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Adolescent and Adult Consumption of Alcohol-Adulterated Gelatin on Impulse Choice, and Alcohol Consumption and Preference in Rats

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Preference for small-immediate reinforcers over large-delayed ones (i.e., impulsive choice) is a hallmark of individuals with substance use disorders (see Bickel et al., 2014). This behavioral pattern is facilitated by a reduced sensitivity to delayed reinforcing and punishing choice outcomes. Epidemiologically, individuals with substance use disorders consistently exhibit greater delay discounting compared to individuals without substance use disorders, suggesting a link between impulsivity and addiction. However, the causal nature of this relationship is unknown. Preclinical modeling of a related phenomenon, risky choice, (e.g., Nasrallah et al., 2009) found adolescent rats who consumed alcoholic gelatin (mean 11.4 g/kg/day) for three weeks preferred a riskier choice alternative (e.g., 4 pellets with a 25% probability over 2 pellets with 100% probability) more than non-alcohol controls. To extend these findings to impulsive choice and investigate whether the age of exposure mattered, rats consumed an alcoholic gelatin (10% v/v) and sucralose (10% w/v), either in adolescence or adulthood. Following alcohol consumption during adolescence (mean 7.1 g/kg/day) and adulthood (mean 3.1 g/kg/day) access periods, impulsive choice was assessed (i.e., one-immediate versus three-delayed pellets [0–24 s]). Contrary to expectations, alcohol consumption did not alter preference for the large-delayed reinforcer in any exposure group, which may have been due to the lower amount of alcohol consumed compared to the Nasrallah. However, given that 7.1g/kg/day of alcohol is equivalent to 35 standard drinks for a 70 kg human, these findings highlight that impulsive choice may be more of a vulnerability factor to alcohol use disorder and not necessarily a causal one.

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Contributions
JRS and MPR conceptualized the study and experimental design and finalized the experimental protocols. JRS was responsible for daily conduct of the study and data collection and supervised conduct of the study, analyzed the data. JRS and MPR drafted the manuscript and provided editorial comments and suggestions. All authors reviewed drafts of the manuscript and approved the final version.

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The effects of excessive alcohol (i.e., ethanol) consumption during adolescence and early-adulthood (i.e., binge-drinking in the teens to early twenties; Courtney & Polich, 2009) is a public health concern due to the acute and long-term health and behavioral issues it creates (Jones et al., 2017; Oesterle et al., 2008). Epidemiological studies have shown that alcohol intoxication increases the likelihood of physical injuries, drowning, burns and falls (Hingson et al., 2009) likely due to the behavioral patterns it causes, such as increased impulsive choice and risk taking (Erickson & Trocki, 1992; Hingson et al., 2009). These impulsive and risky behavioral patterns, however, are not unique to alcohol use disorders but are more broadly a hallmark of substance use disorders.

One mechanism underlying these behavioral patterns is delay discounting or the reduced efficacy of delayed reinforcers and punishers to alter behavior (Ainslie, 1975). For example, when given a choice between a large-delayed versus small-immediate reinforcers, preference for the large alternative diminishes as the delay is increased (Rachlin & Green, 1972). In humans, excessive discounting of delayed reinforcers (i.e., stable access to food, money and shelter; Bickel et al., 2006; Kirby et al., 1999) in the presence of immediate ones (i.e., illicit drugs, alcohol, etc.) is common with substance use disorders. Over time, a consistent preference for the immediate alternative disrupts the ability to maintain a normally functioning life and is often a focus of therapeutic interventions to reduce it (Bickel et al., 2006). However, determining if greater delay discounting is a relatively fixed behavioral pattern (i.e., trait) in these individuals or if it is a transient one (i.e., state) produced by drug dependence/intoxication, has been extremely difficult to disentangle (Bickel et al., 2012). For example, preclinical models have found that the acute intoxicating effect of alcohol (e.g., 1-3 g/kg vs. saline) increases impulsive choice in rats during an intertemporal choice procedure (Evenden & Ryan, 1999; Hellemans et al., 2005; Mejia-Toiber et al., 2014; Olmstead et al., 2006; but see Wilhelm & Mitchell, 2012). However, the reverse relationship has also been observed, wherein greater impulsive choice has been found to be predictive of higher alcohol consumption during free-access periods (Poulos et al., 1995). Preclinical models that examine the acute intoxicating and chronic developmental effects of drug exposure (i.e., alcohol), especially at different developmental time points (i.e., adolescence vs. adulthood; Spear, 2015) has allowed researchers to begin to disentangle the factors involved in this phenomenon.

Adolescence is a critical period for alcohol exposure due to the behavioral and neurobiological changes occurring during this development period (Spear, 2015). During this period, rats are less sensitive to the acute effects of alcohol, including its sedative/hypnotic (e.g., shorter sleep duration and higher blood-alcohol level [BAL] at regaining consciousness; Silveri & Spear, 1998), hypothermic (Brasser & Spear, 2002; Silveri & Spear, 2000), motor impairing (e.g., higher BAL at regaining righting reflex; White et al., 2002) and anxiolytic (e.g., socializing with novel rats more at lower doses; Varlinskaya & Spear, 2002) effects. Additionally, adolescent rats appear to be less sensitive to the anxiogenic effects produced by acute alcohol withdrawal (e.g., increased time on the exposed arms of an elevated plus maze while "hung-over"; Doremus et al., 2003). This decreased sensitivity, combined with a slightly higher rate of alcohol metabolism (Brasser & Spear, 2002; Walker & Ehlers, 2009; but see Silveri & Spear, 2000), enables adolescent rats to actively consume alcohol for longer periods of time, facilitating the increased alcohol consumption common in adolescent rats (see Crews & Boetigger, 2009). Taken together, these findings support the age-dependent differences in the effects of alcohol and suggest adolescent rats, due to their relatively higher level of alcohol consumption, could be more prone to increases in impulsive choice following chronic self-administration.

Preclinical research with rats has modeled the long-term impact of adolescent and early-adulthood (e.g., postnatal day [PND] 28-50; Spear, 2007) alcohol consumption on preference for riskier choice outcomes (Nasrallah et al., 2009; Nasrallah et al., 2011). In three separate experiments, Nasrallah and colleagues had rats self-administer alcohol for 20 days during adolescence, facilitated by mixing alcohol and sucrose in a gelatin matrix (i.e., 10% alcohol and 10% polycose sugar; see Rowland et al., 2005). The average consumption was ~11-12 g/kg/day, which is about double the doses (~5-7 g/kg) typically observed in adolescent *alcohol preferring rats* drinking unsweetened alcohol (Bell et al., 2006). Either three weeks or three months post alcohol exposure, rats were trained to respond on a probability-discounting procedure wherein rats chose between a certain (2 pellets) and risky (4 pellets with a probability of 0.75, 0.50, or 0.25) alternatives, with the probability reduced across three consecutive sessions. Across all experiments, rats that consumed alcohol in adolescence preferred the risky alternative more than controls. These findings are consistent with the previously mentioned human research showing individuals with alcohol use disorder tend to make risky (e.g., Bjork et al., 2004) and impulsive choices (e.g., Vuchinich & Simpson, 1998) as adults. Given this, it stands to reason that adolescent alcohol self-administration could also increase impulsive choice. Additionally, the previous Nasrallah studies do not provide definitive evidence that adolescence is the only period of development where chronic alcohol consumption produces an increase in risky choice. If adolescence is the only critical period, as suggested by Dom et al. (2006), then the findings of Nasrallah and colleagues should not replicate with rats that chronically consume alcohol in adulthood.

Prior work has examined how intermittent experimenter-administered alcohol (e.g., 1 or 3 g/kg: IG on 9 days from PND 31-50) during adolescence impacts impulsive choice (Mejia-Toiber et al., 2014; Passos et al., 2015). Both studies, however, failed to find an impact on impulsive choice, which may have been due to the relatively lower doses of alcohol administered during adolescence compared to those ingested in the Nasrallah studies (11-12 g/kg/day). Another reason may be that the alcohol was administered intermittently, and it was experimenter-, not self-administered, which is a known factor that reduces alcohol tolerance development and behavioral patterns in alcohol use (Weise-Kelly & Siegel, 2001). Thus, the present study attempted to directly replicate the prior self-administered findings from

Nasrallah and colleagues (2009, 2011) and extend them to impulsive choice.

