the formation of somatic state patterns by brainstem nuclei as well as insular and somatosensory cortices. When the stimuli are presented again, memories of the emotional state are elicited by these patterns. The ventromedial prefrontal cortex (VMPFC) seems to be the structure necessary for triggering somatic states from these memories as opposed to the stimuli themselves (Bechara & Damasio, 2005). In individuals with AUD, it is possible that a defect in this system causes the pleasurable somatic states induced by alcohol to be more salient than the aversive states elicited by the negative consequences resulting from alcohol use. Decision-making processes may dysfunctionally put greater emphasis on the immediate effects of the drug than the long-term consequences of its use (Verdejo-García et al., 2006). Whether this impairment is a result of state variables related to the effects of alcohol intoxication or trait variables such as genetic influences

(or some combination of the two) has yet to be determined, and animal models of this type of decision-making are essential to investigating these influences.

Evidence for Trait and State Contributions of Risky Decision-Making

There is substantial evidence that there is a genetic, or trait-specific, component to AUD, with family history of alcoholism contributing to about 50-60% of risk for developing the disorder (McGue, 1999). More specifically, risky decision-making in AUD, such as that observed in the IGT, may be subject to genetic influences. Using a different rodent model of decision-making under risk, Ashenhurst, Seaman, and Jentsch (2012) found that about 55% of the variance in risk-taking behavior was attributable to heritable factors, and a similar finding was observed in adolescent, human males (Anokkhin et al., 2009). Effects of a family history of substance abuse on IGT performance have also been investigated. O'Brien, Lichenstein, and Hill (2013) found that individuals with a family history of substance use disorders exhibited overall impaired performance on the IGT, with significantly poorer performance on the final block of trials, suggesting failures to improve decision-making strategies following repeated task experience. Individuals with a family history of alcoholism also show greater activation of brain regions such as the left dorsal anterior cingulate cortex and caudate nucleus than those with a negative family history of the disorder, suggesting that the neural processes involved in performing the task are impaired in these individuals (Acheson et al., 2009). Evidence points to genetic influences on decision-making in the IGT, but further research regarding these mechanisms is necessary.

Along with possible trait-specific genetic determinants of risky decision-making, there is also evidence of alcohol's state-specific effects. Alcohol intoxication contributes to increases in risk-taking behaviors such as drunk driving and high-risk sexual behaviors (for review see Corte & Sommers, 2005). Increases in risky behavior have also been observed experimentally. For example, Lane et al. (2004) administered different doses of alcohol to subjects performing a risk-taking task, and found dose-dependent increases in selection of risky options. Lyvers, Mathieson, and Edwards (2015) recruited subjects actively drinking at university bars and campus parties with breath alcohol concentrations (BrAC) ranging from .002% to .19%, and administered various assessments including the IGT. Even after controlling for regular alcohol use and trait impulsivity and disinhibition scores, BrACs predicted IGT performance such that individuals

with higher breath alcohol won less money in the task. These results suggest that alcohol intoxication has a negative effect on risky decision-making. Using intravenous alcohol infusions during functional magnetic resonance imaging, Gilman et al. (2012) demonstrated some of these neural effects. A blood alcohol concentration of approximately 0.070 g% increased risk-taking behaviors, and increased activation of the striatum in response to risky choices compared to placebo. Additionally, alcohol reduced response to notification of win and loss outcomes in areas like the thalamus and insula. Further investigation of these state-specific effects of alcohol on decision-making in the IGT is needed, and animal models provide a unique opportunity to do so.

Animal Models of Risky Decision-Making

Animal models of the IGT are immensely valuable for several reasons as described by de Visser et al. (2011). First, these models provide us with opportunities to study more precise mechanisms of decision-making than brain imaging with human studies alone, such as the role of specific brain regions, neurotransmitters, and neurodevelopmental events. Importantly, these animal models also allow for careful manipulation of genetic variation, drug exposure, and other environmental conditions. As such, rodent models of the IGT are an important tool for the investigation of the relationship between decision-making and AUD. Several variations of these models have been developed, ranging in their reward and punishment contingencies, apparatus, forms of reward and punishment, task duration and training procedures (de Visser et al., 2011). However, few studies have utilized rodent versions of the IGT to investigate alcohol-specific questions. In the proposed operant version of the IGT, mice will have choices between a "risky" and a "safe" lever, each with independent reward and time-out contingencies. Risky choices may initially be viewed as appealing due to longer reinforcer times, but are ultimately disadvantageous as they also result in longer, and more frequent time-out penalties. More specific session parameters have been piloted and are detailed in the figure below. Despite evidence suggesting genetic factors contribute to impaired performance on the IGT and impaired risky decision-making in populations with AUD, animal models of these genetic factors have yet to be utilized. Selectively bred high- and low-alcohol preferring (HAP and LAP) mice (Oberlin et al., 2011) provide a useful tool to investigate complex genetic differences in risky decision-making behaviors related to alcohol consumption.

The genetic contributions to AUD are complex, with multiple genes interacting with each other and with the environment. While other animal models such as inbred strains, transgenic mice, or genetic knockouts provide useful contributions to the genetic study of alcoholism, selective breeding provides its own unique perspective. By repeatedly mating heterogeneous animals that exhibit a desirable phenotype, the alleles contributing to this phenotype become fixed. Over time, selection can amplify the phenotype to extremes that may not occur naturally. HAP and LAP mice have been bred from heterogeneous HS/Ibg stock by selecting for high or low alcohol consumption in a two-bottle choice drinking procedure in which mice have 24-hour access to both water and unsweetened 10% ethanol. Over multiple generations, this has resulted in HAP mice that drink in excess of 25 g/kg of ethanol per day, and LAP mice that drink less than 1 g/kg/day (Oberlin et al., 2011). The fixation of the divergent alleles in these lines importantly allows us to investigate their relation to other behavioral phenotypes, such as risky decision-making.

Specific Hypotheses

If genetics related to alcohol-drinking behaviors play a role in impaired IGT performance, HAP mice will make riskier, and ultimately more disadvantageous, choices compared to LAP mice. HAP mice will voluntarily drink alcohol to intoxicating levels, allowing us to use alcohol as a reinforcer in an operant version of the task. Using alcohol as a reinforcer, I hypothesize that alcohol intake and intoxication will correlate with riskier choices.

METHODS

Subjects

A total of 36 LAP3 mice (18 male) and 24 HAP3 mice (11 male) were used in Experiment 1. The same 24 HAP3 mice were used for Experiment 2. All animals were single housed in standard Plexiglas cages with pine bedding and acclimated to a 12-hour reverse light cycle (lights off at 0700) at least 7 days prior to the first day of magazine training. To encourage responding for the liquid reinforcer in the operant box, mice were water deprived for 22 hours prior to operant conditioning. Mice received two hours of water access each day directly after operant training. Both experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Indiana University-Purdue University Indianapolis and conducted according to the NIH Guide for the Care and Use of Laboratory Animals.

Apparatus

Twelve operant chambers (Med Associates, St. Albans, VT) were used for the operant testing in this experiment. Each chamber measured 21.6 x 19.7 x 12.7 cm and was located inside a light- and sound-attenuating box. The operant boxes were equipped with two levers on each side of a center sipper-tube opening. Green lights were positioned above both levers. Additionally, a nose-poke hole with a green light was located above the sipper tube opening. Upon a correct response, the 10-mL sipper-tube containing the appropriate reinforcer descended into the chamber's opening. For Experiment 1, the reinforcer was 0.1% saccharin solution (S). For Experiment 2, the sipper contained either the saccharin solution or a solution of 10% ethanol mixed with 0.1% saccharin (E+S), depending on group assignment. Session duration, lever presses, nose-pokes, rewards, and time-outs were recorded using MED-PC IV software (Med Associates, St. Albans, VT).

