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Increased impulsivity in rats as a result of repeated cycles of alcohol intoxication and abstinence

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Abstract

Impulsivity is a risk factor for alcoholism and long-term alcohol exposure may further impair impulse control in a manner that propels problematic alcohol use. The present study employed the rat 5-Choice Serial Reaction Time Task (5-CSRTT) to measure behavioral inhibition and attentional capacity during abstinence from repeated 5d cycles of alcohol liquid diet consumption. Task performance was not disrupted following the first cycle of alcohol exposure, however, evidence of impaired behavioral inhibition emerged following the third cycle of alcohol exposure. In comparison with controls, alcohol rats exhibited deficits in inhibitory control during cognitively challenging 5-CSRTT tests employing variable inter-trial intervals (varITI). This behavioral disruption was not present during early abstinence (3d) but was evident by 7d abstinence and persisted for at least 34d. Interestingly, renewed alcohol consumption ameliorated these disruptions in impulse control, though deficient behavioral inhibition re-emerged during subsequent abstinence. Indices of increased impulsivity were no longer present in tests conducted after 49 days of abstinence. Alcohol-related impairments in impulse control were not evident in sessions employing highly familiar task parameters regardless of abstinence period and control experiments confirmed that performance deficits during the challenge sessions were unlikely to result from alcohol-related disruption in the adaptation to repeated varITI testing. Together, the current findings demonstrate that chronic intermittent alcohol consumption results in decreased behavioral inhibition in rats that is temporally similar to clinical observations of disrupted impulsive control in abstinent alcoholics performing tasks of behavioral inhibition.

Keywords

abstinence; alcoholism; attention; 5-Choice Serial Reaction Time Task; cognitive impairment; impulsivity

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Author contributions

LP and CI designed the experiments. CI and IP performed the experiments. JW, TdV, TP provided key tools and help with 5-CSRTT training. CI and LN analyzed the data. LN provided expertise regarding statistical analyses. CI and LP wrote the paper with help from JW, LN, TdV and TP.

INTRODUCTION

Alcohol dependence is associated with significant impairments across cognitive domains such as inhibitory control/impulsivity, attention, working memory and other executive functions (De Wit, 2008; Stavro et al., 2012). Deficits in cognitive processing are considered to be central to addiction as they likely underlie difficulty in reversing behaviors related to drug taking that propel continued drug use despite negative consequences (Bowden-Jones et al., 2005; Duka et al., 2011a; Rubio et al., 2008).

Impulsivity is a multi-dimensional construct that includes poor inhibition of automatic or reward-driven responses (impulsive action) and impaired choice processing (impulsive choice) (Dalley et al., 2011; De Wit, 2008; Eagle and Baunez, 2010; Evenden, 1999; Pattij and Vanderschuren, 2008). Impaired impulse control is a hallmark of addictive behavior (Goldstein and Volkow, 2011) and is an important predictor of relapse (Bowden-Jones et al., 2005; MacKillop and Kahler, 2009). A body of clinical research demonstrates a substantial association between increased impulsivity and alcoholism (Lejuez et al., 2010). For example, alcohol dependent subjects exhibit higher impulsivity on the Barratt Impulsivity Scale (BIS) (Mitchell et al., 2005) and detoxified alcoholics show poor inhibitory control in the Continuous Performance Task (CPT) (Bjork et al., 2004), Go/No-Go task (Kamarajan et al., 2005) and Stop Signal Serial Reaction Task (SSSRT) (Lawrence et al., 2009).

A persistent question in alcohol research is the relative influence of pre-existing cognitive impairment that may confer vulnerability to problem drinking versus cognitive deficiencies that result from alcohol-induced neurophysiological disruptions. Several lines of evidence suggest that premorbid impulse disorders contribute to the initiation of problem drinking. Adolescents exhibiting behavioral disinhibition are more likely to use drugs and alcohol and to begin drinking at an earlier age (McGue et al., 2001). Early-onset drinking and subsequent alcohol use disorders are significantly correlated with high impulsivity on the BIS (Von Diemen et al., 2008) and impaired inhibitory control is more prevalent in Type II vs. Type I alcoholics (Bjork et al., 2004). Rodent lines selectively bred for high alcohol consumption are characterized by impaired inhibitory control (Wilhelm et al., 2007) and greater impulsive choice behavior (Oberlin and Grahame, 2009) relative to low drinking rodent lines. Studies in outbred rats also reveal a correlation between deficient inhibitory control and high alcohol intake (Logue et al., 2008).