The present study examines the casual nature of alcohol exposure during different developmental phases and the impact on impulsive choice in rats. Specifically, this study alternates between alcohol consumption and impulsive choice assessments from adolescence through adulthood and thus, is well-suited to replicate the bi-directional relationship of alcohol consumption and impulsive choice. To accomplish this, whenever alcohol consumption preceded an assessment of impulsive choice, the average dose of alcohol consumed during gelatin and bottle (acute pretreatment) self-administration was correlated with preference for the impulsive alternative and vice-versa. Such self-administration models alcohol consumption in humans and it produces a wider dose-effect range to correlate the exposure dose with behavioral outcomes. Additionally, to determine if the Nasrallah effects are indeed age-dependent, a second group of rats was included that only consumed alcohol in adulthood. Specifically, different groups of rats consumed an alcoholic gelatin for three weeks in adolescence (early-drinkers) or adulthood (late-drinkers), while the other experimental and control group (non-drinkers) consumed an alcohol-free gelatin. Following each access period, rats were assessed for between-group differences in impulsive choice in a delay-discounting procedure. Following the adolescent and adulthood gelatin access periods, all rats self-administered alcohol from a bottle prior to experimental sessions to determine how the acute effects alter impulsive choice and if it was related to the period of alcohol exposure. Lastly, the reinforcing properties of alcohol were determined using a two-bottle choice assay.

Table 1. Timeline.

Postnatal Day →							
30-50	51-55	56-76	80-100	101-106	107-124	129-142	146-147
AA-I	Lever Training (↓90% FFW)	IA-I	AA-II	Break	IA-II (↓90% FFW)	IA-III	ARA

AA: Alcohol Access (Free home cage access to chow, water and an (non-)alcoholic gelatin)
IA: Impulsivity Assessment (@ 90% FFW)
ARA: Alcohol Reinforcer Assessment (2-bottle choice @ 90% FFW; includes bottle training)

Procedure

Method

Subjects. Sprague-Dawley male rats (N = 36; Charles River Laboratories, Portage, MI) were housed individually in polycarbonate tubs with free access to water in a room with a reverse day/night cycle (lights on at 2000 h). Upon arrival (PND 21; ~75 g), rats were assigned to one of three groups (n = 12) based upon weight to minimize between-group differences. Rats were provided free access to food (Harlan-Taklad Laboratory Rodent Diet) from the day they arrived in the lab through the first alcohol access (AA-I; see Timeline and Experimental Design below) and again during the second alcohol access (AA-II). Rats were food restricted to 90% of their ad-libitum free-feeding weights (FFW) during the three impulsive choice assessment testing phases (IA-I, II, & III) and the Alcohol Reinforcer Assessment (ARA); FFW was increased 1.5% each week of food restriction. During food restriction, rats were weighed daily and provided supplemental feeding to maintain body weights at 90% FFW. FFWs were recalculated following the period of free-food access in AA-II. All experimental phases were conducted 7 days/week. Protocols were approved by Central Michigan University's Animal Care and Use Committee and were conducted in accordance with NIH guidelines set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Apparatus. Impulsive choice sessions were conducted in eight Med-Associates (Georgia, VT) operant chambers enclosed in sound-attenuating boxes with exhaust fans that provided masking noise. The chambers measured 27 cm wide by 22 cm deep by 20 cm high and had sidewalls and ceilings of Plexiglas, front and rear walls of aluminum, and floors constructed from stainless steel rods. The front panel of each chamber was equipped with two levers (0.25 N per response), 7.5 cm above the floor, and 8 cm from the midline of the panel on center. Three cm above each lever, a 28-V DC light bulb was recessed behind a translucent plastic cover. A recessed pellet hopper, measuring 5 cm wide by 4 cm tall, was located 3 cm above the floor between the two levers and delivered 45-mg Bio-Serv® Dustless Precision Pellets (Product #F0021). A 28-V DC house light, located 3 cm below the ceiling on the rear panel, provided general illumination. A computer running Windows XP® and Med-PC IV® software orchestrated environmental control of operant chambers and recorded data.

Experimental design. Three groups of rats (n = 12; Early-, Late- & Non-Drinkers) were either assigned to have free-access to an alcoholic gelatin during adolescence (Early-Drinkers [ED]; AA-I: PND 30-50), early-adulthood (Late-Drinkers [LD]; AA-II: PND 80-100) or not at all (Non-drinkers [ND]). If alcohol was not provided, that group was given an alcohol-free gelatin during access periods. Following gelatin access periods, rats were reduced to 90% FFW for lever training (PND 51-55) and the impulsive choice assessments (IA-I: PND 56-76; IA-II: PND 107-124).

Following IA-II, rats were given access to an alcohol-free or alcohol solution prior to experimental sessions (IA-III: PND 131-143) to determine how various histories of alcohol consumption alter the acute effects of alcohol on impulsive choice. Once bottle trained, rats self-administered the alcohol-free and alcohol (10% polycose/10% ethanol) solutions prior to experimental sessions employing a 6-s large alternative delay (this delay was chosen because it produced intermediate levels of impulsive choice in most rats). Rats initially self-administered the alcohol-free solution for the first six-session block and then the alcohol solution for the second six-session block.

Following IA-III two 2-bottle choice sessions were conducted

for three days to assess between-group differences in the reinforcing properties of alcohol (ARA: PND 146-147), wherein rats were given 20-m of access to two bottles, one containing an alcohol-free (10% polycose) and the other containing an alcohol solution (10% polycose/10% alcohol).

Gelatin preparation. Rats were given access to either an alcohol (10% ethanol/ 10% polycose) or alcohol-free gelatin (10% polycose) in their home-cages during adolescence (AA-I: PND 30-50) and adulthood (AA-II: PND 80-100). Gelatins were continuously available for 21 days and were provided in 4 oz jars (2" tall, wide mouth glass canning jars). Gelatins during AA-I and II (see Experimental Design below) were filled to allow for a maximum dose of approximately 18 g/kg/day of alcohol (~35 ml for a 150 g rat); an equivalent amount, sans alcohol, was provided to the non-drinking rats. Each day at 1300 h, the remaining gelatin was weighed, discarded and replaced with a new gelatin jar. Differences in jar weight from the previous day were used to calculate the dose of alcohol self-administered each day as well as the total percentage consumed. To monitor evaporation rates, sentinel jars weighing the average amount of the alcohol-free and alcoholic gelatins were kept in an empty polytub with bedding. Average daily evaporation rates were lower during AA-I (alcohol-free gelatin: 2.1 g/day & alcoholic gelatin: 2.7 g/day) than AA-II (alcohol-free gelatin: 2.9 g/day & alcoholic gelatin: 3.5 g/day; i.e., *~0.6 g/day of ethanol evaporated daily*). Since it was not possible to determine when gelatin consumption occurred, consumption calculations were not adjusted to account for evaporation.

To prevent spillage, jars were stabilized on day 13 of AA-I by securing them (~ 4 cm from the bottom along the glass band) within machine cloth (15 cm wire-mesh square, with a central jar hole, folded down 5 cm on opposite sides). For AA-II, the jar holder was updated by stapling them to a composite decking (Ultra-Deck®) 14 cm × 15 cm base (~650 g).

To make the gelatin, filtered water was boiled, then a gelatin powder (3 g/100 ml; *NOW*® Gelatin) and Polycose® (10% w/v) were added. After the gelatinous solution had cooled but before it had set, the solution was split to add alcohol (10% v/v) and then the jars were filled. The jars were covered to minimize alcohol evaporation and refrigerated to set overnight. In the morning, jars were removed from the refrigerator and allowed to reach room temperature before being given to the rats.