Risky Decision-Making Pre-Training – Experiments 1 & 2

Prior to completing the risk task, mice went through approximately seven stages of operant pre-training, during which they responded for 0.1% saccharin solution (see Table 1). For

Stages 1-3, levers and their corresponding lights were removed from the operant box, leaving just the nose-poke hole and sipper opening. Stage 1 consisted of magazine training in one, 30-minute session. All nose-pokes were rewarded on a fixed ratio one (FR1) schedule with 20 seconds of sipper access. Additionally, regardless of nose-poke responses, 20 seconds of sipper access was presented every 2 minutes, shaping the mice to drink from the sipper tube. The criterion to advance from Stage 1 was drinking at least 0.2 mL from the sipper tube. The advancement criterion for all subsequent stages was completing at least 15 trials and consuming at least 0.2 mL in 30 minutes. For Stage 2, nose-pokes were reinforced with 10 seconds of sipper access on a FR1 schedule, and the nose-poke light remained lit during the 30-minute session. The same reinforcement schedule was used in Stage 3, with the addition of a 5-second intertrial interval (ITI). The ITI began following sipper tube retraction, and the nose-poke hole was inactive and the light was extinguished. Levers were returned to the operant box for Stage 4 and all subsequent stages. In this stage, mice were trained to chain a nose-poke response with a lever press. A nose-poke extinguished the center light and subsequently illuminated both lever lights. Responding on either lever resulted in a 5-second sipper access reward, followed by a 5-second ITI. Mice had an unlimited time after the nose-poke to lever press.

After four days of training on Stage 4, 75% of animals were completing the correct response chain and receiving reinforcement, but were not consuming the reinforcer. All equipment and programs were confirmed to be functioning properly, and mice completed an extra session starting with the sipper descended to ensure they had learned to drink following presentation of the sipper. Animals that had not met Stage 4 criterion were trained on two intermediate stages with the nose-poke removed. The first stage reinforced responses on either lever on an FR1 schedule with 5 seconds of sipper access. The second stage consisted of cued lever press training, with the right and left lever each illuminated and active 50% of the time. Criterion for advancement remained the same as all other training stages. After over 80% of mice had passed these intermediate stages, all animals advanced to Stage 5. This stage requires a center nose-poke to initiate each trial, followed by a cued lever press, in which the lever light above the right or left lever signaled which was active. After a nose-poke, the animal had 10 seconds to press the active lever. A correct response resulted in 5 seconds of sipper access, while failure to respond within 10 seconds was counted as an omission. Omissions triggered the start of the 5-second ITI, followed by initiation of a new trial. The final stage of training was identical to

Stage 5, except reward time was decreased to 1 second of sipper access. This stage was intended to train mice to reach the sipper quickly after completing a lever press, which would be necessary once the risk task began.

Risky Decision-Making Task

Parameters for the task differed between Experiment 1 and 2, but the general procedure remained the same for both (see Figure 1). The original parameters for this task were based on the Rat Gambling Task developed by Zeeb, Robbins, and Winstanley (2009) and a pilot study using HAP and LAP mice from the second replicate line. Results from this pilot study indicated choosing the risky option resulted in decreased sipper access time for both lines. Each 30-minute session began with illumination of the center nose-poke light. A response in the center nose-poke within 10 seconds initiated the trial by extinguishing the center light and illuminating both lever lights. Failure to respond within 10 seconds resulted in the trial being scored as an omission. Following an omission, the center light extinguished, and a new trial began after the 5-second ITI. A response on either lever extinguished both lever lights and resulted in either a reward of sipper access or a time-out punishment with the house light illuminated. A 5-second ITI followed the reward or punishment, after which the nose-poke was illuminated and a new trial began. To prevent perseverative behavior, after 3 consecutive choices on the same lever, a forced choice on the opposite lever was presented. During these trials, the light above the forced lever remained illuminated until the animal responded on this lever. After the proper response, the lever press would be rewarded or punished following the same contingencies, followed by the 5-second ITI and initiation of a new choice trial.

The two levers differed in both probability and duration of rewards and time-outs. The "risky" lever, similar to the "bad" decks of the IGT, frequently resulted in long time-out punishments, but infrequently resulted in long sipper rewards. Responding on the "safe" lever, similar to the "good" decks of the IGT, often resulted in smaller rewards of short sipper access times. Time-outs resulting from selecting the safe lever were shorter and less frequent.

Experiment 1

In Experiment 1, HAP3 and LAP3 mice all responded for 0.1% saccharin. Animals were counterbalanced for family, sex, and left or right risk lever assignment. After pre-training, mice

completed 11 days of the risk task with the risky lever being rewarded 30% of the time with 6 seconds of saccharin access and punished 70% of the time with an 80-second time-out. The safe lever was rewarded 80% of the time with 0.5 seconds of saccharin access and punished 20% of the time with a 10-second time-out. Including rewards from forced-choice trials, a mouse singularly choosing the risky lever would be almost twice as advantageous as choosing the safe lever exclusively. However, pilot data from the second replicate line of HAP and LAP mice suggested these parameters resulted in the risky option being disadvantages. Yet, after the first 11 days it was determined that the risky option was ultimately advantageous for both lines (Figure 2). That is, the riskier an animal's behavior, the greater their intake. As such, parameters were altered to decrease the reward of the risky option while increasing it for the safe option, in hopes that this would result in a negative relationship between risk preference and intake. For the remainder of the task, the risky lever was rewarded 20% of the time with only 4 seconds of saccharin access, and punished 80% of the time with the same 80-second time-out. The safe lever was still rewarded and punished with the same probabilities, but the reward was increased to 1-second saccharin access. With these updated parameters, exclusively choosing the risky or safe option would be equally advantageous, but the updated regressions indicated the risky option was now disadvantageous. The mice completed 11 additional 30-minute sessions using the updated parameters.

Experiment 2

The parameters used in Experiment 1 resulted in the HAP3s nearing a 0% risk preference. As the same animals were to be used in Experiment 2, and the aim of this experiment was to detect an effect of ethanol in either direction, parameters were changed to increase HAP risk preference from about 0%, hitting floor. The probability of reward and punishment for the risky lever was thus changed to 0.5. The 24 HAPs were counterbalanced based on family, sex, left or right risk lever assignment, and order of S/E+S presentation. Half of the animals responded for E+S for five days and switched to S for the remaining five, while the other half was given the opposite reinforcer order. Following the 10 days of testing, all HAPs were given 2 additional days of responding for E+S to ensure they adapted to the ethanol reinforcer. After these additional days, all HAPs completed an additional 30-minute session responding for E+S. Immediately following session completion, retro-orbital blood samples were taken using 25*u*l

heparinized capillary tubes. Samples were centrifuged to separate plasma, which was stored at -20°C until analysis. Plasma ethanol concentrations (BECs) were assessed using gas chromatography as described in Lumeng et al. (1982).

Statistical Analysis

Data were analyzed using SPSS software (SPSS, Version 25, Chicago, IL) and graphed and analyzed with GraphPad Prism software (GraphPad Prism, v. 7.0b, La Jolla, CA). All significance α-values were set at 0.05 unless otherwise stated. All risk preference values were calculated by dividing the total number of choice risk trials by the total number of choice trials for each session. Mice would be removed from risk analysis if their choice lever presses totaled fewer than 10, as this may improperly represent the animal's true risk preference. Fluid intake per second was calculated by dividing the total seconds of access for each 30-minute session by intake for that session. Free choice intake values were then calculated by multiplying each mouse's intake per second by seconds of sipper access granted from only free choice trials. Theoretical values (T) for complete preference of the safe lever were obtained with the formula:

$$T_{safe} = P_{risk}*D_{risk}*0.25 + P_{safe}*D_{safe}*0.75$$

where P is the probability of reward and D is the duration of the reward. The choice lever was multiplied by 0.75 and the opposite lever by 0.25 due to forced-choice trials. Every time the same lever was pressed 3 times, the opposite lever was required to be pressed the fourth. When the safe lever is assumed to be chosen for all free-choice trials, the risky lever would thus have been pressed 25% of the time. Theoretical values for the risky lever were obtained with a similar formula:

$$T_{risk} = P_{risk}*D_{risk}*0.75 + P_{safe}*D_{safe}*0.25$$

Regressions were carried out using general linear regression after confirming residuals were normally distributed. To evaluate possible differences between HAP3s and LAP3s, the median for each dependent variable was calculated across sessions and analyzed using a non-parametric Mann-Whitney U test. Non-parametric Friedman's tests were used to assess changes across sessions for each line separately. For Experiment 2, Mann-Whitney U tests ruled out order effects. As in Experiment 1, median values across sessions were used in Mann-Whitney U tests to investigate differences between reinforcers, and across-session effects were analyzed via Friedman's tests.