Less is known about disruptions in impulse control resulting from persistent alcohol-induced neuroadaptation. Frontal structures including the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC) and orbital frontal cortex (OFC) control impulsive action and impulsive choice behaviors (Eagle and Baunez, 2010; Winstanley, 2010) and these are among the most heavily disrupted regions in the alcoholic brain (Sullivan and Pfefferbaum, 2005). Alcoholics have lower grey and white matter volumes as compared with age-matched controls in cortical structures including the PFC, ACC and OFC (Duka et al., 2011b; Fein et al., 2006a), and abstinent alcoholics are characterized by disrupted neural activation in these structures during performance of tasks challenging behavioral inhibition and impulsive choice (Boettiger et al., 2007; Li et al., 2009). These disruptions appear to derive in part from alcohol exposure as they are aggravated by multiple detoxifications (Duka et al., 2003;

Duka et al., 2011b; Loeber et al., 2009) and progressive recovery of cognitive performance and regional brain volume deficits is evident over the course of long-term abstinence (Bendszus et al., 2001; Fein et al., 2006b; Sullivan et al., 2000). Consistently, preclinical studies demonstrate that an ethanol (EtOH) dosing regimen that mimics binge drinking results in cortical damage (Crews and Nixon, 2009). Collectively, these findings point toward persistent alcohol-induced dysregulation of cortical function that could contribute to impaired impulse control. However, while several studies have probed the effects of acute alcohol intoxication on cognitive function, the consequences of long-term alcohol exposure on cognitive function are largely unexplored.

The present study characterized the effects of long-term alcohol exposure on attentional capacity and impulsive behavior in rats using the 5-Choice Serial Reaction Time Task (5-CSRTT) (Robbins, 2002). An EtOH liquid diet procedure was used for alcohol exposure and repeating cycles of 5d EtOH diet consumption and 9d abstinence were employed to mimic the cyclical nature of intoxication and withdrawal experienced by human alcoholics. The first experiment evaluated the emergence and persistence of disrupted 5-CSRTT performance across 3 liquid diet cycles and subsequent 21d of abstinence, testing the hypothesis that increased impulsive action emerges over the course of multiple cycles of intoxication and abstinence. The second experiment tested the hypothesis that abstinence-related deficits in behavioral inhibition will be reduced following re-exposure to chronic EtOH consumption, and that indices of increased impulsive action will re-emerge during subsequent protracted abstinence.

MATERIALS AND METHODS

Subjects

Male Wistar rats (Charles River, Wilmington, MA, USA) weighing 250g at the beginning of the experiments were housed 2 per cage in a humidity and temperature-controlled (22°C) vivarium on a 12 h light/dark cycle (lights off at 10 AM). With the exception of the 5d cycles of liquid diet maintenance described below, rats were maintained at 90% of their free feeding body weight to enable performance of the 5-CSRTT for food reinforcement. All procedures were conducted in strict adherence to the NIH Guide for Care and Use of Laboratory Animals.

5-Choice Serial Reaction Time Task (5-CSRTT)

5-CSRTT training and testing (Bari et al., 2008; Wiskerke et al., 2012) occurred in 6 identical rat five-hole nose poke operant chambers (Med Associates, St. Albans, VT, USA) with apparatus control and data collection performed using MED-PC v4.0 (Med Associates). Each 5-CSRTT session consisted of 100 trials during which subjects scanned the array of 5 apertures for a brief visual stimulus (2 sec) signaling the correct response location. A nose-poke response in the lit aperture either during stimulus presentation or within an additional 2 sec limited hold (LH) period was counted as a correct response and was rewarded by a food pellet delivery. Collection of the food pellet initiated an intertrial interval waiting period (ITI, 5 sec), after which the next trial began. Nose-pokes in non-stimulus apertures (incorrect response) and failure to respond in any aperture during a trial (omission) was

