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Ethanol pre-exposure does not increase delay discounting in P rats, but does impair the ability to dynamically adapt behavioral allocation to changing reinforcer contingencies

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Abstract

Increased subjective discounting of delayed rewards is associated with substance abuse, and individuals tend to discount their drug of choice at a greater rate compared to monetary rewards. While there is evidence indicating that increased delay discounting (DD) is a risk factor for substance abuse, some results suggest that exposure to drugs of abuse also increases DD, but effects are mixed. The current study examined whether ethanol pre-exposure increases DD and if an ethanol reinforcer would be discounted at a greater rate than sucrose. Alcohol preferring (P) rats were pre-exposed to either ethanol or sucrose using an intermittent access protocol (IAP) for 8 weeks. Then animals completed an operant fixed choice procedure where each pre-exposure group was split into either an ethanol or sucrose reinforcer group. Afterwards, animals completed an adjusting delay DD task using the same groups as the fixed choice task. Animals that received access to ethanol in the IAP showed increased delayed reward preference in a delay and session dependent manner. Specifically, ethanol pre-exposed animals took more sessions to decrease their preference for the delayed reward at longer delays. In the adjusting delay task, no differences in mean adjusting delays were seen, but ethanol pre-exposure impaired animals' ability to reach stability criteria. The observed results are not consistent with ethanol pre-exposure causing a change in DD. Rather they indicate ethanol pre-exposure impaired animals' ability to reallocate their behavior in response to a change in reinforcer contingencies. The current findings extend prior results showing alcohol naïve P rats exhibit both increased DD and decreased response inhibition (Beckwith & Czachowski 2014; 2016) by demonstrating that after alcohol exposure they exhibit a form of behavioral inflexibility. Hence, a "two-hit" genetic vulnerability/ environmental acceleration of addictive behavior is supported.

Keywords

Rat; Impulsivity; Selected Line; ethanol exposure; Intermittent Access Protocol; Behavioral Flexibility

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1. Introduction

Delay discounting (DD) refers to the decline in subjective value attributed to an outcome as the delay to its receipt increases. This discounting of value is often examined experimentally by comparing an individual's preference for sooner, smaller rewards over larger, more delayed ones. While discounting rewards as a function of delay is a normal, adaptive process, heighted preference for immediate outcomes is considered maladaptive and a form of impulsivity. Impulsive DD, also known as increased DD, has been repeatedly shown to be associated with various forms of psychopathology, illicit drug use, and alcohol use disorders (Bickel et al., 2012; MacKillop et al., 2011)

Multiple pieces of evidence suggest that increased DD is a risk factor for addiction. In preclinical models using nonselected rodent lines, increased DD has been associated with context induced cocaine reinstatement, intake and responding for nicotine at high fixed ratio requirements, inelastic demand for cocaine, increased cocaine intake when switching from short to long access sessions, greater cocaine self-administration, and an increased likelihood and faster acquisition of cocaine self-administration (Broos et al., 2012; Diergaarde et al., 2008; Koffarnus & Woods, 2011; Anker et al., 2009; Perry et al., 2005; but see Mitchell et al., 2014 and Schippers et al., 2012). Findings using rodent lines bidirectionally selected for homecage ethanol intake suggest that DD may be a genetically correlated phenotype (Beckwith & Czachowski, 2014; Oberlin & Grahame, 2009; Wilhelm & Mitchell, 2008; Perkel et al., 2015; Linsenbardt et al., 2016). While discordant findings on whether DD is a correlated trait for homecage ethanol intake exist (Wilhelm & Mitchell, 2012, Wilhelm et al., 2007), it is likely that DD specifically tracks with alcohol seeking and not consumption per se (Beckwith & Czachowski, 2014; Stein et al., 2015). In humans, DD predicts later levels of smoking longitudinally (Audrain-McGovern et al., 2009) and mediates the protective effects of both working memory and religiousness on later alcohol and substance use, respectively (Khurana et al., 2012; Kim-Spoon et al., 2015). All of this evidence suggests increased DD is a risk factor for substance abuse.

A mutually inclusive possibility is that exposure to alcohol and other drugs of abuse can result in increased DD. Prolonged cocaine exposure has been repeatedly seen to increase DD in nonselected rodent lines (Dandy & Gatch, 2009; Mitchell et al., 2014; Paine et al., 2003; Roesch et al., 2007; Simon et al., 2007). Self-administered heroin (Schippers et al., 2012), but not chronic, non-contingent exposure (Harty et al., 2011), increases DD as does acute administration of morphine (Pitts & Mckinney, 2005; Kieres et al., 2004). Moreover, chronic, non-contingent nicotine exposure has been shown to increase DD (Dallery & Loecy, 2005) but conflicting results exist (Anderson & Diller 2010; Counotte et al., 2009). This discordance in the nicotine literature may be because an increase in DD may only be measurable in formerly low impulsive animals (Kayir et al, 2014; Kolokotroni et al., 2014). Ethanol's effects on DD are mixed, perhaps being related to the specific paradigm and manipulation. In tasks that involve a within-session shift in delay, chronic intermittent ethanol exposure along with a challenge dose has been seen to decrease DD (Mejia-Tober et al., 2014) whereas an acute dose alone causes an increase in DD (Evenden & Ryan, 1999). T-maze paradigms have consistently shown an increase in DD with acute alcohol (Olmstead et al., 2006; Poulos et al., 1998). Finally, an across-session adjusting amount procedure

yielded no effects (Richards et al., 1999). Consequently, more controlled studies investigating alcohol's effects are needed.

Substance abusing individuals tend to discount the value of their drug of choice at an accelerated rate as compared to the discounting that they demonstrate when tested with other reinforcers. In smokers, cigarettes are discounted at a greater rate than delayed monetary and health outcomes (Baker et al., 2003; Bickel et al., 1999; Johnson et al., 2007). Similarly, delayed heroin (Giordano et al., 2002), cocaine (Johnson et al., 2015), and alcohol rewards are discounted at a greater rate than delayed money (Petry, 2001) in drug and alcohol abusing individuals. Interestingly, when money serves as the immediate and cocaine as the delayed option, cocaine is discounted at an even greater rate than when cocaine is an immediate alternative (Bickel et al., 2011). Taken together, alcohol and drugs of abuse tend to be discounted at a greater rate than money in substance abusing individuals.

The current study sought to evaluate whether ethanol exposure is sufficient to cause increased DD. Furthermore, we examined whether alcohol preferring P rats, a genetic model of alcoholism, exhibit increased DD for an ethanol versus a non-drug reinforcer and if such an increase depends on prior ethanol exposure. To this end, P rats underwent an intermittent access protocol (IAP) to pre-expose them to an ethanol or sucrose solution. They then completed two different DD tasks using either ethanol or sucrose solutions as the reinforcer. It was hypothesized that increased DD would result from ethanol exposure, and an ethanol reinforcer would be discounted at a faster rate versus a sucrose reinforcer, contingent upon prior ethanol exposure.

2. Methods

2.1. Subjects

The subjects were 36 male alcohol preferring P rats (79th generation; Indiana University School of Medicine, Indianapolis, IN). At the start of the experiment, subjects were age matched at approximately 52 days with a mean weight of 187.4g (SD=14.6g). Animals had ab libitum access to food and water, however, they were briefly water restricted for operant training using previously described methods (Beckwith et al., 2016). They were singly housed in plastic shoebox cages and maintained on a 12-hour light/dark cycle with lights on at 5am. All behavioral testing was conducted during the light phase. All animal care procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals (2011) and had IACUC approval.

2.2. Operant Chambers

All operant sessions were conducted in modular chambers enclosed in sound attenuating boxes with exhaust fans and electrical inputs and outputs controlled by an IBM compatible PC (Med-Associates, St. Albans, VT; 30 × 30 24.5cm). The chambers were equipped with a nosepoke recess with an internal stimulus light and photocell, centered on the front wall 2cm above a stainless-steel bar floor. A 4,500Hz tone generator was located 18cm above the nosepoke. On both sides of the nosepoke recess were retractable levers with stimulus lights 4cm above each lever. A retractable graduated cylinder tube with a rubber stopper, double

ball bearings, stainless steel spout, and a lickometer was located opposite the nosepoke beneath a house light.

2.3. General Experimental Design

A 2×2 factorial design was used wherein animals received either 10% ethanol (v/v; 10E) or 1% sucrose solution (w/v; 1S) pre-exposure in an eight-week IAP. Two days after the final solution access, animals completed a fixed choice DD task followed by an adjusting delay DD task using either a 10E or 1S reinforcer (DDR). This reinforcer stayed the same in both tasks for each animal. These two different behavioral tasks were chosen because in the fixed choice task, delays remain the same within a daily session but change over days, while in the adjusting task they change across trials within a single daily session. By using both tasks, impairments in the ability to shift choice behavior within a daily session can be identified and ruled out as a potential alternative explanation for a change in delay discounting. Half of the animals in each IAP group were assigned to each DDR type, resulting in 4 final groups: 10E-IAP:10E-DDR, 10E-IAP:1S-DDR, 1S-IAP:10E-DDR , and 1S-IAP:1S-DDR.