RESULTS

Experiment 1

All HAP3 mice completed each stage of training. A total of 17 LAP3 mice did not complete pre-training: four mice were removed for failure to respond after seven days of nose-poke training, when over 90% of mice had advanced. Nine mice were removed for failure to respond after nine sessions of intermediate training, including five sessions during which the levers were baited with small strips of saccharin-soaked paper towels, used to encourage interaction with the levers. Finally, four mice were removed for failure to respond after twelve sessions of training on Stage 5, when over 90% of mice had advanced.

One LAP was removed from all analyses for failure to respond during testing. Thus, 24 HAP3s and 18 LAP3s completed the task. The first set of parameters for the risky and safe levers used in sessions 1-11 was chosen based on previous results using the second replicate line of HAP and LAP mice. When these sipper times were calculated, choosing the safe option was disadvantageous compared to choosing the risky option. This was confirmed when the intake from free-choice trials was averaged across sessions and regressed against risk preference; the riskier animals were achieving greater intakes than those choosing safer options (Figure 2A). Regressions were split by line to account for baseline differences in intake (discussed below). Two HAP outliers and one LAP outlier were removed from regression analyses because their residuals fell more than 2.5 standard deviations outside the mean. Averaged LAP risk preference significantly predicted averaged session intake from free-choice trials, $b = 0.265$, $t(17) = 5.758$, $p < 0.001$. LAP risk preference also explained a significant proportion of the variance in free-choice intake, $R^2 = 0.674$, $F(1,17) = 33.155$, $p < 0.001$ (Figure 2A). HAP risk preference also predicted averaged session intake from free-choice trials, $b = 0.231$, $t(21) = 4.365$, $p < 0.001$. HAP risk preference also predicted a significant proportion of the variance in free-choice intake, $R^2 = 0.488$, $F(1,21) = 19.054$, $p < 0.001$ (Figure 2A). The slopes of the HAP and LAP regressions do not differ, $F(1,36) = 0.230$, $p = 0.634$, suggesting the risky option is equally advantageous for both lines. However, the Y-intercepts of the regression lines were different, signifying a baseline intake difference between lines, $F(1,37) = 99.360$, $p < 0.001$. Theoretical calculations using reward access times and probabilities from risk and safe choices demonstrate

choosing the risky lever should be disadvantageous. However, when empirical intake data are analyzed, choosing the risky option was advantageous for both lines.

Because the risky option was meant to be disadvantageous, the parameters were modified to increase safe reward and decrease risk reward, and an additional 11 days of the task were completed. According to the calculated theoretical maximums, choosing the risky option and safe option should have been equally advantageous. However, when the averaged intake from choice trials and averaged risk preference were regressed against one another, risk preference was negatively related to intake from choice trials for LAP3 mice, b = -0.226, t(17) = -3.449, p = 0.003. A negative relationship between risk preference and choice trial intake was also found for HAP3s, b = -0.432, t(21) = -3.386, p = 0.003 (Figure 2B). Again, LAP3 risk preference also predicted a significant proportion of the variance in free-choice intake, R^2 = 0.426, F(1,17) = 11.8974, p = 0.003, as did risk preference for HAP3s, R^2 = 0.364, F(1,21) = 11.462, p = 0.003. While theoretical calculations suggest a positive relationship between risk preference and sipper access time, the intake data show a significant negative relationship.

Preference for the risky option was advantageous for both lines in sessions 1 to 11. However, risk preference clearly differed between lines. Five LAPs were excluded from risk preference analysis for consistently failing to complete at least 10 free-choice trials within a 30-minute session. Using the median risk preference of sessions 1 through 11, a Mann-Whitney test indicated LAP3 mice had a higher preference for the risky option than HAP3 mice, U = 115, p = 0.024 (Figure 3A). There was no effect of sex in either line for sessions 1-11 or 12-22, ps > 0.05. A non-parametric Friedman's test revealed that LAP3 mice did not significantly change their risk preference across the 11 sessions, $X^2(10)$ = 15.749, p = 0.107 (Figure 3B). However, HAP3 risk preference decreased across sessions, $X^2(10)$ = 31.000, p = 0.001 (Figure 3B). Differences in risk preference between the lines were even more pronounced with the changed parameters in sessions 12 through 22. A non-parametric Mann-Whitney test comparing median risk preference across sessions again indicated LAP3s preferred the risky option more than HAP3s, U = 83.000, p < 0.001 (Figure 3C). With the parameters updated, a Friedman's test revealed LAP3 risk preference decreased across sessions, $X^2(10)$ = 81.388, p < 0.001 (Figure 3D). HAP3 risk preference also declined, $X^2(10)$ = 45.979, p < 0.001 (Figure 3D). I hypothesized that HAP3 mice would exhibit riskier behavior, but LAP3 mice consistently preferred the risky lever to a

greater extent than HAP3s, although both lines' preference decreased over time, indicating an ability to learn the long-term advantage of choosing the safe lever as the task progressed.

Fluid intake and completed free-choice trials were examined to further explore contributions to differential task performance between lines. To evaluate differential fluid intake between lines, the median intake in mL was calculated for each animal. A non-parametric Mann-Whitney test revealed that HAP3s drank significantly more fluid than their LAP3 counterparts in sessions 1 through 11, $U = 9.000$, $p < 0.001$ (Figure 4A), and in sessions 12 to 22, $U = 6.500$, $p < 0.001$ (Figure 4B). Mann Whitney tests revealed no sex differences for LAP3s for either parameter set, ps > 0.05, but female HAP3 mice drank more than male HAP3 mice in sessions 1-11, $U = 36.500$, $p = 0.041$. To evaluate if the individual lines' intakes changed across sessions, non-parametric Friedman's tests were used. HAP3 intake both differed across sessions 1-11, $X^2(10) = 65.096$, $p < 0.001$, and across sessions 12-22, $X^2(10) = 43.540$, $p < 0.001$. LAP3 intake did not differ across sessions 1-11, $p = 0.122$, but did differ across sessions 12-22, $X^2(10) = 35.815$, $p < 0.001$. The tendency of HAP mice to drink more fluid may suggest increased motivation and overall eagerness to respond.

Along with fluid intake, total free-choice trials completed were compared between lines and across sessions. Similar to what was seen with intake, a Mann-Whitney test confirmed HAP3s completed more free-choice trials in sessions 1 to 11, $U = 32.000$, $p < 0.001$ (Figure 5A), and in sessions 12 to 22, $U = 6.500$, $p < 0.001$ (Figure 5B). There was no effect of sex on completed free-choice trials for either line, ps > 0.05. Non-parametric Friedman's tests were used for each line to determine if the number of free-choice trials completed changed across sessions. In sessions 1-11, free-choice trials differed across sessions for LAP3s, $X^2(10) = 38.418$, $p < 0.00$, and HAP3s, $X^2(10) = 71.044$, $p < 0.001$. Similarly, in sessions 12-22 with the parameters changed, free choice trials differed across sessions for LAP3 mice, $X^2(10) = 78.450$, $p < 0.001$, as well as HAP3 mice, $X^2(10) = 68.106$, $p < 0.001$. As with total fluid intake, HAP3's propensity to complete more trials than LAP3s suggests an overall behavioral avidity that may be related to alcohol consumption.