punished by withholding of food reward and a brief period of darkness (time out (TO), 5 sec), after which the next trial started. Failure to withhold responding during the ITI (premature response) was punished by a 5s TO, after which the trial restarted. Additional nose-pokes during a TO were counted as perseverative responses. Task performance was indexed by: (1) choice accuracy (100*correct/(correct+incorrect) responses, an index of attentional capacity); (2) premature responses, an index of impulsive action and poor inhibitory control; (3) total perseverative responses (index of behavioral disinhibition—here we counted any responses made during the punished TO as perseverative errors (Murphy et al., 2012)); (4) total omissions; (5) latency to correct response (index of information processing speed); (6) feeder latency (index of motivation). All 5-CSRTT indices were interpreted in conjunction with changes in other relevant 5-CSRTT parameters. Because alcohol-related impairments in executive function may be most evident under conditions of enhanced cognitive load we increased the 5-CSRTT difficulty in distinct "challenge tests" employing within-session randomized presentation of altered ITIs (Bari et al., 2008; Robbins, 2002). The difficulty of the variable ITI challenge tests stems from the unpredictability of the stimulus presentation that precludes the use of timing strategies to solve the task. In our experiments, the difficulty of variable ITI challenge sessions was additionally increased by reducing the SD to 1 sec. Disruptions in inhibitory control (premature & perseverative responses) were probed in sessions using long ITI challenges (5 – 32 sec) and attentional capacity was probed in sessions using short ITI challenges (1 – 5 sec). In each challenge session 4 distinct ITIs were pseudo randomly presented across trials such that each ITI was presented 25 times. Further details on 5-CSRTT training and testing may be found in the supplemental materials. The results from the short ITI challenge sessions are also in the supplemental material.

Alcohol exposure

A liquid diet paradigm was employed for alcohol exposure as described in our previous publications (Alvarez - Jaimes et al., 2009). The Ethanol group received liquid diet containing 10% (w/v) EtOH and the Control group received an equicaloric EtOH-free diet. Alcohol consumption was indexed by calculating the daily intake in g EtOH/kg/rat and blood alcohol levels (BALs) were determined once a week (further details are described in the supplemental materials). During diet exposure, the animals did not have access to an alternative diet.

Experimental protocol

A pictorial representation of the experimental design is shown in Figure 1.

Experiment 1—The effect of repeated cycles of EtOH consumption and withdrawal on measures of impulsive action and attention.

The goal of this experiment was to characterize the development of disruptions in impulsive behavior and attentional capacity that may result from repeated cycles of alcohol intoxication and abstinence, and to evaluate the persistence of these disruptions over the course of 21d of EtOH abstinence. Rats were trained in the 5-CSRTT and divided into two groups of equal task performance (indexed by accuracy, omissions, premature and

perseverative responses). These groups were subsequently given either an EtOH-containing (n=12) or control liquid diet (n=11) for three 14d cycles comprised of 5d on liquid diet, 2d on standard chow, 5d of 5-CSRTT testing consisting of 3d with standard 5-CSRTT parameters, one short ITI challenge session (1, 2, 3 & 5 sec ITI; abstinence day 6) and one long ITI challenge session (5, 6, 7 & 9 sec ITI; abstinence day 7). Animals were then given 2d ad libitum chow prior to the subsequent cycle of liquid diet. Following the 3rd cycle of diet exposure the persistence of behavioral disruptions was evaluated by 19d 5-CSRTT testing with standard task parameters followed by short- and long-ITI challenge tests on abstinence days 20 & 21, respectively. Because habituation to repeated varITI challenges occurs (Murphy et al., 2008; Walker et al., 2011) each type of challenge (e.g. short or long ITI) was presented no more than once every two weeks in an effort to maintain the test difficulty across repeated measures.

Experiment 2: The effect of renewed EtOH consumption and subsequent abstinence on measures of impulsive action and attention—This experiment further characterized the temporal profile of EtOH-related disruptions in 5-CSRTT performance by evaluating earlier and later abstinence time-points than were probed in Experiment 1. Clinical studies demonstrate a temporal profile for the emergence and abatement of impulsivity during prolonged abstinence (Bendszus et al., 2001; Fein et al., 2006b; Stavro et al., 2012; Sullivan et al., 2000), with the severity of these and related cognitive impairments increasing with multiple detoxifications (Duka et al., 2011b). This suggests that renewed alcohol exposure may “reset the clock” for the re-emergence of impulsivity during subsequent abstinence, and this possibility was also evaluated in this experiment. Lastly, because of the substantial training required for rodent models of cognitive function most preclinical studies evaluate the effects of drug exposure on performance of previously established, highly familiar behavioral tasks. This contrasts with clinical studies in which a subject’s prior drug history likely influences both the *acquisition* and performance of cognitive tasks (which typically occur in a single session). Thus, to more closely mimic the clinical setting we evaluated the effect of prior exposure to repeating cycles of EtOH intoxication and withdrawal on acquisition and subsequent performance of the 5-CSRTT.