2.4. Intermittent Access Protocol (IAP)

For eight weeks, animals underwent an IAP (Simms et al., 2008) using either 1S or 10E solution. Animals received three, 24-hour periods of free access two-bottle choice (MWF; 1S or 10E versus water). All access periods began one hour after the start of the dark cycle, and animal weights were recorded at this time. During the last access period of each week, intake was measured at 1 hour in addition to the 24hr intake measurement. The change in volume was determined by the change in bottle weight divided by the density of the solution. Bottle sides were alternated every period to account for any possible side preference. Two “leak” cages per solution, which were on the same racks as experimental animals but did not contain any rats, were used to estimate spillage. The mean change in the two leak cages for each day was subtracted from the intake of every rat receiving the same solution daily. When there was a clear perturbation of a subject’s intake measure for any reason (e.g., excessive leakage), the average of the period before and after was used instead.

2.5. Fixed Choice Delay Discounting (DD) Task

Operant training began with 60 minutes of free access to the sipper tube containing water in the operant box. In the next session, animals were presented with non-contingent, 15-second presentations of the sipper tube on a variable time 15-second schedule. Animals were then hand-shaped to press either lever on a FR1 schedule for 20-second presentations of the sipper tube for two sessions and then 15-second presentations for another 2 sessions. On each trial, both stimulus lights were illuminated signaling the availability of reinforcement, and extinguished during reinforcer presentation along with the levers retracting. Next, a 5-second inter-trial interval was implemented during which the levers were retracted, and all stimulus lights were extinguished. Once animals acquired lever pressing (≥20 trials in 60 minutes), forced choice trials were introduced. If the animal pressed the same lever 2 consecutive times, on the subsequent trial only the opposite lever and light were extended and illuminated signaling the only available choice. After two sessions, each new trial started with the illumination of the nosepoke’s internal stimulus light, and then a nosepoke was required to extend the levers and turn on the stimulus lights. On all trials, no limited

holds, for either trial initiation nor lever pressing, were in place. Then the sipper tube presentation was decreased to 5 seconds (3 sessions) and then 4 seconds (3 sessions) to complete training, and lever preference was assessed.

Water restriction was then removed, and either 10E or 1S became the reinforcing solution. The preferred lever was reinforced with an immediate, 2-second sipper tube presentation and the non-preferred lever was assigned a delayed, 4-second sipper tube presentation. The initial delay was 0 seconds (0sD). Daily sessions concluded when 60 minutes had elapsed or when animals completed 90 total trials. To ensure animals could discriminate the reward magnitudes, a new delay was not implemented until subjects demonstrated an average preference for the delayed option, the primary dependent measure, of ≥ .85 across a block of 5 sessions. Once this criterion was reached, delays of 2, 4, 8, and 16 seconds (2-, 4-, 8-, and 16sD) were implemented for 10 daily sessions each in ascending order.

2.6. Blood Ethanol Concentration (BEC)

After the 16-second delay was completed, a second 0sD was added for measuring blood ethanol concentrations. Tail blood samples were taken using heparinized capillary tubes at the end of the session and after 10 free choice trials had been completed (in separate animals). Samples were immediately stored on ice, centrifuged, and frozen. The concentration of ethanol was determined from a 5μL volume of plasma using an AM1 analyzer (Analox Instruments, Lundenburg, MA). Bloods samples were taken from both 1S and 10E reinforced animals to control for any stress resulting from the procedure.

2.7. Adjusting Delay DD Task

For each animal, the adjusting delay paradigm used the same delayed and immediate reward lever assignment as the fixed choice paradigm as well as the same reinforcing solution. The delay to the larger, delayed reward started at 0 seconds and then changed following each subject's choices. A delayed reward selection increased the delay on the next trial by 1 second, and an immediate reward selection decreased the delay by 1 second. Forced choice trials occurred in the same fashion as in the fixed choice paradigm. The mean adjusting delay (MAD) scores were calculated by taking the mean of the adjusting delays on each trial inside a session, and the MAD was carried over as the starting delay of the next session. A minimum of 10 sessions were conducted. Once MAD scores did not vary by 5 seconds or show a significant linear trend (α=.9) across 5 days, the average of the MAD scores from that period was taken as a measurement of DD and was the primary dependent measure from the task. A performance criterion of 20 trials per session was used. Once this stability criterion was reached, a delay of 4 and then 8 seconds was placed on the smaller, immediate reward. The delay to the larger, normally delayed reward continued to titrate in the same fashion. For all immediate reward delays, the same stability criteria applied.

2.8. Data Analyses

Log 10 transforms were used when data were determined to deviate from normality based on inspection of q-q plots, histograms, and a shapiro-wilks test; the transformed variables included free choice trial completion, median trial initiation latencies, and median choice latencies. All preference data were arcsine transformed to mitigate normality deviations as

well as any floor and ceiling effects. IAP intake was analyzed separately by solution with a mixed factorial ANOVA with the future DD reinforcer (DDR) and access period as factors. Effects of session were followed up with polynomial curve fitting to describe the effect. Polynomial curve fitting was conducted by starting with centered 0 order polynomials and working up until a higher order polynomial no longer provided a significantly better fit.

The fixed choice procedure was analyzed with a mixed factorial ANOVA with factors of IAP solution, DDR, delay, and session. Post hoc testing following significant 3-and 4-way interactions with delay used subsequent mixed ANOVAs inside of each delay with Bonferroni corrected alpha levels. Additional characterization utilized polynomial curve fitting to describe the effect of session inside each delay. Due to their very conservative nature, Scheffé tests were used as post hoc tests for the follow-up ANOVAs and to examine the effects of delay and how said effects may be changing across levels of other factors. Inside the 8sD, delayed lever preference (DLP) inside the first 10 free choice trials was compared to DLP for the last 10 trials. Animals that failed to reliably demonstrate at least 20 free choice trials (>2 sessions with <20 free choice trials) were excluded from this analysis, and the mean trial completion at the 8sD was used as a covariate. A linear regression analysis was used to determine the relationship between BEC and 10E intake, and a t-test was used to compare BECs taken after 10 free choice trials to those taken at the end of the session.

For the adjusting delay paradigm, a mixed ANOVA with factors of IAP, DDR, and immediate reward delays was used to examine MAD scores. The number of animals meeting criteria was compared with Mantel-Cox survival curves, and the number of sessions to reach criteria was analyzed with a mixed ANOVA with IAP solution and DDR as factors. To determine whether the reasons for failing to reach stability criteria (trial completion vs. MAD score variability) differed between the groups, Fishers exact tests were used to compare the proportion of animals not reaching criteria for each reason between the groups.

3. Results

3.1. IAP

Ethanol intake (g/kg; Fig. 1A) was not different between groups that would subsequently have different DDRs, $F(1,16)=0.5$, $p=.51$ but did change across access period tending to increase, $F(23,368)=6.3$, $p<.001$. Ethanol intake inside 1 hour showed a parallel pattern with no effect of DDR, $F(1,16)=0.4$, $p=.84$, and an effect of access period, $F(7,112)=6.2$, $p<.001$. Similarly, 24-hour sucrose intake (g/kg; Fig. 1B) was not different based on DDR, but after an initial increase, it decreased and leveled off. Specifically, 1S intake showed no difference based on DDR, $F(1,16)=0.8$, $p=.39$, and an effect of access period, $F(23, 368)=20.4$, $p<.001$, Sucrose intake inside 1 hour also showed no difference with regard to DDR, $F(1,16)=2.7$, $p=.12$, and an effect of access period, $F(7,112)=7.3$, $p<.001$.

3.2. Fixed Choice Task

Only one animal (10E-IAP:10E-DDR) failed to reach magnitude discrimination and was excluded from all further analyses. This left final group sizes of 9 for each combination of

IAP and DDR except for 10E-IAP:10E-DDR which had 8. The main dependent variable from the fixed choice delay discounting task was the arcsine transformed delayed lever preference (DLP). A mixed factorial ANOVA on DLP found significant main effects of delay, $F(4,124)=271.0$, $p<.001$, session, $F(9,279)=9.0$, $p<.001$, and IAP, $F(1,31)=11.0$, $p<.01$. Significant two-way interactions of delay by IAP, $F(4,124)=3.7$, $p<.01$, and delay by session, $F(36,1116)=47.4$, $p<.001$ were found as was a significant three-way interaction of delay by session by IAP, $F(36, 1116)=1.5$, $p<.05$. Finally, the four way interaction of delay by session by IAP by DDR was significant, $F(36,1116)=1.5$, $p<.05$.