Lever Training & Impulsive Choice Assessment. Rats were initially trained to lever press on the two levers in the week prior to IA-I (see the Timeline). During these five training sessions, rats were trained to respond on both levers on a fixed-ratio 1 schedule. Initially, both levers were active, signaled by their associated stimulus light being illuminated. Once 25 reinforcers were earned on a lever, the associated stimulus light was darkened and the lever became inactive. Rats completed the training session once earning 25 pellets on both levers. Once trained, levers were assigned (counterbalanced across rats) to deliver 1 pellet immediately or 3 pellets after a delay of *t* sec, signaled by the stimulus light flashing at 1 Hz. Sessions began with 24 sample trials, followed by 24 choice trials, and these trials were signaled by the onset of the house light and the stimulus light(s). Sample trials required rats to respond on one of the two levers and thus, experience both alternatives across trials. Choice trials allowed rats to choose between the two alternatives. Between trials, the lights were extinguished during a 45-s inter-trial interval (ITI) that maintained rates of trial presentation by being compensated for the length of the delay (i.e., a trial with a 12-s delay would have a 33-s ITI). Sessions concluded following 24 choice trials or 45-m.

Initially, rats were trained with no delay and once preference for the large-alternative was established (> 88% preference averaged across two sessions), the delay to the large alternative was increased every session (3, 6, 12, & 24 s). After the 24-s delay, two no-delay sessions occurred, after which the delay cycle was repeated. During IA-I and II, this sequence continued until 3 cycles were completed. To create representative group delay gradients, each subject's average large preference at each delay across the three delay cycles were averaged (preference at the 0-s delay was calculated using the two session that initiated each delay cycle). During IA-III, the large alternative delay was maintained at a 6-s delay for six sessions per bottle alcohol self-administration manipulation (12 days total; see Experimental Design below).

Bottle alcohol self-administration and two-bottle choice assessment. In a darkened room (two 60-watt light bulbs provided weak illumination), rats were placed in polytubs without bedding and had access to bottles containing an alcoholic (10% polycose/10% ethanol; left side of the polytub) and/or an alcohol-free (10% polycose; right side of the polytub) water solution. To train the rats to drink from these bottles, they were given two 1-hr training sessions (PND 129-130) with an alcohol-free bottle (about half the rats from each group required supplemental overnight bottle access to complete training and these rats drank similar amounts to those without this training). Once trained, rats were allowed to self-administer from one of these bottles for 20-m prior to experimental sessions (IA-III). After 20 min, the bottles were removed, and the rats were given an additional 20 min to allow for alcohol absorption. In the two-bottle choice condition (the alcohol reinforcement assessment [ARA]) following IA-III, rats were given 20 min access to both the alcohol (left) and alcohol-free bottle (right), and preference was quantified by the total amount consumed from both bottle types as well as the percentage of total consumption from the bottle containing alcohol. Since rats were no longer running in experimental conditions during this phase, rats were immediately placed back in their home cages following the 20-min access period.

Data Analysis. Between-group differences in gelatin consumption during AA-I and AA-II were determined using Mixed 3 (drinking group) × 3 (drinking week) analysis of variance (ANOVA; SPSS® 20.0 for Mac) of individual average weekly percent gelatin consumption. Weekly averages, instead of daily, were chosen because of missing data resulting from gelatin spillage (see Gelatin Preparation above). In addition, between-group differences in body weight gain across AA-I and AA-II were also assessed using 3 (drinking group) × 21 (access day) Mixed ANOVA.

Following IA-I and II, between-group differences in the proportion of large alternative choices across delay were quantified in two ways. First, a Mixed 3 (drinking group) × 5 (delay) ANOVA was used to determine if between-group differences in individual average delay gradients were significant. Second, between-group

differences in individual average delay gradient shape were quantified via the area-under-the-curve (AUC; see Myerson, Green & Warusawitharana, 2001) method in Graph Pad Prism© 5.0 for Mac. With this measure, a decrease in the AUC indicates an increase in impulsive choice (i.e., preference for the larger, delayed alternative decreased). AUC, in addition to quantifying the change in proportion of large alternative choices, was used to 1) characterize changes in impulsive choice produced by various histories of chronic and acute alcohol consumption and also to predict alcohol consumption and 2) assess the relationship (Graph Pad Prism® 4.0 - Pearson's product-moment correlation coefficient) between impulsive choice and alcohol consumption at various points throughout the study. The same analysis was also used to assess the relationship between impulsive choice and alcohol consumption in IA-III were assessed using the average proportion of large choices during the six 10% polycose sessions and subsequent average dose of alcohol consumed.

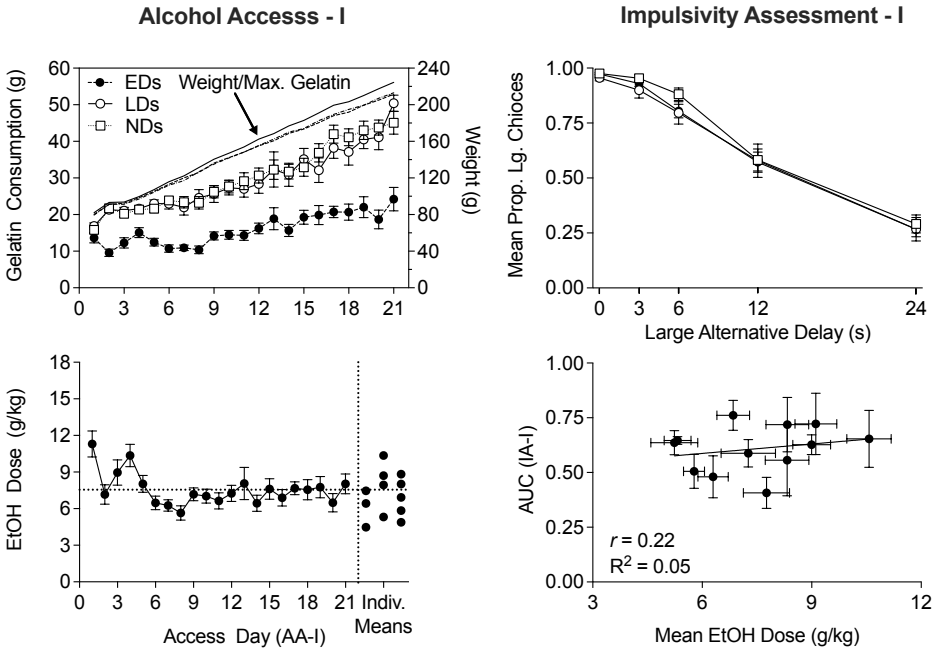

Figure 1. **Top Left**: Mean group (± SEM) consumption of raw gelatin (symbols), gelatin amount provided (lines; left y-axis) and weights (lines; right y-axis) across access days to an alcoholic (EDs) or alcohol-free gelatin (LDs & NDs). **Bottom Left**: Mean dose of alcohol consumed (EDs only). **Top Right**: Mean proportion of large alternative choices (± SEM) across delay during the three gradients of IA-I. **Bottom Right**: EDs individual and average (± SEM) AUC from IA-I plotted against individual average (± SEM) self-administered dose (g/kg) in AA-I.

After completing IA-III, between-group differences in consumption and self-administered dose were assessed visually and quantified using a 3 (drinking group) × 6 (drinking session) Mixed ANOVA. For the ARA, between-group differences in the amount consumed from each bottle type were assessed visually and with 2 (solution type) × 3 (drinking group) Mixed ANOVA. Differences in the percent consumption from the alcoholic bottle were assessed with a One-Way ANOVA.