Rats were given three 7d cycles of liquid diet exposure consisting of 5d of EtOH (n = 12) or control diet (n = 11) and 2d of regular chow. Subsequently, the animals were trained in the 5-CSRTT, achieving stable baseline performance over the course of 27d. On day 28 after the last liquid diet exposure rats were given a single long ITI challenge session (5, 6, 7 & 9 sec ITI) to probe for group differences in impulsive action. Each group was subsequently given an additional cycle of liquid diet exposure, followed by a long ITI challenge session (5, 6, 7 & 9 sec ITI) on the 3rd day of abstinence from liquid diet. To evaluate the persistence of altered 5-CSRTT performance during protracted withdrawal a subsequent long ITI challenge test was conducted on abstinence day 34. To minimize the influence of adaptation to the ITI challenge procedure, this challenge employed a different battery of ITI durations (5, 7, 9, 11 sec). Following an additional cycle of liquid diet exposure rats were given long ITI challenge tests with a distinct collection of ITIs (5, 11, 21, 30 sec) after both acute (3d) and prolonged (49d) periods of abstinence.

Statistical analyses

The dependent measures analyzed were accuracy, latency to correct response, premature responses, perseverative responses, errors of omission and feeder latency. All rats completed all trials in every session, including the variable ITI challenges, obviating the need to normalize data by the number of completed trials.

In Experiment 1, we examined the effect of three repeated cycles of EtOH exposure on 5-CSRTT performance. Baseline performance during abstinence days 3 – 5 was analyzed using repeated measures ANOVA with group (EtOH and CON) as the between-subjects factor and diet cycles (4 levels, baseline and 3 cycles) as the within-subjects factor. Performance during the three variable ITI challenges was examined using a 3-way repeated measures ANOVA with group (CON, EtOH) as between-subjects factor, and diet cycles (3 levels; cycles 1 - 3) and ITI (4 levels, 5, 6, 7 and 9 sec) as within-subjects factors. Significant interactions were followed by simple effects ANOVA and Student t-tests when appropriate. Following the 3rd cycle of diet exposure, we examined the effect of 3 weeks of protracted abstinence on 5-CSRTT performance. Repeated measures ANOVA was employed to analyze baseline performance with standard task parameters with group (EtOH and CON) and abstinence time (3 levels; averaged behavior for each of the 3 weeks of abstinence) as factors. Variable ITI challenges were presented at 7 and 21 days of abstinence, and these were analyzed using a 3-way ANOVA with group (CON, EtOH), abstinence time (2 levels) and ITI (4 levels, 5, 6, 7 and 9 sec) as factors.

In Experiment 2, we analyzed the effect of 3 cycles of prior diet exposure on 5-CSRTT acquisition using repeated measures ANOVA with group as between-subjects and time (first 12 sessions) as within-subjects factors. Baseline performance with standard task parameters was evaluated following two additional diet cycles using repeated measures ANOVA with group and time as factors (performance indices were averaged for specific abstinence periods as detailed in Table 2). Variable ITI challenge sessions administered at different abstinence period were analyzed by 2-way mixed factorial ANOVA with group as between-subjects and ITI duration as within-subjects factors. Potential performance adaptations resulting from repeated varITI testing were evaluated using 3-way ANOVA with group, challenge test (2 sessions, immediately preceding and after the 4th diet cycle) and ITI as factors. The Greenhouse-Geisser correction was used for all repeated measures ANOVAs when sphericity assumptions were violated. The level of significance was set at $p < 0.05$.

RESULTS

Experiment 1: The effect of repeated cycles of EtOH consumption and abstinence on measures of impulsive action and attention

Following 5-CSRTT training, rats were separated into two groups that did not differ on measures of accuracy, omissions, premature or perseverative responses (Table 1). During the 3 cycles of diet exposure rats in the EtOH group consumed an average of 10.1 ± 0.43, 12.0 ± 0.48 and 14.0 ± 0.51 g/kg/day EtOH, resulting in BALs of 211 ± 16, 258 ± 13 and 378 ± 23 mg% for each of the 3 cycles, respectively.