To follow up the significant 3-and 4-way interactions, ANOVAs were conducted inside each delay with Bonferroni corrected alpha levels (.01). These post hoc analyses revealed that the effects of session were significant inside of all delays, $p<.001$. DLP tended to increase across sessions at the 0sD as animals went through magnitude discrimination and then began decreasing as a function of delay and session (Fig 2A & 2B). However, there was no significant effect or interaction with DDR at any delay (Fig. 2B), but there was an effect of IAP at the 8sD where 10E-IAP animals had increased DLP, $p<.001$, and an interaction of IAP and session at the 16sD, $p<.001$ (Fig 2A). All other comparisons did not yield any significant results. In sum, it appears that the IAP solution was primarily having its effect on DLP at the 8-and 16sD.

To further describe these effects, polynomial curve fitting was conducted inside of both the 8-and 16-second delays independently for 1S-and 10E-IAP animals collapsing across DDR as this factor had no significant effects or interactions at these delays. This analysis revealed that the change as a function of session took on a different form as a function of IAP. Inside the 8-second delay (Fig. 2C), ethanol IAP animals displayed a linear decrease in DLP across sessions, $F(1,998)=105761$, $p<.001$, $a=0.76$, $b=-0.05$, but 1S-IAP animals' decrease was best described by a quadratic function, $F(1,177)=7.0$, $p<.01$, $a=0.12$, $b1=-0.032$, $b2=0.008$. Inside the 16-second delay (Fig. 2D), 1S-IAP animals preference showed a floor effect at 0, and ethanol IAP animals showed a decrease best described by a quadratic function, $F(1,167)=5.1$, $p<.05$, $a=0.032$, $b1=-0.013$, $b2=0.001$.

To investigate the possibility that DLP may change within the session, the DLP in the first 10 trials of a session were compared to the last 10 trials inside the 8sD. These trial blocks were selected a priori as they capture the start and end of each session. In the omnibus mixed ANOVA, significant effects of IAP, $F(1,25)=7.1$, $p<.05$, and IAP by trial block, $F(1,25)=4.8$, $p<.05$, were observed. Two-way RM-ANOVAs inside each IAP were used as follow-up tests with Bonferroni pairwise comparisons for inside each session. Inside the 10E-IAP, effects of session, $p<.001$, and trial block by session, $p<.05$, were observed. Bonferroni follow up tests revealed that the last 10 trials displayed greater DLP relative to the first 10 trials in sessions 3, 5, 6, and 8, but there were no other differences. In sum, 10E-IAP subjects tended to switch back to the delayed lever in the last 10 trials relative to the first 10 from the 3rd session onward (Fig. 2E). Inside 1S-IAP, effects of session, $p<.001$, and trial block by session, $p<.01$, were significant. 1S-IAP animals only displayed decreased DLP in the last 10 trials relative to the first 10 in session 1. Therefore, unlike 10E-IAP animals, 1S-IAP animals showed a greater DLP only on the first 10 trials of the first session and no difference in DLP as a function of trial block for the remainder of the sessions (Fig. 2F).

Free choice trial completion was non-normally distributed and subsequently log transformed. The omnibus ANOVA found significant main effects of session, $F(9,279)=15.8$, $p<.001$, and DDR, $F(1,31)=6.3$, $p<.05$, and two way interactions of delay by DDR, $F(4,124)=8.0$, $p<.001$, and delay by session, $F(36,1116)=4.4$, $p<.001$. Post hoc ANOVAs inside each delay with Bonferonni corrected alpha (.01) levels revealed that 10E-DDR animals completed fewer trials at the 2sD, $p<.001$, and 4sD, $p<.01$ (Fig 3B). Across delays, Scheffé tests indicated 10E-DDR animals exhibited no differences in trial completion by delay, but inside of 1S-DDR animals, there were fewer trials completed at the 16sD compared to the 0sD, p<.05, 2sD, p<.01, 4sD, p<.001, and 8sD, p<.01. In sum, DDR affected free choice trial completion with 10E-DDR animals tending to complete fewer trials. However, 10E-DDR animals' trial completion did not vary by delay, whereas 1S-DDR animals showed decreased trial completion at longer delays.

Median trial initiation latencies (Fig. 3C & 3D) from each session were positively skewed, and were log transformed. We observed significant effects of delay, $F(4,124)=14.1$, $p<.001$, and session, $F(9,279)=4.5$, $p<.001$, but not IAP, $F(1,31)=0.5$, $p=.50$, or DDR, $F(1,31)=2.6$, $p=.11$. Two-way interactions of session by DDR, $F(9,279)=3.5$, $p<.001$, and delay by session, $F(36,1116)=4.2$, $p<.001$, were significant. Following up the effect of delay, Scheffé tests revealed initiation latencies tended to increase. Latencies were longer at the 16sD compared to the 0sD, $p<.001$, 2sD, $p<.001$, 4sD, $p<.001$, and 8sD, $p<.01$. The 8sD had significantly longer initiation latencies than the 2sD, $p<.01$. Bonferroni corrected ANOVA's inside of each delay were used to examine the interactions and showed latencies decreased across sessions at the 0sD, $p<.001$, and increased across sessions overall at the 4sD, p<.001, with 10E-DDR animals having shorter initiation latencies before increasing to equalize 1S-DDR rats, $p<.01$.

The median choice latencies (Fig. 4) per session were log transformed to correct a positive skew, significant effects of delay, $F(4,124)=33.6$, $p<.001$, and session, $F(9,279)=13.5$, $p<.001$, were observed. Two-way interactions of session by DDR, $F(9,279)=2.3$, $p<.05$, and delay by session, $F(36,1116)=5.3$, $p<.001$, were significant, and delay by IAP, $F(4,124)=2.3$, $p=.06$, was trending. We observed a significant 3-way interaction of delay by IAP by DDR, $F(4,124)=2.5$, $p<.05$.

For post hoc analysis, Bonferroni corrected ANOVA's inside each delay were used to follow up the significant interactions as were Scheffé tests across delays (Fig 3). These tests revealed that choice latencies decreased as animals went through magnitude discrimination, and then decreased at the longer delays. However, this delay-dependent decrease in choice latency was right shifted in 10E-IAP animals relative to 1S-IAP animals. For all animals, choice latencies decreased across sessions at the 0sD, $p<.001$. During the 4sD, choice latencies increased before decreasing, $p<.01$, with the increase in latency lasting longer in 10E-DDR animals, $p<.01$. At the 8sD, choice latencies decreased across days overall, p<.001, and 10E-IAP animals trended towards longer latencies, $p=.051$, and at the 16sD, 10E-IAP animals had longer latencies initially but not after several sessions, $p<.01$ (Fig 3A). Comparing across delays with Scheffé tests (Fig. 3B), choice latencies were shorter at the 8sD versus the 0sD, p<.001, 4sD, $p<.001$, trending at the 2sD, p=0.055., and the 16sD had shorter choice latencies compared to every other delay, p<.001. However, this pattern did not

hold across the different IAPs. Inside the 10E-IAP animals, only the 16sD had shorter choice latencies, p<.001 versus all other delays. The 1S-IAP animals showed shorter choice latencies at the 8sD versus the 0sD, p<.001, 2sD, p<.05, and 4sD, p<.01, and shorter latencies at the 16sD versus the 0sD, p<.001, 2sD, p<.001, and 4sD, p<.001.

In 1S-DDR animals, sucrose intake in g/kg (Fig. 5A) increased as animals went through magnitude discrimination, but then decreased as function of delay. The mixed factorial ANOVA revealed a main effect of delay, $F(4,64)=24.0$, $p<.001$, and session, $F(9,144)=4.8$, $p<.001$, but not IAP, $F(1,16)=0.5$, $p=.471$. A significant two-way interaction of delay by session was observed, $F(36, 576)=4.3$, $p<.001$. ANOVAs inside each delay determined that at the 0sd intake increased across session, $p<.001$, but at the 4sD, $p<.001$, 8sD, $p<.001$, and 16sD, $p<.001$, intake decreased across sessions. Animals' sucrose intake tended to decrease with increases in delay. Intake was significantly less at the 16sD versus the 2sD, $p_{\cdot}<.001$, 4sD, $p<.001$, and 8sD, $p<.01$.