Experiment 2

Parameters for the risky and safe levers were updated for Experiment 2 to ensure the HAP3 mice that were nearing 0% risk preference would increase their preference for the risky

option. With risk preference closer to 50%, either a positive or a negative effect of ethanol on risk preference could be detected. Non-parametric Mann-Whitney tests were used to confirm that no order effects were present for S or E+S with median risk preference, intake, and completed trials, *p*s > 0.05. Regressions were split by reinforcer type. When risk preference was averaged across sessions, it significantly predicted a positive relationship with choice trial intake for ethanol plus saccharin, $b = 0.378$, $t(23) = 9.355$, $p < 0.001$, and for saccharin alone, $b = 1.086$, $t(23) = 5.781$ $p < 0.001$. Ethanol plus saccharin risk preference also explained a significant proportion of the variance in free-choice intake, $R^2 = 0.799$, $F(1,23) = 87.523$, $p < 0.001$, as was the case with saccharin alone, $R^2 = 0.603$, $F(1,23) = 33.418$, $p < 0.001$. The slopes of the S regression and the E+S regression did not significantly differ, $F(1,44) = 0.025$, $p = 0.875$ (Figure 6A), suggesting that ethanol does not change how advantageous or disadvantageous risky decisions are in regards to intake. However, baseline intake did differ between E+S and S, as evidenced by different Y intercepts, $F(1,45)= 68.48$, $p<0.001$.

To examine if reinforcer type had an effect on preference for the risky option, the median risk preferences for E+S and S were taken for 5 days and analyzed using a non-parametric Wilcoxon Signed Ranks test. There was no difference in median risk preference between E+S and S, $Z = -1.486$, $p = 0.137$. However, when split by sex with a Bonferroni adjusted alpha level of 0.025, males showed a higher preference for the risky option when reinforced with E+S than when working for S alone, $Z = -2.491$, $p = 0.013$, while females did not differ in their preference for the risky option between reinforcers, $Z = -0.420$, $p = 0.675$ (Figure 6B). A non-parametric Mann-Whitney U test was used to compare median g/kg ethanol intake between sexes, and females achieved significantly higher median g/kg intake than males, $U = 28.000$, $p = 0.012$. Non-parametric Friedman's tests showed no difference in risk preference across days with either sex for both solutions, *p*s > 0.05.

As in Experiment 1, intake in mL and number of free-choice trials completed were evaluated to characterize overall motivation to drink and respond. While there was no difference between ethanol plus saccharin and saccharin alone in risk preference, HAP3 mice consumed more saccharin than ethanol plus saccharin, $Z = -4.303$, $p < 0.001$ (Figure 7A), and completed more choice trials when responding for saccharin alone, $Z = -4.204$, $p < 0.001$ (Figure 7B). These effects were significant for both sexes, *p*s < 0.05. *U* Friedman's tests were used to determine if intake in mL and completed choice trials differed across days. E+S intake did not differ across

sessions, $X^2(4) = 5.441$, $p = 0.245$, and neither did choice trials completed when responding for E+S, $X^2(4) = 5.360$, $p = 0.252$. Saccharin intake did differ across days, $X^2(4) = 27.569$, $p < 0.001$, as did completed choice trials when responding for saccharin alone, $X^2(4) = 16.017$, $p = 0.003$.

An additional session was completed in which all animals responded for E+S, and retro-orbital blood samples were collected. Across the five E+S sessions, a median ethanol intake of 2.02 g/kg was reached in 30 minutes. On the day of blood collection, the mice reached a median of 1.79 g/kg in the 30-minute session, resulting in a median BEC of 126.64 mg//dL. Ethanol intake in g/kg predicted BEC, $b = 69.751$, $t(23) = 6.481$, $p < 0.001$ (Figure 8A). There was no difference in BEC between males and females, $U = 61.000$, $p = 0.543$. Intake also explained a significant proportion of the variance in BEC, $R^2 = 0.656$, $F(1,23) = 41.999$, $p < 0.001$. Four HAP3s were removed from risk preference analysis because of unrepresentative risk preference values due to low intake and/or choice trials completed. Similar to the averaged test days, risk preference significantly predicted choice trial intake, $b = 0.252$, $t(19) = 2.280$, $p = 0.035$. Risk preference also explained a significant proportion of the variance in choice trial intake, $R^2 = 0.224$, $F(1,19) = 5.198$, $p = 0.035$ (Figure 8B). However, there was no significant relationship between risk preference and BEC, $t(19) = -1.846$, $p = 0.081$.

DISCUSSION

General Discussion

These experiments utilized a novel operant task to examine if a preference for making risky and overall disadvantageous decisions is influenced by genetics related to alcohol drinking and state intoxication. Based on IGT performance in humans, it was hypothesized that riskier decisions would be made by mice bred to drink high amounts of alcohol, and that alcohol intoxication would heighten preference for these decisions. Genetics do appear to be involved in the decisions made in this task, but in the opposite direction of expectations. Mice bred to drink low amounts of alcohol consistently showed a higher preference for the "risky" lever. However, they displayed this preference regardless of the actual cost or benefit of the risky option, suggesting their behavior was motivated by something other than net reward differences between the risky and safe option. Findings from Experiment 2 suggest that alcohol intoxication may potentiate risky decision-making, but only in males. Results from these experiments point to both state and trait influences on risky decision-making and alcohol use, but further research may be needed to characterize these behaviors in regards to overall differences in avidity and differences between males and females in effects of alcohol intoxication.

Genetic Influences on Decision-Making and Risk

HAP3 mice had a lower preference for the risky lever than LAP3 mice. While these results contradict expectations, they may be explained in a number of ways. In the sessions 12-22, when examining actual behavior instead of strictly examining theoretical maximum values as is standard practice in many rodent gambling tasks (e.g. Zeeb, Robbins, & Winstanley, 2009; Peña-Oliver, Sanchez-Roige, Stephens, & Ripley, 2014; Pais-Viera, Lima, & Galhardo, 2007, Koot et al., 2012, & Miller et al., 2017), choosing the safe option resulted in greater saccharin reward time. The first explanation of HAP3 preference for the safe lever relates to increased wanting of the sweet reinforcer. There is a considerable amount of literature in both rodents and humans suggesting that genes related to high alcohol consumption may also contribute to increased sensitivity to sweet rewards (for review see Kampov-Polevoy, Garbutt, & Janowsky,

1999). For example, Oberlin et al. (2011) found a strong genetic correlation between free-choice alcohol drinking and saccharin consumption, with HAP2 and HAP3 mice consistently drinking more saccharin than their LAP2 and LAP3 counterparts. Similar results have been found in humans with a family history of alcoholism. When rating how much they liked or disliked a series of sucrose solutions, individuals with a positive family history of alcoholism were 2.5 times more likely to exhibit a pleasurable response to sweet tastes than those without a family history of AUD (Kampov-Polevoy, Garbutt, & Khalitov, 2003). It is therefore possible that HAP mice chose the safe option in order to gain the most sweet rewards possible, while the LAP mice have a lower preference for sweet solutions and thus had a weaker preference for the option with the greatest theoretical gain of these solutions. However, this interpretation alone seems unlikely, as HAPs consistently preferred the safe option, regardless of its effect on intake (see discussion below).