The effect of repeated cycles of alcohol exposure and abstinence on 5-CSRTT performance with standard task parameters—As compared with pre-liquid diet baseline, repeated cycles of liquid diet did not significantly alter most performance indices measured 3 – 5d following diet exposure (Table 1). The EtOH and CON groups did not differ in terms of response accuracy, premature responding, perseverative responding, correct response latency, omissions and latency to retrieve food rewards (group-effects: $F_{1,21}<2.751$, *NS* for all parameters; time × group effects: $F_{3,63}<2.140$, *NS* for all parameters except perseverative responding, $F_{3,63}=3.014$, $p<0.05$). Similarly, there were no significant group differences in most performance indices when rats were tested during three weeks of abstinence from the final liquid diet cycle. The two groups had similar response accuracy, premature and perseverative responding, correct response latency and feeder latency (group: $F_{1,21}< 2.252$, *NS* for all parameters; time × group, $F_{2,42} <2.00$, *NS* for all parameters). Interestingly, the EtOH group exhibited significant reductions in omissions during the 3 week abstinence period (group, $F_{1,21} = 10.609$, $p < 0.01$; time × group, $F_{2,42} <1$, NS). These data suggest that with the exception of diminished omissions, repeated cycles of alcohol intoxication and abstinence do not substantially alter 5-CSRTT performance under highly familiar, well-trained task parameters.

Effects of repeated cycles of alcohol exposure and abstinence on 5-CSRTT performance under conditions of enhanced cognitive load (variable ITI challenges)—Three-way ANOVA of the varITI challenges administered after each diet cycle (Figure 2) indicated that accuracy significantly improved in both groups over repeated testing (challenge test: $F_{2,42}=6.011$, $p<0.01$; group: $F_{1,21} <1$, NS; challenge test × group: $F_{2,42}=3.807$, $p<0.05$; ITI: $F_{3,63}=2.250$, NS; ITI × group: $F_{3,63}<1$, NS; challenge × ITI × group: $F_{6,126}=1.507$, NS; simple effects ANOVA on the effect of repeated varITI challenges within each group: CON, challenge test: $F_{2,20}=5.229$, $p<0.05$; EtOH, challenge: $F_{2,22}=4.046$, $p<0.05$). Premature responses, an index of impulsive action, was also influenced by repeated varITI testing (challenge test, $F_{2,42}=4.738$, $p<0.05$) though there were no significant alterations in group differences, or the interactions between group and ITI duration over the repeated challenge testing (group: $F_{1,21}=1.794$, NS; challenge, $F_{2,42}=4.738$, $p<0.05$; challenge test × group: $F_{2,42}<1$, NS; ITI, $F_{3,63}=81.262$, $p<0.0001$; ITI × group: $F_{3,63}=2.004$, NS; challenge test × ITI × group: $F_{6,126}<1$, NS; simple effects ANOVA on the effect of repeated varITI challenges within each group: CON, challenge: $F_{2,20}=3.919$, $p<0.05$; challenge × ITI: $F_{6,60}=2.055$, NS; EtOH, challenge: $F_{2,22}=1.640$, NS; challenge × ITI, $F_{6,66}<1$, NS). Perseverative responses also changed with repeated testing, and despite an interaction between group and challenge test on this index, group differences did not reach significance (challenge test, $F_{2,42}=3.267$, $p<0.05$; group: $F_{1,21} <1$, NS; challenge test × group: $F_{2,42}=3.344$, $p<0.05$; ITI: $F_{3,63}=26.637$, $p<0.001$; ITI × group: $F_{3,63}<1$, NS; challenge test × ITI × group: $F_{6,126}=1.507$, NS; simple effects ANOVA on the effect of repeated varITI challenges within each group: CON, challenge, $F_{2,20}=13.621$, $p<0.0001$, challenge × ITI, $F_{6,60}<1$,NS; EtOH, challenge, $F_{2,22}<1$, NS; challenge × ITI, $F_{6,66}=1.132$, NS). Repeated challenge testing also altered omissions, with EtOH rats making significantly fewer omissions during the challenges (challenge test, $F_{2,42}=3.819$, $p<0.05$; group: $F_{1,21}=5.940$, $p<0.05$; challenge test, × group: $F_{2,42}<1$, NS; ITI: $F_{3,63}=1.633$, NS; ITI × group: $F_{3,63}=1.835$, NS; challenge test, × ITI × group: $F_{6,126}=1.658$, NS; simple effects

ANOVA on the effect of repeated varITI challenges within each group: CON, challenge, $F_{2,20}<1$, NS, challenge × ITI, $F_{6,60}<1$,NS; EtOH, challenge, $F_{2,22}=3.409$, NS; challenge × ITI, $F_{6,66}=1.554$, NS).