In 10E-DDR animals, ethanol intake in g/kg (Fig. 5B) increased across sessions at the 0sD as animals went through magnitude discrimination, resulting in slightly lower total intake at the 0sD compared to other delays. Otherwise, ethanol intake did not change as delay increased. The overall ANOVA showed a significant effect of delay, $F(4,60)=3.9$, $p<.01$, and session, $F(9,135)=2.4$, $p<.05$. The two-way interaction of delay by session was significant, $F(36,540)-3.9$, $p<.01$. Follow up ANOVAs inside each delay revealed at the 0sD there was a trend for intake to increase across days, $p=.032$. At the 4sD, p<.01, and 16sD, p<.001, intake varied from session to session in a see-saw pattern such that it was increased on the first day of each week after 2 days without ethanol. Across delays only the 0sD showed decreased intake relative to the 2sD, p<.05, and the 8sD, p<.01.

3.3. BECs

Ethanol intake (g/kg; Fig. 5C & 5D) was significantly associated with BEC when animals were allowed to complete the full session, $F(1,7)=12.8$, $p<.01$, $a=-13.95$, $b=59.53$, but not when subjects only finished 10 free choice trials, $F(1,7)=2.9$, p=.17. After the full session, BECs were at "pharmacologically relevant" [i.e., at levels shown to be discriminate in rats (Hodge et al., 2001)] ($M=53.34$mg/dl, 50=40.97). When samples were collected after only 10 free choice trials were completed, BECs were lower $t(16)=2.9$, $p<.05$, and likely not "pharmacologically relevant" ($M=13.16$, $SD=8.89$).

3.4. Adjusting Delay Task

Due to the surprising findings in the fixed choice task, animals then subsequently completed an adjusting delay DD task. In this task, no differences were found in the primary measure of DD. In animals that achieved stability, MAD scores did not systematically vary based on any factor except immediate reward delays (Fig. 6). There was no effect of IAP, $F(1,29)=0.3$, p=.60, DDR, $F(1,29)=0.3$, p=.59, or IAP by DDR, $F(1,29)=0.2$, p=.65. For animals which reached stability through the 4 second immediate reward delay, MAD scores increased with immediate reward delays, $F(1,23)=25.7$, $p<.001$, but did not differ based upon IAP, $F(1,23)=0.8$, $p=.39$, or DDR, $F(1,23)=0.3$, $p=.59$. Two-way interactions of immediate reward delays by IAP, $F(1,23)=0.2$, p=.68, immediate reward delays by DDR, $F(1,23)=0.4$, p=.53,

and DDR by IAP, $F(1,23)=0.8$, p=.37, were not significant; nor, was the three-way interaction significant, $F(1,23)=0.1$, p=.74.

More 10E-IAP animals failed to reach stability criteria, primarily due to variability in their MAD scores . Across immediate reward delays of 0, 4, and 8 seconds 88%, 71%, and 12% of 10E-IAP animals reached stability criteria as oppose to 100%, 83%, and 56% for 1S-IAP animals. For DDR, 89%, 82%, and 35% of 10E-DDR animals and 94%, 72%, and 33% of 1S-DDR animals reached stability criteria for immediate reward delays of 0, 4, and 8 seconds respectively (Fig 7A & 7B). Mantel-Cox survival curve analysis revealed that 10E-IAP animals were less likely to reach stability criteria, $\chi^2(1)=6.2$, $p<.05$, but the survival curves were not significantly different based on DDR, $\chi^2(1)=0.1$, $p=.72$. Among animals that reached stability criteria, 10E-IAP animals also appeared to require more sessions compared to 1S-IAP animals (Fig. 7C). Looking at the number of sessions required to reach stability (only through the 4 second delay to the immediate reward), there was an effect of immediate reward delays, $F(1,23)=124.1$, $p<.001$, IAP, $F(1,23)=9.2$, $p<.01$, and the two way interaction of delay by IAP was significant, $F(1,23)=8.6$, $p<.01$. Scheffé follow up tests revealed 10E-IAP animals took more sessions to reach criteria, and this effect was driven by 10E-IAP animals taking more sessions at the 4sD, $p<.01$. The causes for animals failing to reach criteria were classified dichotomously as either MAD score variability or trial completion (Fig 7D). A Fishers exact test revealed the reasons for failing to meet criteria did not differ between 1S-IAP and 10E-IAP animals, $p=.60$. However, across all animals, failing to reach criteria tended to be due to MAD score variability, $\chi^2(1)=8.9$, $p<.01$.

4. Discussion

Overall, these findings do not indicate that ethanol pre-exposure increased DD as originally hypothesized. Rather, our findings as a whole point to ethanol pre-exposure causing a deficit in subjects' ability to quickly switch and maintain their behavioral allocation in response to changing reinforcer contingencies. While 10E-IAP animals surprisingly show a delay-and session-dependent *increase* in preference for the delayed option as compared to the 1S-IAP animals, the pattern of change in DLP was indicative of 10E-IAP animals taking longer to change their preference over sessions (Fig 2). For instance, at the 8sD, 10E-IAP animals showed a steady linear change in their preference for the immediate reward. By contrast, the 1S-IAP animals had a curvilinear change with a steeper initial slope followed by a shallower one. This slower change in DLP was also seen at the longest delay (16sD) where 10E-IAP animals took several sessions for DLP to eventually reach 0, but 1S-IAP animals essentially reached the zero floor in the first session. In addition, increased MAD scores were not observed in the adjusting delay task. In this second task, fewer 10E-IAP animals reached stability criteria and they took longer to do so. If both delay discounting paradigms had not been conducted, and if a careful analysis of both within and across session responding had not been conducted, it would have appeared that P rats pre-exposed to ethanol were actually less impulsive than those without ethanol pre-exposure. However, the big picture indicates that all animals eventually demonstrated a similar preference for the immediate reward at long delays across sessions, but that ethanol pre-exposure resulted in animals taking more sessions to ultimately "switch" to the immediate choice.

The within session change in DLP also corroborates the conclusion that ethanol pre-exposure causes a deficit in subjects' ability to quickly switch and maintain their behavioral allocation when the demands of the task change. In 10E-IAP animals, no difference in DLP was observed between the first and last 10 free choice trials in the first 2 daily sessions of the 8sD. Alternatively, 1S-IAP animals showed such a decrease during the first daily session. This suggests that sucrose pre-exposed subjects have an ability to quickly detect changed reinforcer contingencies and adjust their behavior that ethanol pre-exposed animals do not possess. For subsequent sessions where delay remained unchanged within the session, 1S-IAP animals showed no difference between the trial blocks, but in 10E-IAP animals, DLP was actually greater in the last versus first 10 trials. This difference suggests that ethanol exposed animals may exhibit a form of regressive error wherein they return to behaving based upon prior contingencies over the course of the session.

The combination of findings with regard to trial completion, initiation latencies, and choice latencies also support an impairment in 10E-IAP animals' ability to dynamically reallocate their behavior when the reinforcement contingencies change. The IAP solution did not affect trial completion or initiation latencies at any delay. However, the IAP caused longer choice latencies for the 10E-IAP animals at the 8sD, and at early sessions of the 16sD. Moreover, the decrease in choice latencies as a function of delay did not occur as quickly in 10E-IAP animals as compared to 1S-IAP animals (i.e., the effect of delay inside 10E-IAP subjects was right shifted relative to 1S-IAP animals). A decrease in motivation is insufficient to explain this pattern, since one would expect a corresponding decrease in trial completion and initiation latencies paralleling the choice latencies, which was not observed. Alternatively, the longer choice latencies in 10E-IAP animals could indicate that 10E-IAP animals needed more time to process their choice and may have had more difficulty making decisions at the 8-and 16sD. These longer delays are when differences in DLP were seen between the IAP groups, and when subjects should have been changing their choice strategy. If animals were impaired in their ability to switch their behavioral allocation in response to these changing contingencies, these choices would be more difficult for them, and an increase in choice latencies, similar to what was observed, would be predicted.

The adjusting delay task also provides additional evidence for a behavioral flexibility deficit in 10E-IAP animals and not a difference between groups in DD, per se. No difference in MAD scores, the task's primary measure of DD, was seen as a function of any factor. Rather, fewer 10E-IAP animals met stability criteria and among those that did, it took them more sessions. The primary reason for not reaching stability criteria was MAD score variability, not trial completion. The increased time and inability to meet stability criteria could result from animals not being able to appropriately modify their behavior in response to the changing delay to the larger reward, thereby leading to increased variability in MAD scores.