Results

Figure 1 (top left) displays the EDs, LDs, and NDs average raw gelatin consumption (symbols), total gelatin provided (lines; left Y-axis) and body weight (lines; right Y-axis) across AA-I. In general, total gelatin consumption was higher for the alcohol-free gelatin (LDs & NDs) than the alcoholic gelatin (EDs) and tended to increase across access day, however, this increasing pattern was likely the result of ceiling effects since the amount of gelatin provided increased to accommodate weight gain; no group differences in weights were observed. The relative consumption of gelatin vs. the total amount provided appeared to start near 100% and then trended downward slightly before stabilizing at either 40% (EDs) or 80% (LDs & NDs). For the EDs, this produced daily ethanol doses (Figure 1 – bottom left) during the first week of access that were modestly higher than those of the final two weeks where average doses stabilized. Overall, the average self-administered dose was approximately 7.1 g/kg/day. These observations were supported by a 3 (drinking group) × 3 (access week) Mixed ANOVA that found a significant main effect of drinking group, $F(2, 33) = 34.69$, $p < .05$ and access day, $F(2, 66) = 13.77$, $p < .05$, but not their interaction, $F(4, 66) = 0.165$, p = n.s.

Figure 1 (top right) also displays the average proportion of large alternative choices across delays to the large alternative for the EDs, LDs, and NDs during each delay gradient, and their average, in IA-I. In general, preference for the large alternative decreased linearly with increases in delay and this pattern was similar across the three gradients. No between-group differences were observed. A 3 (drinking group) × 5 (delay) Mixed ANOVA of the rats' average gradients revealed a main effect of delay, $F(2.31, 76.34) = 316.13$, $p < .05$, but no effect of drinking group, $F(2, 33) = 0.46$, p = n.s., or interaction of delay and drinking group, $F(4.63, 76.34) = 0.46$, p = n.s. after correcting for Mauchly's sphericity violation using the Huynh-Feldt method (except the main effect of drinking group).

To assess the relationship between the EDs alcohol consumption and their level of impulsive choice, the AUC of their individual gradients, including pooled individual average, from IA-I were plotted (Figure 1 – bottom right) with their average self-administered dose of alcohol during AA-I. In general, there did not appear to be a relationship between dose and AUC in averaged individual gradients ($r[10] = .23$, p = n.s.) from IA-I.

Following IA-I rats were again given access to food and an alcohol-free (EDs & NDs) or alcoholic gelatin (LDs). Figure 2 (top left; lines) displays the EDs, LDs, and NDs average raw gelatin consumption (symbols), total gelatin provided (lines; left Y-axis) and body weight (lines; right Y-axis) across AA-II. In general,

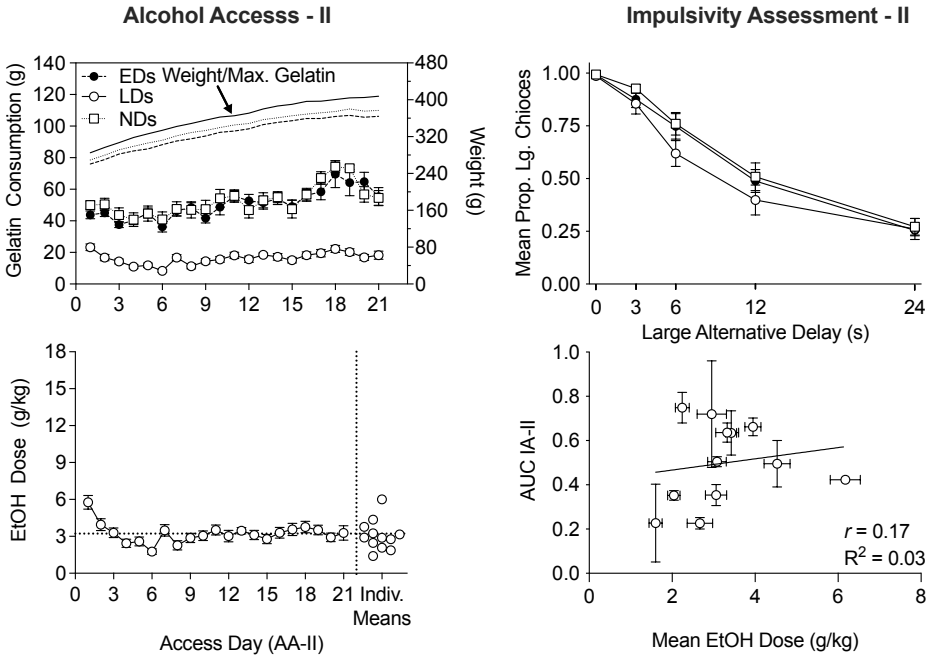

Figure 2. **Top Left**: Mean group (± SEM) consumption of raw gelatin (symbols), gelatin amount provided (lines; left y-axis) and weights (lines; right y-axis) across access days to an alcoholic (LDs) or alcohol-free gelatin (EDs & NDs). **Bottom Left**: Mean dose of alcohol consumed (LDs only). **Top Right**: Mean proportion of large alternative choices (± SEM) across delay during the three gradients of IA-II. **Bottom Right**: LDs individual and average (± SEM) AUC from IA-II plotted against individual average (± SEM) self-administered dose (g/kg) in AA-II.

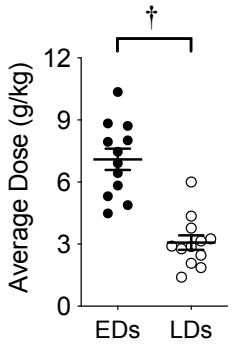

Figure 3. Mean individual dose consumed by EDs during AA-I and LDs during AA-II, with group mean (± SEM) denoted by the bar. † denotes a significant ($p < 0.0001$) between-group difference in mean ethanol dose consumed.

weight tended to increase in a negatively accelerating function that was close to asymptotic levels by the end of AA-II. Throughout the AA-II access period, the LDs weighed more on average than the rats in the other two groups. A 3 (group) × 21 (access day) Mixed ANOVA that found a main effect of access day, $F(1.86, 61.29) = 727.07$, $p < .05$, drinking group, $F(2, 33) = 5.02$, $p < .05$., but not the interaction of access day and drinking group, $F(3.71, 61.29) = 1.38$, p = n.s., after correcting for Mauchly's sphericity violation using the Huynh-Feldt method (except the main effect of drinking group). A bonferroni post hoc ($a < .166$) found the LDs were significantly heavier than the EDs ($M = 33.84$, $SEM = 10.87$), but not the NDs ($M = 22.57$, $SEM = 10.87$).

Figure 2 (top left; symbols) displays the EDs, LDs, and NDs average gelatin consumption and total amount of gelatin provided (lines; left Y-axis). In general, total consumption of the alcohol-free gelatin (EDs & NDs) was higher than the alcoholic gelatin (LDs) and tended to decrease across the first six days of access before increasing slightly (LDs) or modestly (EDs & NDs) across the remaining access days. The relative percentage of gelatin consumption versus the total gelatin provided tended to follow a similar decreasing pattern initially but then tended to increase slightly across the remaining access days. The average dose self-administered by the LDs also followed this pattern and resulted in average consumption levels around 3.1 g/kg/day (Figure 2; bottom left), which was significantly lower and slightly less than half of what was observed with the EDs during AA-I, $t(22) = 6.45$, $p < .0001$ (Figure 3). Similar to AA-I, consumption of the alcohol-free gelatin was significantly higher than the alcoholic gelatin, which was supported by a 3 (drinking group) × 3 (access week) Mixed ANOVA that found a significant main effect of drinking group $F(2, 33) = 63.94$, $p < .05$, but not of access week $F(2,66) = 1.92$, p = n.s. or their interaction, $F(4, 66) = 0.31$, p = n.s.

Following AA-II, rats were again tested for differences in impulsive choice in the delay discounting paradigm. Figure 2 (top right) displays the average proportion of large alternative choices across large alternative delay for the EDs, LDs, and NDs during each delay gradient, and their average, in IA-II. As before, preference for the large alternative decreased linearly with increases in the delay to the large alternative. A 3 (drinking group) × 5 (delay) Mixed ANOVA of the rats' average gradients supported these conclusions finding a main effect of delay, $F(2.57, 84.79) = 276.11$, $p < .05$, but not of drinking group, $F(2, 33) = 0.93$, p = n.s., or the interaction of delay and drinking group, $F(5.14, 84.79) = 1.28$, p = n.s., after correcting for Mauchly's sphericity violation using the Huynh-Feldt method (except the main effect of drinking group).