While most research supports a connection between alcohol use and riskier decision making, this relationship is not absolute. For example, using the Balloon Analogue Risk Task, humans who endorsed more problems related to alcohol use exhibited fewer pumps per trial, indicating safer behavior. However, this negative relationship was attenuated when accounting for participants' IQ and age (Ashenhurst, Jentsch, & Ray, 2011). Similar to the line comparisons in Experiment 1, Sanchez-Roige, Ripley, and Stephens (2015) compared two inbred strains: C57BL/6J mice (C57), often used for their relatively high levels of alcohol consumption, and DBA2/J mice (DBA), consistently found to be ethanol avoiding. Using a mouse version of the IGT, DBA mice exhibited riskier, and thus poorer, decision making than C57s, just as LAP3s exhibited poorer decision-making than HAP3s. Comparing Sardinian alcohol-preferring and non-preferring rats on performance in the multivariate concentric square field (MCSF) test, Roman and Colombo (2009) found that animals selectively bred to drink alcohol displayed less risky behavior than non-preferring animals. It should be noted, however, that the MCSF test likely measures a different type of risk-taking behavior than the task used in these experiments. While the majority of human studies point towards a genetic link between alcohol use and risky decisions, there are limited animal studies available, and the few that examine genetics and risk do not appear to support this relationship.

Interpreting Risky Decision-Making Tasks

While there is some support for a connection between low alcohol preference and risky, maladaptive decisions, the relationship may be more complex in these types of tasks. In Experiment 1, theoretical values for sessions 1-11 suggest the risky option is advantageous, and analysis of the actual relationship between risk preference and intake indicates the same. That is, riskier animals achieved higher fluid intake, and this relationship is consistent across lines. When reward and time-out parameters were altered to account for this positive relationship, predicted values indicated that the risky choice would result the same amount of sipper access time as the safe alternative. However, actual behavior analysis revealed a different relationship; animals that preferred the safer option achieved higher intakes for both lines. The contradictory nature of the theoretical and actual intake values brings forth two concerns.

First, it is standard practice in risky decision-making tasks utilizing different schedules of reinforcement and punishment to rely on theoretical maximum values. These theoretical values are used as proof that the risky option is disadvantageous, and making risky decisions is maladaptive. However, data from this experiment suggest that individual differences in actual consummatory behavior can alter the advantage or disadvantage of an animal's choices, and theoretical values are not always accurate. Without analyzing animals' actual behaviors in the task, choices may be considered suboptimal when in fact they actually result in greater reward. While gain and loss is straightforward in the traditional human version of the IGT, translating monetary loss into animal studies is a significant challenge. Similarly, most rodent gambling tasks use pellet rewards, which limits the amount of inter-animal variability in consummatory behavior. When sipper access time is used as a reward, individual differences in drinking efficiency can alter how rewarding access times may be. Regardless of the reward type being used, it is important to evaluate these complex tasks on actual behavior in order to avoid mislabeling behavior as disadvantageous.

The second concern relates to the overall motivation behind making risky or safe choices and the use of time-outs as a punishment in this task. In decision-making tasks like what was used in this experiment, it is assumed decisions are made strictly based on their long-term benefit. However, Busemeyer and Stout (2002) used computational modeling to form the Expectancy-Valence Learning model, in which decisions in the IGT are made by integrating attention to gains and losses, attention to recent outcomes, and how sensitive an individual is to

the outcome contingences. This model suggests the factors that go into making decisions in this task may be more complex than the initial assumption suggests. HAP and LAP behavior following contingency manipulations supports more complex decision-making processes. Actual intake data from sessions 1-11 indicate the risky option is the more advantageous choice. These data alone suggest that because the HAP3 mice exhibit a higher preference for the safe option, they are behaving disadvantageously. However, when the parameters were updated, switching the benefit of the risky and safe choices, HAP3 and LAP3s' behavior did not follow suit. That is, even though the safe option changed from disadvantageous to advantageous, the HAP mice did not switch their preference, nor did the LAPs. This lack of flexibility suggests the animals were not making choices based on their long-term outcomes, but on some other characteristic or combination of characteristics.

Following the processes outlined in the Expectancy-Valence model, sensitivity to outcome contingencies may especially factor into behavioral differences between lines. HAPs consistently completed more trials and consumed more fluid than LAPs, suggesting choices may have been driven by overall avidity to respond. So, the tendency of HAPs to choose the safe lever may have been less related to long-term benefit, and more related to the aversiveness of the long time-outs experienced with the risky option. Delay discounting data from the first and second replicate lines of HAP and LAP mice support this theory, showing that HAP mice prefer smaller, sooner rewards rather than waiting for larger rewards (Oberlin & Grahame, 2009). Similarly, alcohol preferring P rats show worse performance on a differential reinforcement of low-rate responding (DRL) task than their low drinking counterparts (Steinmetz et al., 2000). The DRL task measures an animal's ability to withhold responding for a set length of time in order to obtain a reward. Poor performance on this task indicates difficulty waiting before receiving a reward, and possibly even finding long waiting periods aversive. In order to accurately compare risky decision-making between HAPs and LAPs, the reward and the punisher (in this case a time-out) should be equally aversive to both lines. Data from these experiments suggest this is not the case, and HAPs exhibit safer behavior to avoid the long time-outs in the risky option, while LAP mice may not find this punisher as unfavorable. Therefore, this task may not be accurately measuring risky decision-making in these animals, but instead measuring overall avidity to respond and deficits in waiting impulsivity.

Alcohol Intoxication, Sex Differences, and Decision-Making

In Experiment 2, preference for the risky option was equally advantageous when HAPs were rewarded with saccharin alone and when the reward was ethanol and saccharin combined. However, using E+S as a reinforcer did reduce intake and the number of choice trials completed. This effect could be explained by alcohol's depressant effects reducing animals' ability to continue responding once intoxicated, consequently limiting the number of trials completed and fluid consumed in a single session. In 30 minutes, a median BEC of 126.64 mg/dL was reached, well above the 80 mg/dL definition of binge intoxication. Fritz, Grahame, and Boehm (2013) demonstrated that injections of 1.75 g/kg significantly impaired HAP3 mice as measured by a static dowel assessment, a dose similar to the median of about 2.0 g/kg that was self-administered in this task. Therefore, it is reasonable to assume the mice experienced some level of motor impairment due to alcohol intoxication. Another possibility is that mice were still able to respond under the influence of alcohol, but had reached their desired level of intoxication and thus chose to withhold responding.

It initially appeared that while alcohol intoxication was achieved in a 30-minute session, there was no difference in risk preference when animals were intoxicated or sober. However, when sex was taken into account, it was found that alcohol intoxication increased risk preference in males, but had no effect on females, even though females achieved higher median g/kg intakes. While research is limited on sex differences in ethanol intoxication's effects on risky decision-making, a similar effect was found in Long-Evans rats completing a probability discounting task (Wallin-Miller, Chesley, Castrillon, & Wood, 2017). Injections of both 0.5 and 1.0 g/kg ethanol increased male preference for the lever resulting in a large, but uncertain reward, while the same ethanol doses had no effect in females. In the same study, gonadectomized males with testosterone replacement exhibited a greater preference for the large/uncertain lever after saline injection when compared to gonadectomized males without hormone replacement, suggesting an influence of gonadal hormones on risk preference. Similarly, in human subjects, both men and women with higher testosterone levels have been found to choose more frequently from the disadvantageous decks in the Iowa Gambling Task, again implicating testosterone's involvement in risk-taking (Stanton, Liening, & Schultheiss, 2011). Tobiansky et al. (2018) review that not only are androgen receptors present in mesocorticolimbic brain regions involved in the IGT such as the nucleus accumbens and medial

prefrontal cortex, but androgen hormones are synthesized in these regions. These hormones may help regulate executive functioning primarily through their modulation of dopaminergic signaling.