Alcohol exposure causing a behavioral flexibility deficit is consistent with the body of literature examining the effects of ethanol exposure on attentional set-shifting and reversal learning tasks. Chronic intermittent ethanol has been linked to disruptions in both reversal learning and extradimensional shifts, potentially via an increase in both regressive and perseverative errors (Badanich et al., 2011). This disruption also occurs when animals

undergo intermittent ethanol vapor exposure as adolescents (Gass et al., 2014). Forced ethanol exposure, by including alcohol in animals' drinking water and via oral gavages, has also been seen to cause similar behavioral flexibility deficits (Fernandez et al., 2017; Vedder et al., 2015). Therefore, it is possible that the 10E-IAP animals had more difficulty switching to and maintaining a new choice strategy when delays to reinforcement increased. Alternatively, our findings could be argued to be consistent with chronic ethanol consumption impairing goal-directed response systems and facilitating habitual behavior as reviewed by Vandaele and Janak (2017). This possibility was not explicitly tested, and this explanation fails to account for the within session regression to the delayed outcome on the last 10 versus first 10 trials. However, increased insensitivity to outcome value could result in a slower transition away from a response when its outcome value is decreased. In paradigms where animals are concurrently responding for two different flavored solutions and one of the flavors is paired with lithium chloride, habitual behavior is evidenced by animals not transitioning away from the devalued solution (Colwill & Rescorla, 1985). Increasing the delay to a reward essentially lowers its outcome value. Thus, the 10E-IAP animals' slower transition away from the delayed outcome with increases in delay may result from an impairment in goal-directed responding.

With regard to the effects of the two different reward solutions in the DD task, there was a differential pattern in the secondary variables as a function of DDR. However, the pattern is consistent with the 10E-DD animals titrating to a specific dose of ethanol versus a behavioral allocation impairment. For instance, 10E-DDR subjects completed fewer trials than 1S-DDR animals, but 10E-DDR trial completion stayed constant across delay. On the other hand, 1S-DDR trial completion decreased as delay increased. In other words, 10E-DDR intake stayed relatively constant across all delays at an average of 1g/kg whereas sucrose intake decreased with increases in delay. Initiation latencies increased as a function of delay, but 10E-DDR animals consistently trended towards lower initiation latencies versus 1S-DDR animals at the 4sD and higher. Finally, 10E-DDR and 1S-DDR animals showed no difference in choice latencies at the earlier delays. However, once preference started to shift, 10E-DDR animals showed greater increases in choice latencies across sessions at the 4sD and trended towards greater choice latencies at the 16sD versus 1S-DDR animals. The pattern seen across these four variables can be explained by 10E-DDR animals titrating to a specific dose of ethanol. This interpretation accounts for why 10E-DDR animals do not decrease trial completion as a function of delay, but rather critically maintain a constant level of intake that does not decrease as delay increases. 1S-DDR animals, that are presumably not attempting to titrate to a specific "dose", decrease their trial completion, increase initiation latencies, and decrease their intake as the delay increases. The increased choice latencies in the 10E-DDR versus 1S-DDR animals, despite decreased initiation latencies, may be explained by 10E-DDR animals contending with a dissonance caused by continuing to respond to obtain a specific ethanol dose despite the overall task becoming less reinforcing. In sum, the results suggest 10E-DDR animals responded until they reached a certain level of ethanol intake, regardless of other factors.

There is a body of literature suggesting that alcohol and other drugs of abuse are discounted at a faster rate than non-drug outcomes (Bickel et al., 1999; Petry, 2001; Giordano et al., 2002; Baker et al., 2003; Johnson et al., 2007; Johnson et al., 2015). We failed to find such a

commodity effect for an ethanol versus a sucrose reward. However, the aforementioned studies utilized monetary rewards as the standard for comparison, which are secondary reinforcers. When the discount rates for alcohol and drugs of abuse are compared to other primary reinforcers and directly consumable rewards, no differential rate of discounting is observed and these directly consumable rewards (e.g., chocolate) are discounted faster than money as well (Odum & Rainaud, 2003; Odum et al., 2006; Estle et al., 2007; Odum & Baumann et al., 2007; Jiga-Boy et al., 2013; Friedel et al., 2014). Hence, rather than ethanol and other pharmacological reinforcers having an accelerated rate of DD, it may be the case that secondary reinforcers, or at least monetary outcomes, are actually discounted at a slower rate.

In conclusion, the primary finding of this study is that eight weeks of IAP ethanol pre-exposure induced a deficit in animals' ability to reallocate their behavior in response to changing reinforcer contingencies. While the observed impaired dynamic allocation of behavior precludes strong conclusions about alcohol exposure's effects specifically on delay discounting, we did not find evidence for ethanol increasing this measure of impulsivity. These findings add to a body of work showing that alcohol preferring P rats exhibit elevated levels of impulsivity prior to any exposure to ethanol as measured using DD (Beckwith & Czachowski, 2014; Perkel et al., 2015; Linsenbardt et al., 2016) and stop signal reaction time (Beckwith & Czachowski, 2016). Combined, these findings support a "two-hit" genetic vulnerability/environmental acceleration of addictive behavior in that the same rats that showed excessive DD and SSRT also show inflexibility in adjusting their behavior if they have prior alcohol exposure. Our findings suggest that chronic alcohol use can impair an individual's ability to adapt to new circumstances which could explain why alcoholics find it difficult to stop drinking even in the absence of the previously reinforcing effects of alcohol that were present early in their drinking history. This inability to cut back or reduce substance use is a hallmark of addiction. Overall, findings revealing both a preexisting heightened impulsivity as a risk factor as well as a vulnerability to alcohol's effects in subjects with a "family history" of alcohol have implications for prevention as well as treatment of alcohol use disorders.

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Highlights

- The current study examined whether ethanol pre-exposure increases delay discounting and if an ethanol reinforcer would be discounted at a greater rate than sucrose.
- Alcohol preferring (P) rats were pre-exposed to either ethanol or sucrose for 8 weeks, then completed an adjusting delay discounting task using ethanol or sucrose (resulting in four separate groups by pre-exposure and delay discounting reinforcer).
- Animals that received pre-exposure to ethanol took more sessions to decrease their preference for the delayed reward at longer delays.
- The observed results are not consistent with ethanol pre-exposure causing a change in delay discounting, rather they indicate that ethanol pre-exposure impaired animals' ability to reallocate their behavior in response to a change in reinforcer contingencies.

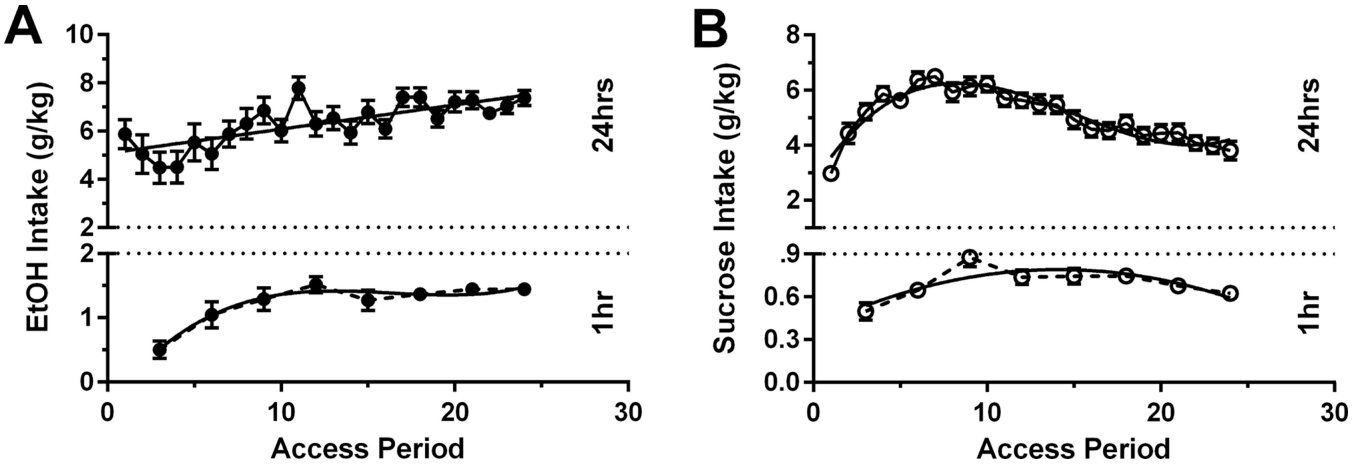

Figure 1:

Ethanol and sucrose intake during the 8-week IAP. Both 24hr (top panels) and 1hr (bottom panels) showed no differences as a function of future DD reinforcer and were collapsed accordingly. However, significant effects of access period were seen for all time periods and solutions. **A)** Mean (±SEM) ethanol intake (g/kg) plotted as a function of access period along with the best fitting polynomial functions. **B)** Mean (±SEM) sucrose solution intake graphed by access period with best fitting polynomial functions.