An additional analysis was conducted to determine if there was a relationship between the LDs alcohol self-administered and their level of impulsive choice, average LD doses in AA-II were plotted with their obtained AUC from each gradient and their pooled average from IA-II in Figure 2 (bottom right). There was a positive, but non-significant relationship between dose and AUC in the average gradient ($r[10] = .17$, p = n.s.) from IA-II.

The LDs slightly higher impulsive choice levels observed,

especially at the 6- and 12-s delays in IA-II (see Figure 2; top right), suggests there could have been a dose-dependent effect of alcohol consumption during AA-II on impulsive choice. Table 2 presents the percentage change in AUC from IA-I to IA-II for each group and for the LDs, the average self-administered dose from AA-II. In general, AUC tended to decrease (impulsive choice increased) from IA-I to IA-II for all groups, but the percent change was not significantly different across groups according to a one-way ANOVA nor was the change in AUC related to dose in the LDs.

Table 2. Average AUC (± SEM) from IA-I, IA-II, the percent change in AUC from IA-I to IA-II and, for the LDs, the average dose (g/kg; ± SEM) self-administered during AA-II.

	EDs			LDs				NDs		
ID	AUC (IA-I)	AUC (IA-II)	% of IA-I	AUC (IA-I)	AUC (IA-II)	% of IA-I	AA-II Dose (g/kg)	AUC (IA-I)	AUC (IA-II)	% of IA-I
01	0.76 (.04)	0.66 (.04)	86.4	0.69 (.05)	0.42 (.03)	61.2	6.2 (1.5)	0.68 (.06)	0.63 (.03)	92.5
02	0.72 (.07)	0.56 (.02)	78.2	0.73 (.03)	0.66 (.02)	90.3	3.9 (0.9)	0.79 (.02)	0.87 (.01)	110.1
03	0.65 (.01)	0.66 (.01)	102.1	0.42 (.02)	0.23 (.02)	53.8	2.7 (1.4)	0.62 (.02)	0.5 (.01)	80.9
04	0.72 (.08)	0.66 (.06)	91.8	0.62 (.04)	0.63 (.06)	102.9	3.4 (0.6)	0.58 (.02)	0.57 (.01)	97.9
05	0.59 (.04)	0.43 (.04)	73.6	0.68 (.13)	0.75 (.04)	110.4	2.2 (0.7)	0.6 (.60)	0.71 (.40)	118.2
06	0.51 (.04)	0.31 (.05)	60.8	0.25 (.08)	0.23 (.10)	91.4	1.6 (0.7)	0.8 (.03)	0.68 (.02)	84.4
07	0.63 (.03)	0.7 (.05)	111.9	0.54 (.10)	0.35 (.03)	65.8	3.1 (1.2)	0.59 (.05)	0.35 (.03)	59.7
08	0.41 (.04)	0.29 (.02)	71.2	0.65 (.01)	0.5 (.01)	77.6	3.1 (1.0)	0.45 (.02)	0.4 (.04)	87.9
09	0.64 (.03)	0.7 (.06)	110.2	0.55 (.05)	0.5 (.06)	90.2	4.5 (1.4)	0.57 (.06)	0.57 (.04)	100.5
10	0.56 (.09)	0.59 (.03)	106.4	0.84 (.06)	0.72 (.14)	85.9	3.0 (1.6)	0.78 (.04)	0.73 (.04)	93.7
11	0.65 (.07)	0.71 (.02)	108.9	0.76 (.02)	0.64 (.03)	83.2	3.3 (1.3)	0.65 (.04)	0.46 (.09)	70.4
12	0.48 (.06)	0.42 (.04)	88.0	0.4 (.04)	0.35 (.01)	88.7	2.0 (0.7)	0.52 (.08)	0.48 (.06)	93.0
Avg	.61 (.03)	.56 (.04)	90.8 (5.0)	.59 (.05)	.50 (.05)	83.5 (4.8)	3.3 (0.4)	.64 (.03)	.58 (.04)	90.8 (4.6)

To investigate whether acute doses of alcohol altered impulsive choice (see Figure 4), rats given 20-m access to a bottle containing 10% polycose (sessions 1-6) or 10% polycose/10% alcohol (sessions 7-12) prior to experimental sessions. For the NDs, this was their first exposure to alcohol. Figure 4 displays pre-session drinking amounts of an alcohol-free (10% polycose) and an alcoholic (10% polycose/10% ethanol) solution (top) prior to impulsive choice assessment sessions, the resulting dose of alcohol (middle) and the proportion of large alternative choices (bottom left) during those test sessions for the EDs, LDs and NDs during IA-III. In general, consumption of the alcohol-free solution increased in a similar fashion for all groups across the first six days of drinking and decreased once alcohol was added. The resulting doses were different across groups according to a 3 (drinking group) × 6 (drinking session) Mixed ANOVA that showed a main effect of drinking group $F(2, 33) = 11.11$, $p < .05$, drinking session $F(4.14, 136.67) = 5.173$, $p < .05$ and their interaction, $F(8.28, 136.67) = 2.17$, $p < .05$, after correcting for Mauchly's sphericity violation using the Huynh-Feldt method (except the main effect of drinking group). Specifically, the resulting doses of alcohol consumed tended to be higher for the EDs and LDs compared to NDs until the third session where LD doses dropped to ND levels and ED doses continued higher. Across the remaining sessions, doses increased similarly for all groups with ED doses remaining significantly higher than the LDs according to a post-hoc analysis of the third ($t[22] = 2.70$, $p < .05$), fourth ($t[22] = 3.28$, $p < .01$), fifth ($t[22] = 3.51$, $p < .01$) and sixth sessions ($t[22] = 3.10$, $p < .05$).

There were no acute effects of alcohol on impulsive choice with any group. This conclusion was confirmed by analyzing average individual proportion large choices for each drinking solution and conducting a 2 (drinking solution) × 3 (drinking group) Mixed ANOVA that found no main effect of drinking solution, drinking group, nor their interaction. Additionally, there was no significant relationship between the self-administered dose and the percent change in impulsive choice (Table 2) from the 10% polycose baseline (not figured).

Poulos et al. (1995) showed that the degree of impulsive choice in a delay-discounting procedure was positively related to future alcohol consumption. To examine this, the relationship between the average proportion of large choices in IA-III (sessions 1-6) and the average dose in IA-III was assessed (see Figure 4 – bottom right). Results of a Pearson product-moment correlation coefficient analysis found these measures to be unrelated, $r(34) = .11$, p = n.s.

Figure 5 displays the average total consumption (top) of an alcohol-free and alcoholic bottle, the average percent consumption (middle) from the alcoholic bottle and the relationship between this percent preference and average IA-III dose (bottom) for each group during the two-bottle choice alcohol Reinforcer assessment. In general, most rats consumed substantially more from the alcohol-free solution compared to the alcoholic one, reflected in a 2 (bottle solution) × 3 (drinking group) Mixed ANOVA that found a significant main effect of solution, $F(1, 33) = 431.20$, $p < .05$ and the interaction of bottle solution and drinking group, $F(2, 33) = 3.30$, $p < .05$, but not of drinking group, $F(2, 33) = 2.48$, p = n.s. The interaction of solution type and drinking group was due to the NDs consuming slightly less of the alcohol-free solution and slightly more of the alcoholic one compared to the other two groups. However, when converted to the percentage of total consumption form the alcoholic bottle (middle), individual percent consumption averages were similar and typically between 1-7% except for two rats (one rat consumed about 20% [ND03] and the other consumed about 70% [ND07] from the alcoholic bottle). A one-way ANOVA found no significant differences in the percentage consumption from the alcoholic bottle between groups. The relationship between the individual average percent consumption from the alcoholic bottle and their average IA-III dose (bottom) were not significant for EDs, LDs, NDs, nor their combined data (regression line not plotted for clarity).