If rodents and humans with higher levels of testosterone show a higher preference for risky options in the IGT, and males drinking ethanol increased their preference for the risky option in this study, a logical assumption is that ethanol exposure may increase testosterone levels in males, thus increasing their preference for riskier options via interaction with mesocorticolimbic structures. Research conducted by Alomary et al. (2003) suggests this may be a possibility. When 2 g/kg of ethanol was injected into male Wistar rats, brain concentrations of testosterone increased 4-fold. Furthermore, adrenalectomized-gonadectomized rats exhibited a 95% reduction in brain testosterone concentrations following ethanol injection, suggesting the increase in testosterone was dependent on synthesis in the periphery. However, this result does not seem to be consistent across studies. For example, Apter and Eriksson (2003) measured testosterone levels in selectively bred alcohol preferring (AA) and alcohol non-preferring (ANA) rat lines under control conditions and after alcohol injection. Contrary to the previously mentioned results, in this study a high dose of alcohol (1.5 g/kg) significantly decreased blood testosterone levels for both lines. However, a lower dose (0.75 g/kg) lowered testosterone levels in the ANA line only. Moreover, under control conditions AA rats exhibited higher basal serum levels of testosterone than ANA rats. This study suggests that while there is a relationship between testosterone and alcohol consumption, it may not always be clear cut or consistent across individuals. Further research is necessary to determine the effects of acute alcohol administration on testosterone levels in different populations, and what effects these changes may have on risky decision-making as was observed in this study.

Conclusions and Future Directions

Future experiments involving possible trait differences in risky decision making may opt to utilize forms of punishment other than time-out, such as bitter tasting substances like quinine or mild footshock, in order to more accurately measure decision-making without the confound of differences in waiting impulsivity. Similarly, further investigation of risky and safe parameters is necessary to ensure that the risky option is equally disadvantageous for both lines. Furthermore, any further research using tasks that vary in punishment and reward should rely on behavior as

well as theoretical values to ensure the task is measuring the proper behaviors and is working as intended. This is especially important when using sipper access as a reward, as individual differences in drinking efficiency can alter the magnitude of rewards.

Further investigation into the effects of alcohol intoxication on risky decision making should also utilize parameters that result in disadvantage when choosing the risky option, while still allowing a positive or negative effect on risk preference. Possible sex differences should be investigated whenever examining decision-making under risk, and the effect of ethanol on sex hormones such as testosterone may be especially important. Researching the effect of ethanol on testosterone in populations with different alcohol drinking behaviors may show differing effects based on alcohol preference, and these differences may contribute to how alcohol intoxication affects male decision-making behaviors.

These experiments demonstrate that genetic differences in alcohol preference may be related to decision-making under risk, although these conclusions are complicated by overall differences in avidity. Achieving the proper balance between reward and punishment is essential in these types of tasks, but must be evaluated theoretically and with actual behavioral data. Finally, animals bred for high alcohol preference can achieve high BECs in a short amount of time, and intoxication may differentially affect males and females on risk behavior.

TABLE

Table 1. Shaping protocol. FR1 = fixed ratio 1, ITI = intertrial interval

Stage	Description	Criterion to advancement
1	All center nose-pokes reinforced on a FR1 schedule with 15 seconds sipper access. Non-response-contingent reinforcement presented every 120 seconds. The nose poke light is on for the entire session. Levers and lever lights removed	One 30-min session, unless consumption < 0.2 mL
2	All center nose-pokes reinforced on a FR1 schedule. Sipper access time is 10 seconds. The nose poke light is on for the entire session. Levers and lever lights removed.	Completion of 15 trials in 30 minutes and consumption ≥ 0.2 mL
3	Center nose-poke turns off the nose-poke light and is rewarded with 5 seconds sipper access. The next trial begins with the nose-poke light coming on after a 5-second ITI that initiates after sipper tube retraction. Levers and lever lights removed.	Completion of 15 trials in 30 minutes and consumption ≥ 0.2 mL
4	Center nose-poke turns off the nose-poke light followed by illumination of both right and left lever lights. A response on either lever turns off both lever lights and results in 5 seconds sipper access, which is in turn followed by a 5-second ITI. During this period, mice have an unlimited time after the NP response to initiate the lever response.	Completion of 15 trials in 30 minutes and consumption ≥ 0.2 mL
5	Center nose-poke turns off the nose-poke light followed by illumination of both right and left lever lights. A response on either lever turns off both lever lights and results in 5 seconds sipper access, which is in turn followed by a 5-second ITI. During this period, mice have an unlimited time after the NP response to initiate the lever response. During this period, mice have 10 seconds to make a lever response. Failure to do so results in a 5-second ITI with all lights off, followed by an initiation of a new trial	Completion of 15 trials in 30 minutes and consumption ≥ 0.2 mL
6	All trials are cued. After the center nose-poke, the light above either the left or right lever will go on (50% of the time for each), to signify that lever is active. Mice have 10 seconds to make a lever response. Correct lever presses are reinforced with 5 seconds of sipper access, which is in turn followed by a 5-second ITI.	Completion of 15 trials in 30 minutes and consumption ≥ 0.2 mL
7	Identical to Stage 6, but sipper access time is decreased to 1 second to prepare mice for shorter access times.	Completion of 15 trials in 30 minutes and consumption ≥0.2 ml

FIGURES

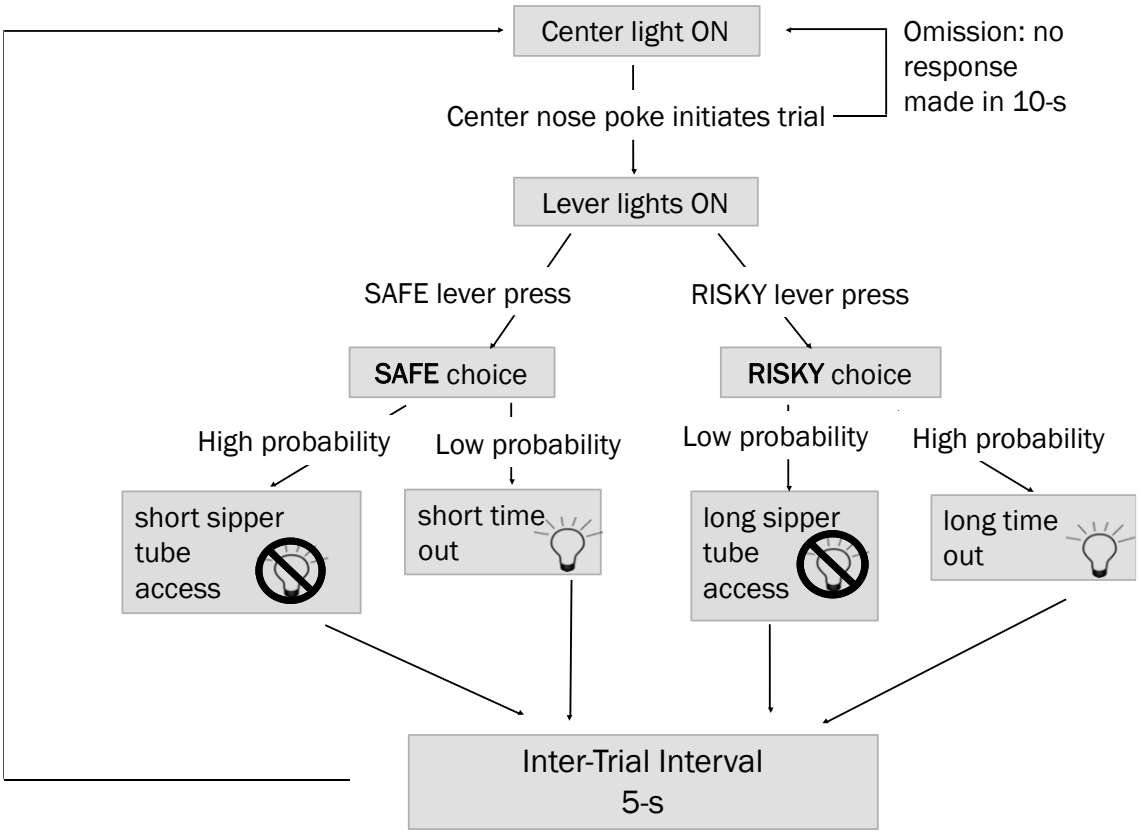

Figure 1. General Procedure

Flowchart depicting general procedure for risky decision-making task across all experiments.