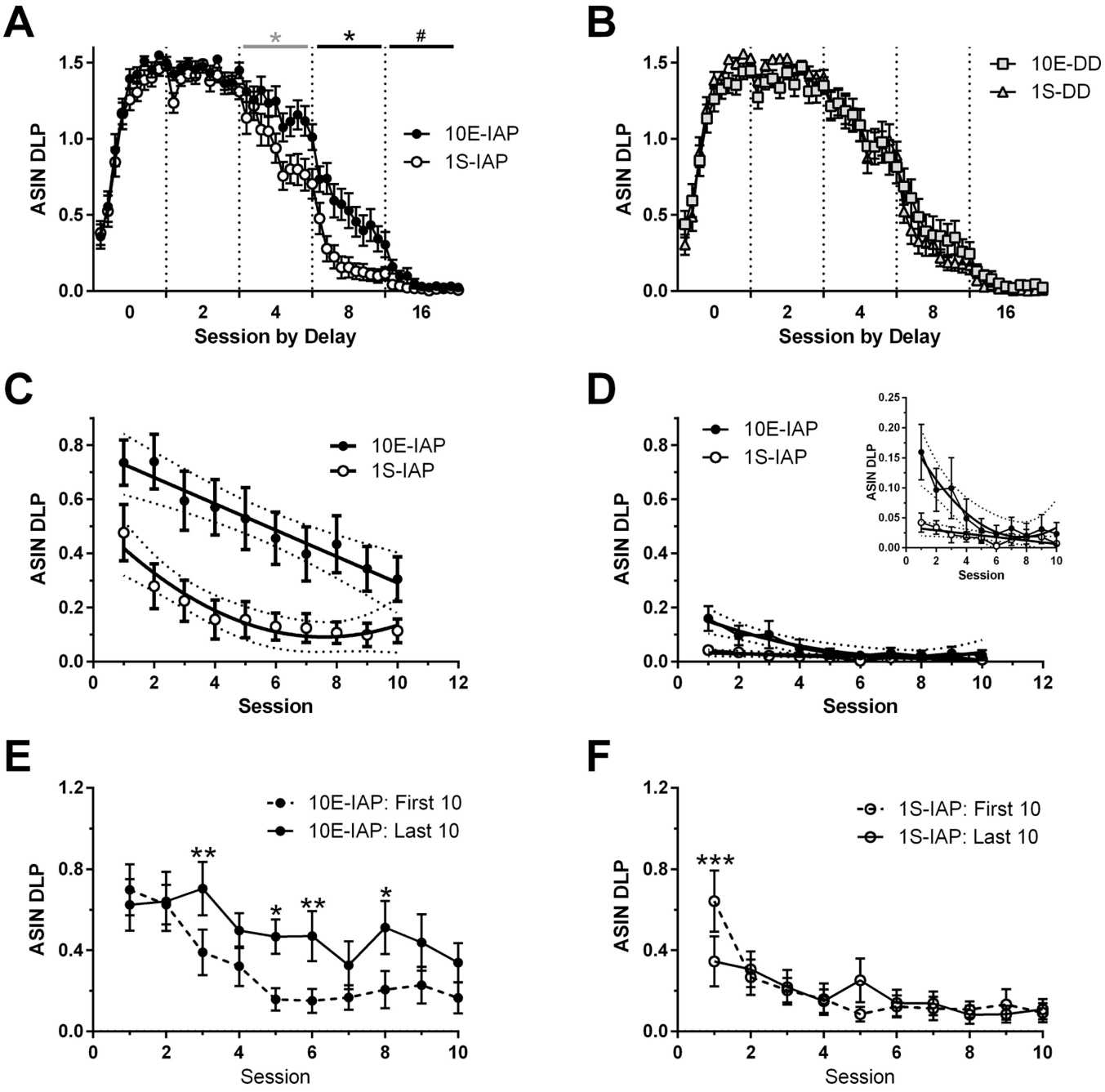

Figure 2:

Arcsine (ASIN) transformed delayed lever preference (DLP) from the fixed choice delay discounting task. Data are collapsed across both DDR and IAP as no significant interactions of these two factors were seen at any individual delay. **A)** Mean (±SEM) ASIN DLP plotted as a function of session by delay by IAP. *(grey) trend for 10E-IAP vs. 1S-IAP; *p<.05 10E-IAP vs. 1S-IAP; #p<.05 for IAP by session interaction. **B)** Mean (±SEM) ASIN DLP plotted as a function of session, delay, and DDR. **C)** Mean (±SEM) plotted as a function of session inside the 8sD along with the best fitting polynomial. **D)** Mean (±SEM) plotted as a function of session inside the 16sD along with the best fitting polynomial. The inset shows the same

data with a different Y axis to aid in visualization. **E)** Mean (±SEM) ASIN DLP in the first and last 10 trials within 10E-IAP animals as a function of session. *p<.05 for first 10 trials vs. last 10 trials; **p<.01 for first 10 trials vs. last 10 trials. **F)** Mean (±SEM) ASIN DLP in the first and last 10 trials within 1S-IAP animals as a function of session. ***p<.001 for first 10 trials vs. last 10 trials.

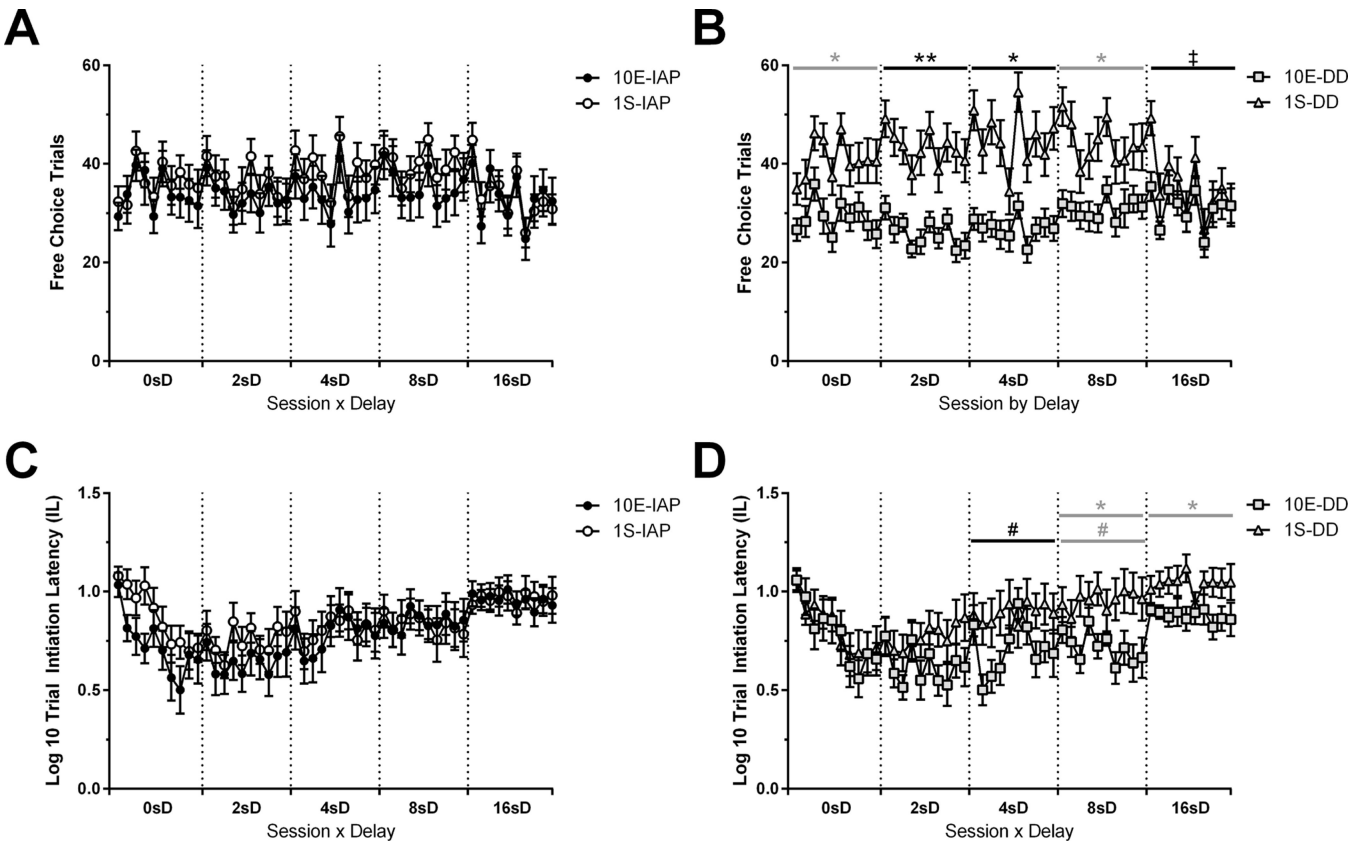

Figure 3:

Free choice trial completion and trial initiation latencies from the fixed choice DD task plotted as a function of session and delay. **A)** Mean (±SEM) free choice trials plotted as a function of IAP. **B)** Mean (±SEM) free choice trials plotted as a function of DDR. *(grey) trend for DDR; *p<.05 for 10E-DD versus 1S-DD; **p<.01 for 10E-DD versus 1S-DD; ‡ $p_{scheffe}$<.05 for 16sD versus all other delays inside 1S-DD. **C)** Mean (±SEM) initiation latencies plotted as a function IAP. **D)** Mean (±SEM) initiation latencies plotted as a function of DDR. *(grey) trend for DDR. #p<.05 session by DDR; #(grey) trend for session by DDR.