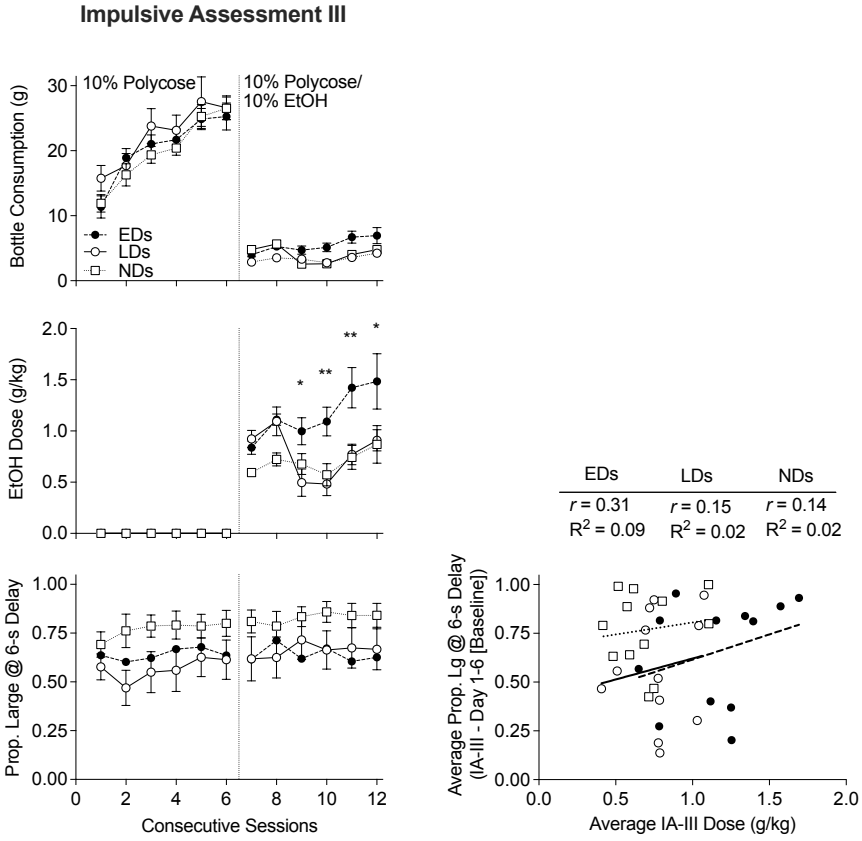

Figure 4. Pre-session consumption of an alcohol-free or an alcoholic solution (top; ± SEM), the resulting dose (middle; ± SEM; * $p < .05$; ** $p < .01$), the proportion of large alternative choices with a 6-s delay (bottom left; ± SEM) and its relationship to dose (bottom right) in IA-III.

Discussion

Chronic alcohol self-administration in adolescent or adult rats did not significantly alter impulsive choice compared to controls, with early-drinkers (EDs) and late-drinkers (LDs) showing similar delay gradients to non-drinkers (NDs). While not significant, LDs did exhibit a slight increase in impulsive choice vs. NDs (Figure 2 – top right). To determine if this was a dose-dependent effect, the relative change in impulsive choice (% change in AUC) from IA-I to IA-II was correlated with alcohol intake in AA-II, but no relationship was revealed (see Table 2). Our findings thus did not extend the results from Nasrallah et al. (2009, 2011), which linked adolescent alcohol consumption to increased risky choice. Besides the difference in what behavior was investigated (impulsive vs. risky choice), the rats in the present study consumed 35% lower doses of alcohol. Nasrallah's 3-day alcohol-free gelatin access prior to alcohol adulteration, may have led to higher gelatin and alcohol consumption. However, it stands to reason that the lack of correlation between AUC and alcohol intake for both EDs and LDs (Figures 1 and 2; bottom right) indicates that chronic alcohol consumption does not alter impulsive choice as prior experimenter-administered alcohol studies have found (Mejia-Toiber et al., 2014; Passos et al., 2015).

Several age-dependent effects were observed. During adolescence, the EDs self-administered doses (~7.3 g/kg/day) were double what the LDs self-administered (~3.3 g/kg/day) in adulthood. This heightened propensity to consume alcohol carried into IA-III where the EDs consumed twice as much of an alcoholic solution than the NDs. There was also an interaction with the dose and/or age of chronic exposure; the LDs pattern of consumption mirrored the EDs for the first two sessions of IA-III then dropped and mirrored the NDs consumption pattern (Figure 4; middle). This decrease in consumption suggests that the LDs could have been more sensitive to the adverse effects of higher doses of alcohol than the EDs. Interestingly, this higher level of drinking by the EDs did not translate into a greater preference for alcohol during the ARA (Figure 5), which suggests consumption may have been primarily determined by access to polycose rather than alcohol, per se. Regardless of the mechanism, the increased alcohol consumption of the EDs replicates and extends previous rodent research showing that: 1) adolescents consume more alcohol than adults during free-access periods (Figure 3; e.g., Broadwater, et al., 2011; Dormeus, et al., 2005; Strong, et al., 2010; Truxell, et al., 2007; Vetter et al., 2007), 2) adolescent

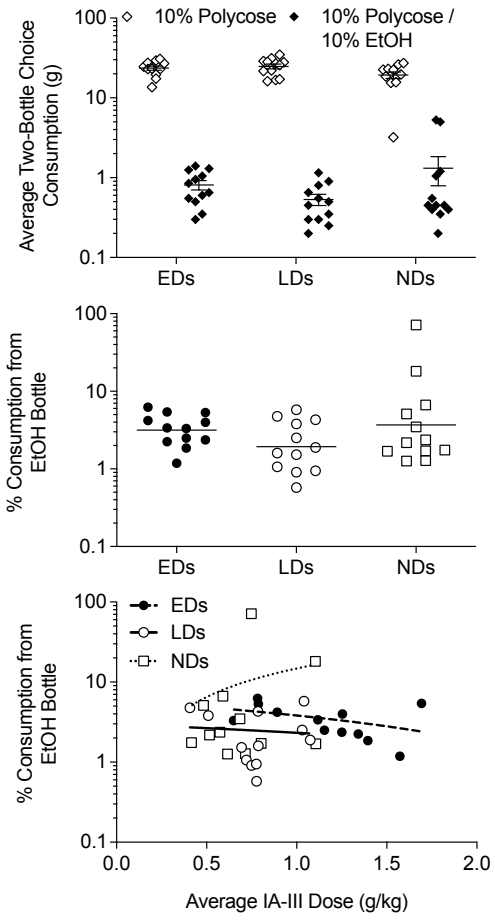

Figure 5. Total consumption (top) from the 10% polycose (open diamonds) and 10% polycose/ 10% ethanol (closed diamonds), the resulting percentage of consumption from the ethanol bottle (middle) across groups during the ARA and the relationship to average IA-III dose (bottom) for EDs ($r = -0.43$, $R^2 = 0.18$), LDs ($r = -0.08$, $R^2 = 0.01$) and NDs ($r = 0.18$, $R^2 = 0.03$).

alcohol consumption produces greater consumption of alcohol in adulthood compared to alcohol naïve adult controls (Figure 4 – middle ED vs. ND; e.g., Pascual, et al., 2009; Siciliano and Smith, 2001) and 3) chronic consumption of alcohol in adolescence produces higher levels of alcohol consumption in adulthood than controls who chronically consumed alcohol in adulthood (Figure 4 - middle ED vs. LD; Rodd-Henricks, et al. 2002a/b; Strong, et al., 2010; but see Vetter, et al., 2007). The present research extends these findings by showing that gelatin, when used as the vehicle for adolescent consumption, produces comparable effects on alcohol consumption in adulthood.

Increased impulsive choice in adulthood has also been predictive of alcohol consumption by previous researchers employing rats (Poulos et al., 1995). In the present study, however, there was no significant relationship between measures of impulsive choice (IA-I AUC [LDs] and IA-II AUC [all groups]) and subsequent alcohol self-administration (in AA-II or IA-III, respectively). One possible explanation for this difference is that alcohol preference in the Poulos study was determined using an unsweetened alcohol whereas the alcohol in the present study was sweetened, which reinforced consumption of the adulterated gelatin. Thus, voluntary consumption of unsweetened alcohol may be a better predictor of delay discounting.