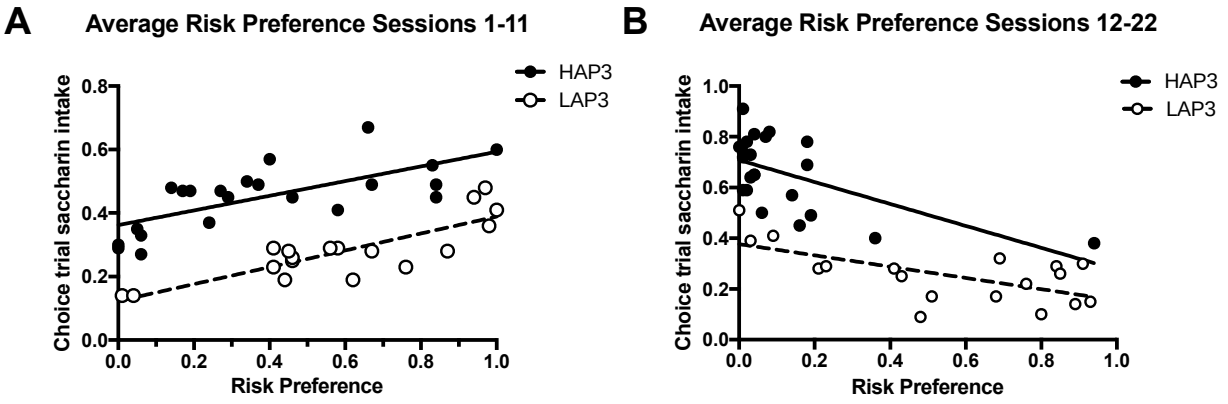

Figure 2. Risk Preference Regressions Split by Line

Experiment 1 regressions of mean risk preference by choice trial saccharin intake. (A) There was a positive relationship between risk preference score and choice trial saccharin intake for both lines in sessions 1-11. HAP3s drank more saccharin than LAP3s when risk preference was 0. (B) In sessions 12-22, there was a negative relationship between preference for the risky lever and choice trial saccharin intake for both lines. Again, HAPs drank more saccharin than LAPs when risk preference was 0.

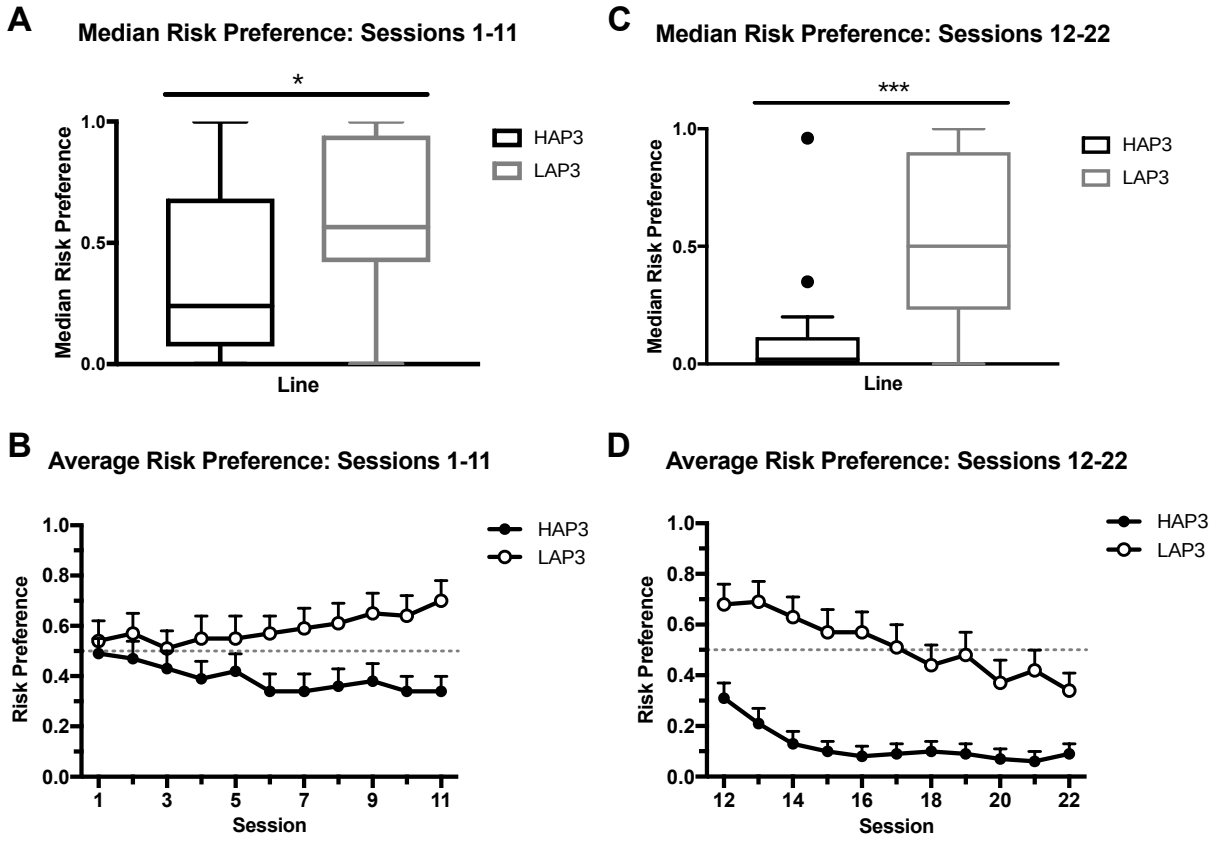

Figure 3. Risk Preference Split by Line

LAP3s exhibited a greater median risk preference for sessions 1-11 (A) and 12-22 (C) than HAP3s. (B) Mean and SEM risk preference split by line for sessions 1-11. (D) Mean and SEM risk preference sessions 12-22, split by line. (*$p < 0.05$, ***$p < 0.001$)

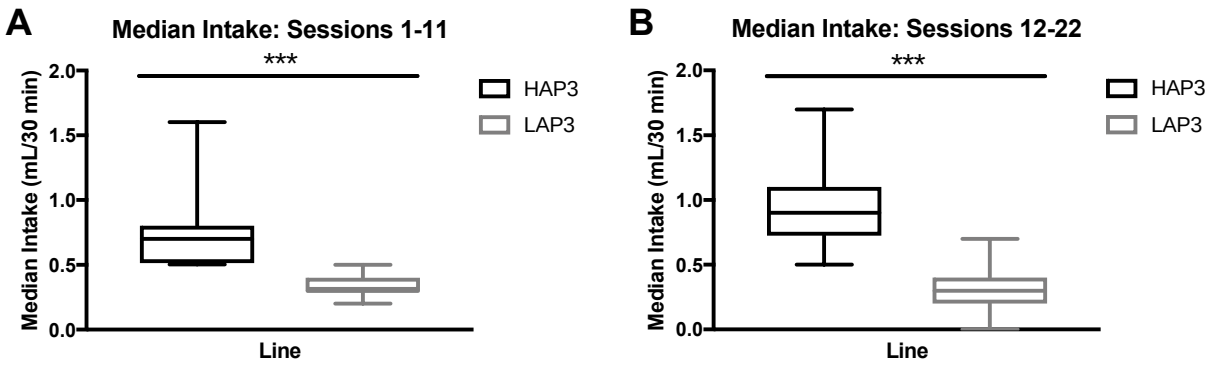

Figure 4. Intakes Split by Line

Experiment 1 median intake in mL/30-minute session split by line. HAP3s drank more saccharin than LAP3s with the first set of parameters in sessions 1-11 (A) and the second set in sessions 12-22 (B). (***$p < 0.001$)