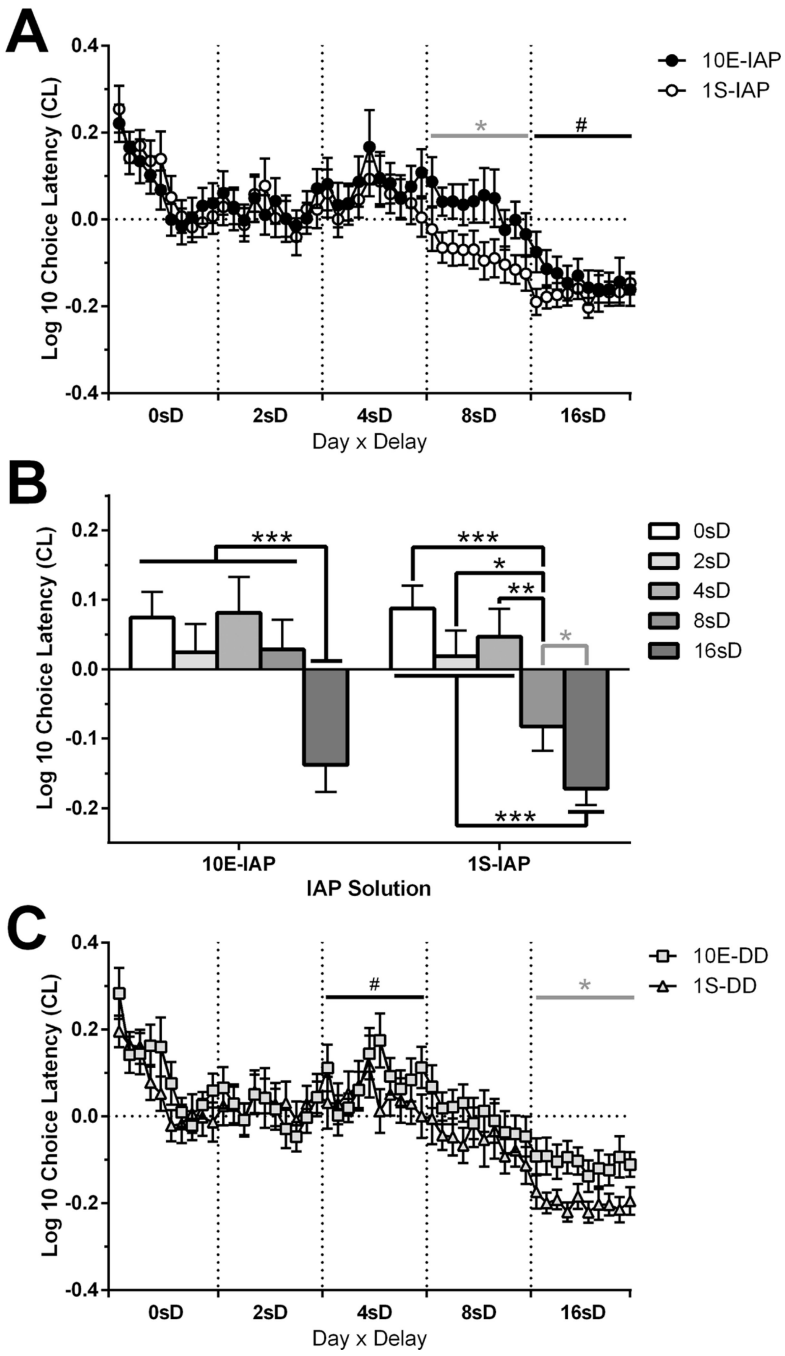

Figure 4:

Log 10 transformed choice latencies from the fixed choice delay discounting task. Data are collapsed across either IAP or DDR due to a lack of an interaction between these factors. **A)** Mean (±SEM) Log 10 choice latencies plotted as a function of session, delay, and IAP. *(grey) trend for 10E-IAP vs. 1S-IAP; # IAP by session interaction p<.05. **B)** Bar graph of mean (±SEM) log 10 choice latencies as a function of delay and IAP. *(grey) trend; *p<.05; **p<.01; ***p<.001. **C)** Log 10 mean (±SEM) choice latencies plotted as function of session, delay, and DDR. *(grey) trend for 10E-DD vs. 1S-DD; # session by DDR p<.05.

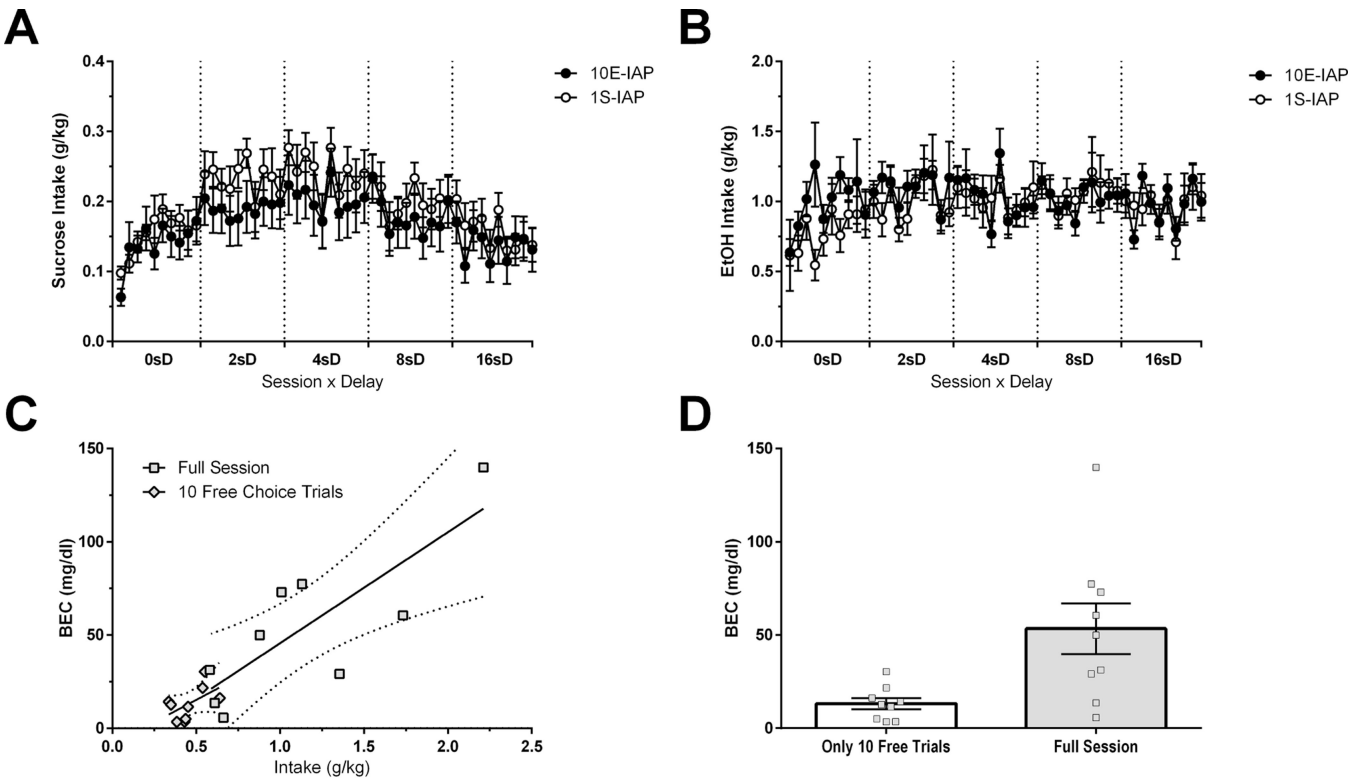

Figure 5:

Sucrose and ethanol intake during the fixed choice delay discounting task and BEC determination. **A)** Mean (±SEM) sucrose intake in g/kg plotted as a function of session, delay, and IAP. **B)** Mean (±SEM) ethanol intake in g/kg graphed by session, delay, and IAP. **C)** Scatter plot of blood ethanol concentration (BEC) plotted as a function of ethanol intake (g/kg) pending whether animals were allowed to complete the whole session or only the first 10 trials. Dotted lines indicate the 95% confidence band. **D)** Mean (±SEM) BEC graphed by when blood was sampled.