Acute doses of alcohol prior to impulsive choice assessment sessions in IA-III did not systematically affect impulsive choice, regardless of the history of chronic alcohol exposure. An analysis of the percent change in impulsive choice from baseline (Table 2) revealed there was no significant relationship to dose. Even doses above 1 g/kg, a dose previously shown to increase impulsive choice (Evenden & Ryan, 1999; Hellemans, et al., 2005; Olmstead, et al., 2006; Poulos, et al., 1998; Tomie, et al., 1998), had no clear systematic effect on impulsive choice. These findings are consistent with several unpublished studies from our lab that also have not been able to replicate that acute alcohol increases impulsive choice when behaviorally active doses (i.e., > 1 g/kg) were orally self-administered prior to experimental sessions. One possible reason for our discrepant findings with the previous literature is the difference in the route of administration between our studies; orally self-administered versus experimenter injected (*i.p.* & *i.g.*).

Despite expectations that higher single-bottle consumption would predict stronger alcohol preference, alcohol preference was unrelated to the dose of alcohol consumed during free access. No correlation was found between alcohol doses self-administered from a single bottle (IA-III) and alcohol preference in a two-bottle choice (ARA) in rats. The use of 10% polycose sweetener in both alcohol and non-alcohol bottles during the preference assessment might have masked alcohol preference with polycose preference. Previous studies using sweetened alcohol (e.g., saccharin, supersac) similarly found no link between daily alcohol doses and two-bottle choice preference (Broadwater, 2001; Vetter et al., 2007), unlike studies with unsweetened alcohol, which typically show this correlation (Rodd-Henricks, 2002 a/b; Siciliano, 2001; Tolliver & Samson, 1991; but see Strong et al., 2010). Thus, sweetened alcohol self-administration does not reliably predict alcohol preference in two-bottle choice scenarios.

The use of gelatin as the vehicle for alcohol self-administration had both advantages and disadvantages. The major advantage of the alcoholic gelatin is it has been shown to facilitate daily alcohol consumption levels in adolescent rats comparable to alcohol-preferring rats (~7 g/kg/day; Bell et al., 2006), a strain used to model alcoholism. This alcoholic gelatin, therefore, allows modeling of early-onset alcoholism without the predisposition of increased impulsive present in alcohol preferring rats (Wilhelm & Mitchell, 2008). Gelatin consumption with the adult rats in the present study, however, produced chronic consumption levels of about half (~3.2 g/kg/day) that of an adult alcohol preferring rats (~7 g/kg/day; Bell et al., 2006) and two-thirds (~5 g/kg/day) that of a Sprague-Dawley rat from the original Rowland et al. (2005) study that developed this alcoholic gelatin matrix. It is still unclear why consumption was markedly lower than that reported by Nasrallah and colleagues. The main disadvantages were that the gelatin was time consuming to make, the alcohol was prone to evaporation, and rats could dig out and manipulate the gelatin.

The latter two issues affected precise measurements of alcohol intake.

The present study attempted to unravel the complex interrelationship between alcohol consumption and impulsive choice. This resulted in several findings, some of which were consistent with previous research whereas others failed to replicate previous research. Consumption of alcohol in adolescence and adulthood had no longer-term effect on choice, suggest the results of Nasrallah et al. (2009, 2011) do not extend to adolescent or adult rats that consume more moderate levels of alcohol. Likewise, acute doses of alcohol did not increase impulsive choice, suggesting that the findings of several previous studies showing acute alcohol to increase impulsive choice (e.g., Evenden & Ryan, 1999; Tomie et al., 1998) are either not replicable or do not extend to orally self-administered alcohol. The present study did, however, demonstrate that adolescents consume higher doses of alcohol and that consuming alcohol during this period may heighten alcohol consumption later in adulthood.

References

Ainslie, G., 1975. Specious reward: A behavioral theory of impulsiveness and impulse control. *Psychological Bulletin, 82*, 463–496.

Bell, R. L., Rodd, Z. A., Sable, H. J. K., Schultz, J. A., Hsu, C. C., Lumeng, L., Murphy, J. M., & McBride, W. J. (2006). Daily patterns of ethanol drinking in peri-adolescent and adult alcohol-preferring (P) rats. *Pharmacology, Biochemistry and Behavior, 83*, 35–46.

Bickel, W. K., Jarmolowicz, D. P., Mueller, E. T., Koffarnus, M. N., Gatchalian, K. M., 2012. Excessive discounting of delayed reinforcers as a trans-disease process contributing to addiction and other disease-related vulnerabilities: Emerging evidence. *Pharmacology & Therapeutics*, *134*(3), 287–297.

Bickel, W. K., Kowal, B. P., Gatchalian, K.M., 2006. Understanding Addiction as a Pathology of Temporal Horizon. *The Behavior Analyst Today*, *7* (1), 32–47.

Bickel, W. K., Miller, M. L., Yi, R., Kowal, B. P., Lindquist, D. M., Pitcock, J.A., 2007. Behavioral and neuroeconomics of drug addiction: Competing neural systems and temporal discounting processes. *Drug and Alcohol Dependence*, *90*, S85–S91.

Bickel, W. K., Odum, A. L., Madden, G. J., 1999. Impulsivity and cigarette smoking: Delay discounting in current, never, and ex-smokers. *Psychopharmacology* (Berl) *146*, 447–454.

Bjork, J. M., Hommer, D. W., Grant, S. J. & Danube, C. (2004). Impulsivity in abstinent alcohol-dependent patients: Relation to control subjects and type 1-/type 2-like traits. *Alcohol*, *34*, 133–150.

Brasser, S. M. & Spear, N. E. (2002). Physiological and behavior effects of acute ethanol hangover in juvenile, adolescent and adult rats. *Behavioral Neuroscience*, *116*, 305–320.

Broadwater, M., Varlinskaya, E. I. & Spear, L. P. Chronic intermittent ethanol exposure in early, adolescent and adult male rats: effects on tolerance, social behavior, and ethanol intake. *Alcoholism: Clinical and Experimental Research*, *35*, 1392–1403.

Courtney, K. E. & Polich, J. (2009). Binge drinking in young adults: data, definitions, and determinants. *Psychological Bulletin*, *135*, 142–156.

Crews, F. T. & Boettiger, C. A. (2009). Impulsivity, frontal lobes and risky for addiction. *Pharmacology, Biochemistry and Behavior*, *93*, 237–247.

Dom, G., D'haene, P., Hulstijn, W. & Sabbe, B. (2006). Impulsivity in abstinent early- and late-onset alcoholics: Differences in self-report measures and a discounting task. *Addiction*, *101*, 50–59.

Doremus, T. L., Brunell, S. C., Varlinskya, E. I. & Spear, L. P. (2003). Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats. *Pharmacology, Biochemistry and Behavior*, *75*, 411–418.

Doremus, T. L., Brunell, S. C., Rajendran, P. & Spear, L. P. (2005). Factors influencing elevated ethanol consumption in adolescent relative to adult rats. *Alcohol: Clinical and Experimental Research*, *29*, 1796–1808.

Erickson, K. P. & Trocki, K. F. (1992). Behavioral risk factors for sexually transmitted diseases in American households. *Social Sciences & Medicine*, *34*, 843–853.

Evenden, J. L. & Ryan, C. N. (1999). The pharmacology of impulsive behavior in rats IV: The effects of ethanol and selective serotonergic drugs on response choice with varying delays of reinforcement. *Psychopharmacology*, *146*, 413–421.

Grant, B. F., Stinson, F. S. & Harford, T. C. (2001). Age at onset of alcohol use and DSM-IV alcohol abuse and dependence: A 12-year follow-up. *Journal of Substance Abuse*, *13*, 493–504.