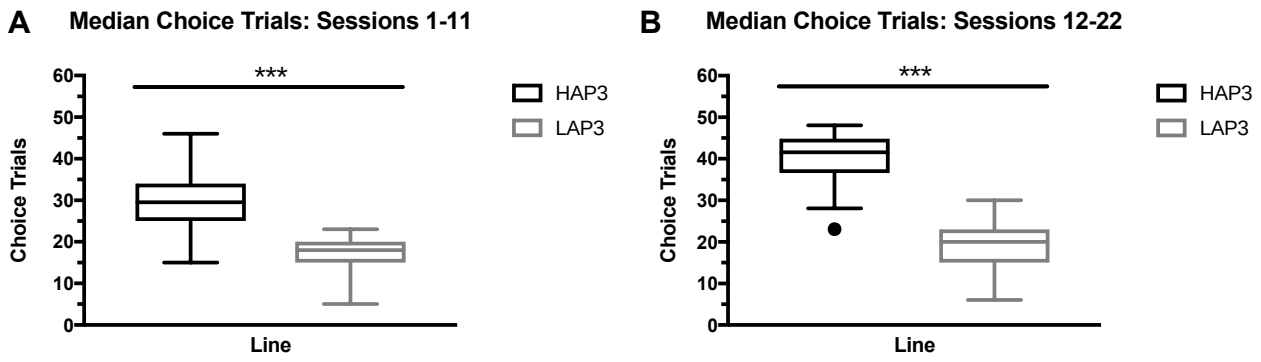

Figure 5. Choice Trials Split by Line

Experiment 1 median choice trials completed split by line. HAP3s completed more trials than LAP3s in sessions 1-11 (A) and sessions 12-22 (B). (***$p < 0.001$)

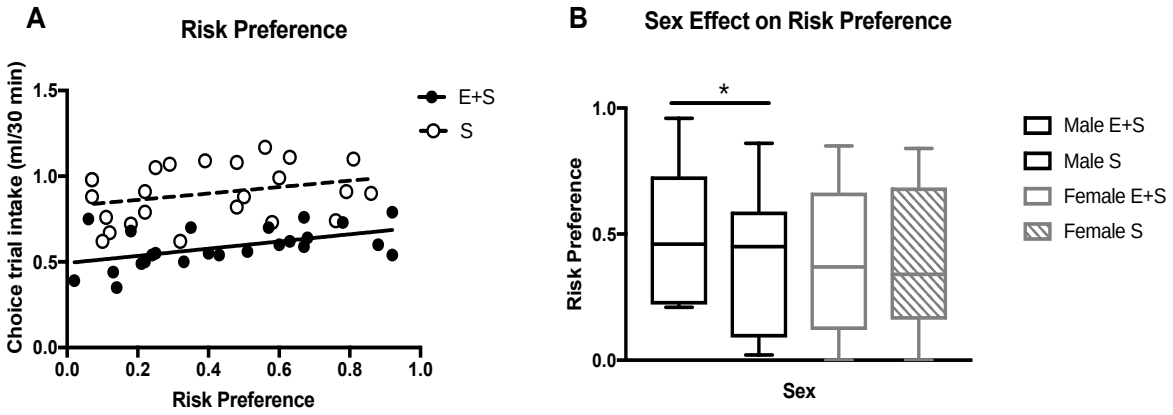

Figure 6. Effect of Reinforcer and Sex on Risk Preference

Experiment 2 HAP3 risk preference. (A) Regression of average risk preference split by reinforcer type. There was a positive relationship between risk preference and choice trial intake for both reinforcers, and the addition of ethanol did not change this relationship. Mice drank more saccharin alone than ethanol plus saccharin when risk preference was 0. (B) Reinforcer type had no effect on risk preference in females, but males exhibited higher preference for the risky lever when responding for ethanol plus saccharin than when responding for saccharin alone. (*$p < 0.05$)

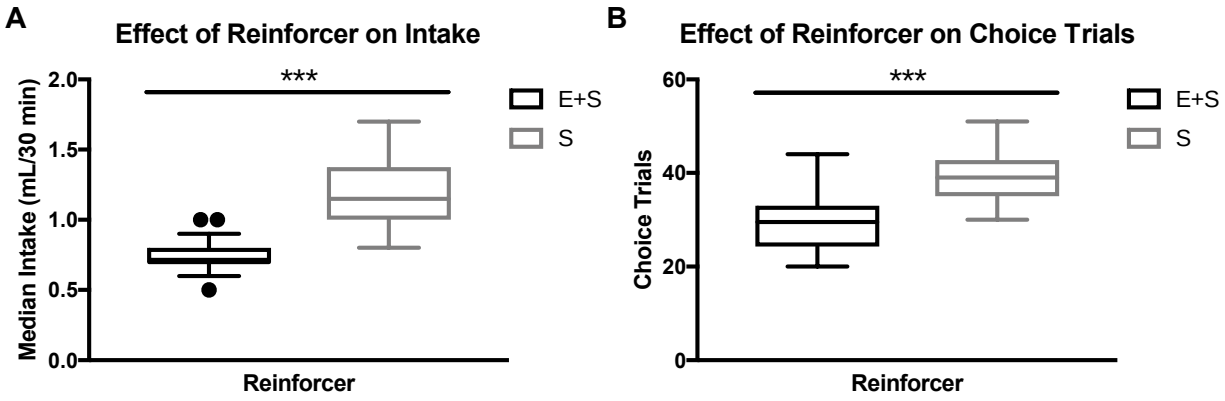

Figure 7. Effect of Reinforcer on Intake and Choice Trials
(A) HAP3s drank less ethanol plus saccharin than ethanol alone. (B) Fewer choice trials were completed when responding for ethanol plus saccharin than when responding for just saccharin. (***$p < 0.001$)

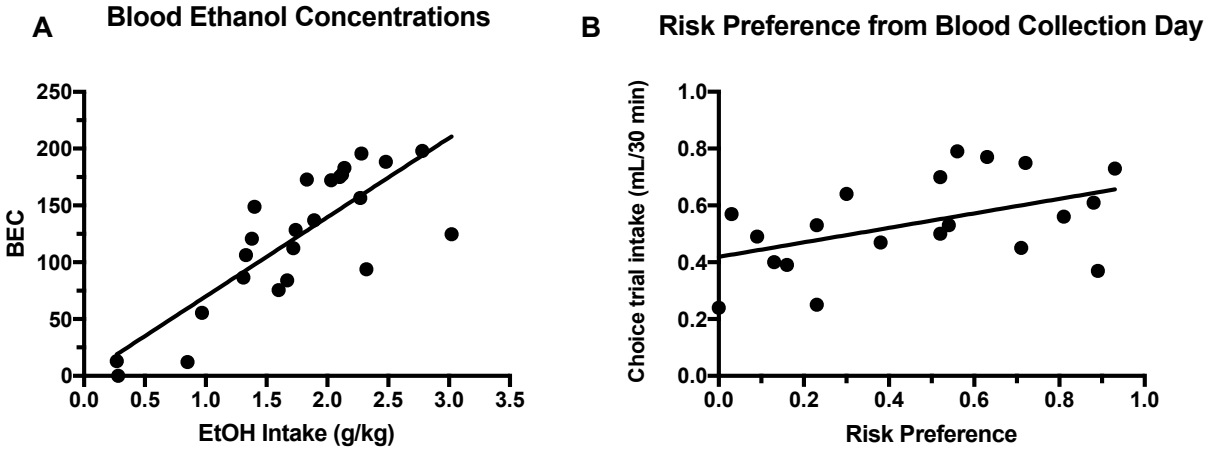

Figure 8. Intake Regressions from Blood Collection Day
(A) Ethanol intake in g/kg had a strong positive relationship with blood ethanol concentration. (B) Higher risk preference predicted higher choice trial intake

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