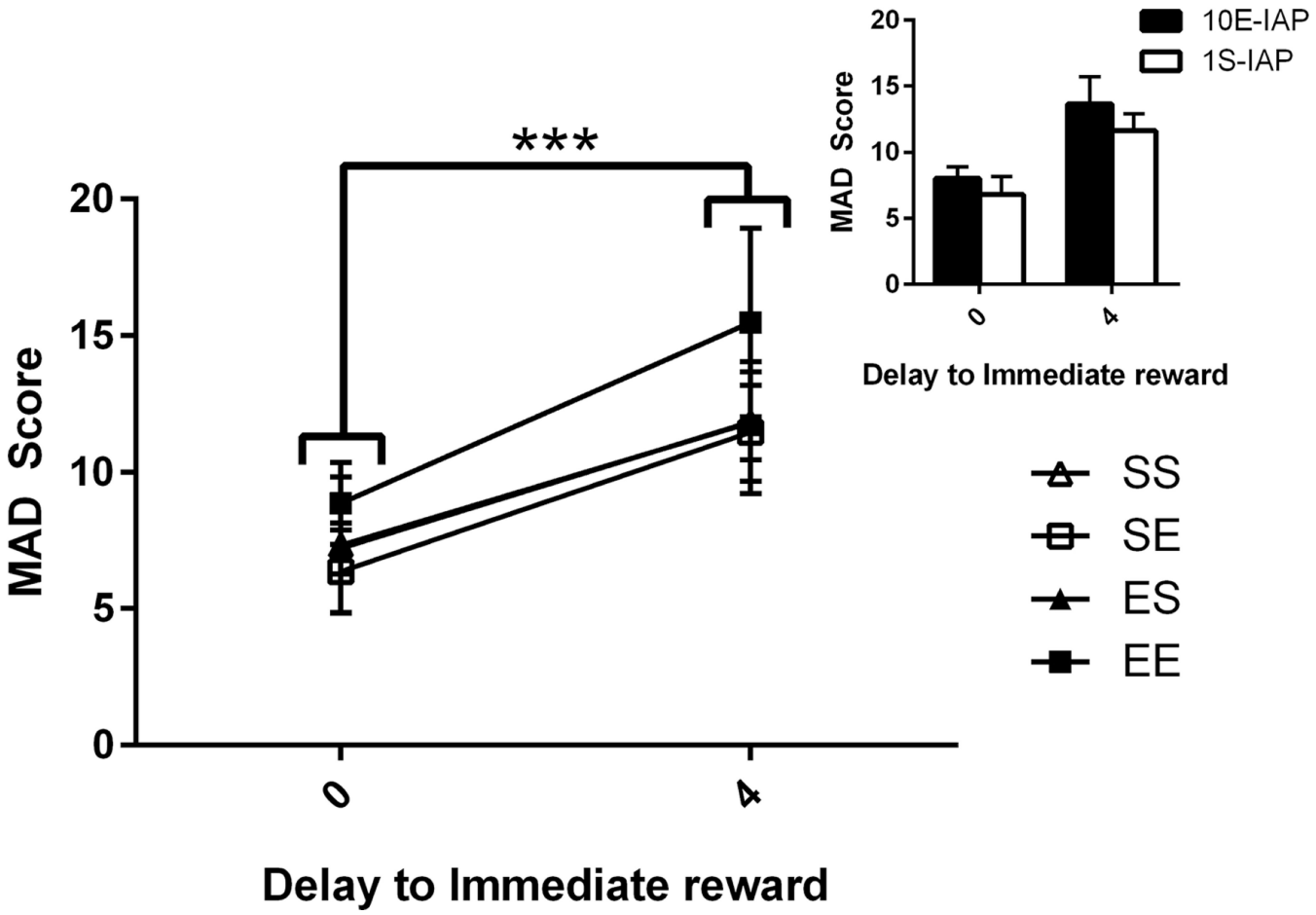

Figure 6:

Mean adjusting delay (MAD) scores from the adjusting delay DD task. Mean (±SEM) scores graphed by group, delay to the immediate reward, and by IAP (inset). ***p<.001 0 vs. 4 second delay to the immediate reward.

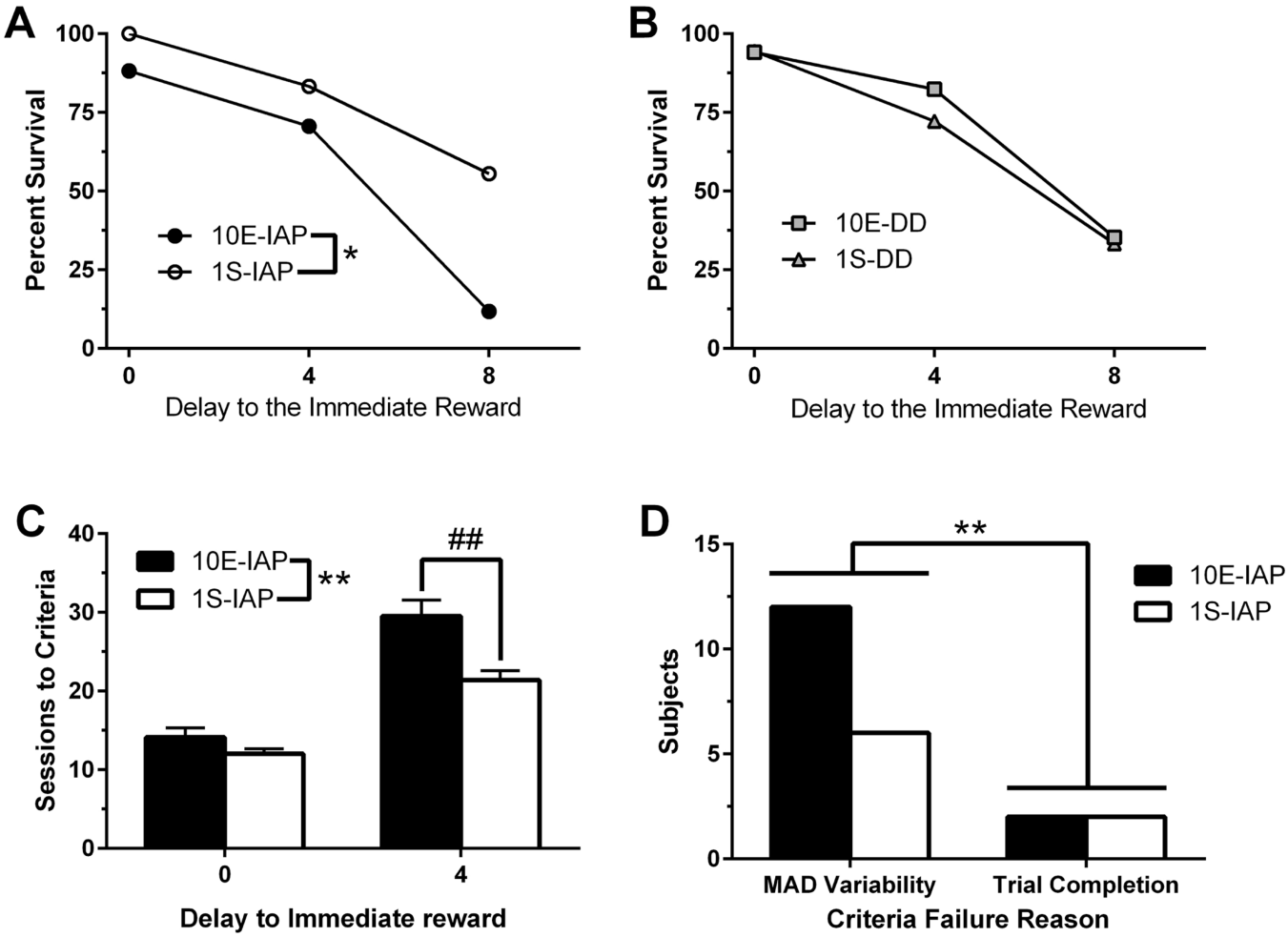

Figure 7:

Analysis of animals' ability to meet stability criteria. **A)** Percentage of animals reaching stability criteria (survival) graphed by delay and IAP. *p<.05 for IAP. **B)** Survival percentage for meeting stability criteria based on DDR and delay to the immediate reward. **C)** Mean (±SEM) number of sessions to reach criteria graphed based on delay to immediate reward and IAP. **p<.01 for main effect of IAP; ## p<.01 for 1S-IAP vs. 10E-IAP at the 4 second delay. **D)** Total subjects that failed to reach stability criteria graphed based on IAP and cause of instability. **p<.01 for MAD score variability vs. trial completion.