Hellemans, K. G. C., Nobrega, J. N. & Olmstead, M. C. (2005). Early environmental experience alters baseline and ethanol-induced cognitive impulsivity: Relationship to forebrain 5-HT1A receptor binding. *Behavioural Brain Research*, *159*, 207–220.

Hingson, R. W., Zha, W. & Weitzman, E. R. (2009). Magnitude of and trends in alcohol-related mortality and morbidity among U. S. college students aged 18-24, 1998-2005. Journal of *Studies on Alcohol and Drugs, Supplement*, *16*, 12–20.

King, K. M., Fleming, C. B., Monahan, K. C. & Catalano, R. F. (2011). Changes in self-control problems and attention problems during middle school predict alcohol, tobacco, and marijuana use during high school. *Psychology of Addictive Behaviors*, *25*, 69–79.

Kirby, K.N., Petry, N.M., Bickel, W.K., 1999. Heroin addicts have higher discount rates for delayed rewards than non-drug-using controls. *Journal of Experimental Psychology: General*, *128*, 78–87.

Mejia-Toiber, J., Boutros, N., Markou, A., Semenova, S., 2014. Impulsive choice and anxiety-like behavior in adult rats exposed to chronic intermittent ethanol during adolescence and adulthood. *Behavioural Brain Research*, *266*, 19–28.

Myerson, J., Green, L., Warusawitharana, M. (2001). Area under the curve as a measure of discounting. *Journal of the Experimental Analysis of Behavior*, *76*, 235–243.

Nasrallah, N. A., Yang, T. W. H. & Bernstein, I. L. (2009). Long-term risk preference and suboptimal decision making following adolescent alcohol use. *Proceedings of the National Academy of Science*, *106*, 17600–17604.

Nasrallah, N. A., Clark, J. J., Collins, A. L., Akers, C. A., Phillips, P. E. & Bernstein, I. L. (2011). Risk preference following adolescent alcohol use is associated with corrupted encoding of costs but not rewards by mesolimbic dopamine. *Proceedings of the National Academy of Science*, *108*, 5466–5471.

Oesterle, S., Hill, K. G., Hawkins, J. D., & Abbot, R. D. (2008). Positive functioning and alcohol-use disorders from adolescence to young adulthood. *Journal of Studies on Alcohol and Drugs*, *69*, 100–111.

Olmstead, M. C., Hellemans, K. G. C. & Paine, T. A. (2006). Alcohol-induced impulsivity in rats: an effect of cue salience? *Psychopharmacology*, *184*, 221–228.

Pascual, M., Boix, J., Felipo, V. & Guerri, C. (2009). Repeated alcohol administration during adolescence causes changes in the mesolimbic dopaminergic and glutamatergic systems and promotes alcohol intake in the adult rat. *Journal of Neurochemistry*, *108*, 920–931.

Passos, J.A.F., Pires, A.V., Scheidt, L., de Almeida, L.A., Ferreira, C.F., Gubert, C., Bizarro, L., de Almeida, R.M.M., 2015. Alcohol use in adolescence, impulsivity, and risk-taking behavior in Wistar rats. *Psychology & Neuroscience*, *8*, 130–142.

Poulos, C. X., Lê, D. A. & Parker, J. L. (1995). Impulsivity predicts individual susceptibility to high levels of alcohol self-administration. *Behavioural Pharmacology*, *6*, 810–814.

Poulos, C. X., Parker, J. L. & Lê, D. A. (1998). Increased impulsivity after injected alcohol predicts later alcohol consumption in rats: evidence for "loss-of-control drinking" and marked individual differences. *Behavioral Neuroscience*, *112*, 1247–1257.

Rodd-Henricks, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., McBride, W. J., Lumeng, L., Li, T. K. (2002a). Effect of ethanol exposure on subsequent acquisition and extinction of alcohol-seeking behavior in adult alcohol-preferring rats: I. periadolescent exposure. *Alcoholism: Clinical and Experimental Research*, *26*, 1632–1641.

Rodd-Henricks, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., McBride, W. J., Lumeng, L., Li, T. K. (2002b). Effect of ethanol exposure on subsequent acquisition and extinction of alcohol-seeking behavior in adult alcohol-preferring rats: I. periadolescent exposure. *Alcoholism: Clinical and Experimental Research*, *26*, 1642–1652.

Rowland, N. E. Nasrallah, N., & Robertson, K. L. (2005). Accurate caloric compensation in rats for electively consumed ethanol-beer or ethanol-polycose mixtures. *Pharmacology, Biochemistry and Behavior*, *80*, 109–114.

Siciliano, D. & Smith, R. F. (2001). Periadolescent alcohol alters adult behavioral characteristics in the rat. *Physiology & Behavior*, *74*, 637–643.

Silveri, M. M. & Spear, L. P. (1998). Decreased sensitivity to the hypnotic effects of ethanol early in ontogeny. *Alcoholism: Clinical and Experimental Research*, *22*, 670–676.

Silveri, M. M. & Spear, L. P. (2000). Ontogeny of ethanol elimination and ethanol-induced hypothermia. *Alcohol*, *20*, 45–53.

Spear, L. P., 2015. Adolescent alcohol exposure: Are there separable vulnerable periods within adolescence? Physiology & Behavior, *148*, 122–130.

Spear, L. P., 2007. Assessment of adolescent neurotoxicity: Rationale and methodological considerations. *Neurotoxicology & Teratology*, *29*(1), 1–9.

Strong, M. N., Yoneyama, N., Fretwell, A. M., Snelling, C., Tanchuck, M. A. & Finn, D. A. (2010). "Binge" drinking experience in adolescent mice shows sex differences and elevated ethanol intake in adulthood. *Hormones and Behavior*, *58*, 82–90.

Tolliver, G. A. & Samson, H. H. (1991). The influence of early postweaning ethanol exposure on oral self-administration behavior in the rat. *Pharmacology, Biochemistry & Behavior*, *38*, 575–580.

Tomie, A., Aguado, A. S., Pohorecky, L. A., Benjamin, D. (1998). Ethanol induces impulsive-like responding in a delay-of-reward operant choice procedure: Impulsivity predicts autoshaping. *Psychopharmacology*, *86*, 376–382.

Truxell, E. M., Molina, J. C. & Spear, N. E. (2007). Ethanol intake in the juvenile, adolescent, and adult rat: Effect of age and prior exposure to ethanol. *Alcoholism: Clinical and Experimental Research*, *31*, 755–765.

Varlinskaya, E. I. & Spear, L. P. (2002). Acute effects of ethanol on social behavior of adolescent and adult rats: Role of familiarity of the test situation. *Alcoholism: Clinical and Experimental Research*, *26*, 1502–1511.

Vetter, C. S., Doremus-Fitzwater, T. L. & Spear, L. P. (2007). Time course of elevated ethanol intake in adolescent relative to adult rats under continuous, voluntary-access conditions. *Alcoholism: Clinical and Experimental Research, 31*, 1159–1168.

Vuchinich, R. E. & Simpson, C. A. (1998). Hyperbolic temporal discounting in social drinkers and problem drinkers. *Experimental and Clinical Psychology*, *6*, 292–305.

Walker, B. M. & Ehlers, C. L. (2009). Age-related differences in the blood alcohol levels of Wistar rats. *Pharmacology, Biochemistry and Behavior*, *91*, 560–565.

Weise-Kelly, L., Siegel, S., 2001. Self-administration cues as signals: Drug self-administration and tolerance. *Journal of Experimental Psychology: Animal Behavioral Processes, 27*(2), 125–136.

White, A. M., Truesdale, M. C., Bae, J. G., Ahmad, S., Wilson, W. A. & Best, P. J. (2002). Differential effects of ethanol on motor coordination in adolescent and adult rats. *Pharmacology, Biochemistry and Behavior*, *73*, 673–670.

Wilhelm, C. J. & Mitchell, S. H. (2008). Rats bred for high alcohol drinking are more sensitive to delayed and probabilistic outcomes. *Genes, Brain and Behavior*, *7*, 705–713.

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