Rats were tested at 7 & 21 days of abstinence from the 3rd diet cycle in order to evaluate the effect of prolonged abstinence on impulsive behavior (Figure 2). At both abstinence times EtOH rats elicited significantly more premature responses than CON rats (group: $F_{1,21}=8.569$, $p<0.01$; challenge test: $F_{1,21}=1.925$, NS; challenge test × group: $F_{1,21}<1$, NS; ITI: $F_{3,63}=58.947$, $p<0.0001$; ITI × group: $F_{3,63}= 6.036$, $p<0.001$; challenge test × ITI × group: $F_{3,63}=1.559$, NS). In contrast, there were no group differences in response accuracy (group: $F_{1,21}=3.328$, NS; challenge: $F_{1,21}=1.293$, NS; challenge × group: $F_{1,21}<1$, NS; ITI: $F_{3,63}=2.312$, NS; ITI × group: $F_{3,63}<1$, NS; challenge × ITI × group: $F_{3,63}=1.859$, NS) or perseverative responding (group: $F_{1,21}=2.139$, NS; challenge test: $F_{1,21}<1$, NS; challenge test × group: $F_{1,21}<1$, NS; ITI: $F_{3,63}=20.473$, $p<0.0001$; ITI × group: $F_{3,63}=1.715$, NS; challenge test × ITI × group: $F_{3,63}<1$, NS). EtOH rats made significantly fewer omissions (group: $F_{1,21}=5.930$, $p<0.05$) and this was not influenced by the abstinence period (challenge: $F_{1,21}<1$, NS; challenge test × group: $F_{1,21}=1.230$, NS; ITI: $F_{3,63}<1$, NS; ITI × group: $F_{3,63}<1$, NS; challenge test × ITI × group: $F_{3,63}<1$, NS).

Experiment 2: The effect of renewed EtOH consumption and subsequent abstinence on measures of impulsive action and attention

Effect of EtOH exposure on acquisition and performance of the 5-CSRTT with standard task parameters—During the 3 cycles of liquid diet exposure prior to 5-CSRTT training, rats in the EtOH group consumed an average of 10.2 ± 0.43, 11.1 ± 0.89 and 11.6 ± 0.48 g/kg/day EtOH/kg/day resulting in BALs of 180 ± 29, 246 ± 19 and 379 ± 21mg%. 5-CSRTT training began on abstinence day 8, and the profile of 5-CSRTT acquisition during the initial 12d of training is shown in Figure 3. Repeated measures ANOVA indicated that EtOH-exposed rats exhibited diminished accuracy (group, $F_{1,21}=7.997$, $p < 0.05$; group × time, $F_{3.6,73.9}= 5.031$, $p <0.01$, $\varepsilon=0.329$), however group differences were significant only during the initial phase of acquisition (Student t test, $p<0.05$ for d1, d2, d4, d6; NS all other days). Similar trends were observed for behavioral inhibition (premature responses, group: $F_{1,21}= 3.696$, NS; group × time: $F_{3.77,79.27}= 4.09$, $p < 0.01$, $\varepsilon=0.343$), perseverative responses (group: $F_{1,21}= 4.311$, NS; group × time: $F_{1.6,34.28}= 5.39$, $p < 0.05$, $\varepsilon=0.15$) and errors of omission (group: $F_{1,21}= 3.95$, NS; group × time: $F_{4.12,86.53}= 2.87$, $p < 0.05$, $\varepsilon=0.37$), although group differences were only present during the first days of training (Premature & perseverative responses, $p<0.05$ for, d1, d2; NS all other days; omissions, NS all days). There were no group differences in terms of motivation for food (feeder latency, group: $F_{1,21}<1$; group × time: $F_{2.1,44.2}<1$, $\varepsilon=0.19$) or correct response latency (group: $F_{1,21}= 3.22$, NS; group × time: $F_{2.7,58.6}= 2.87$, NS, $\varepsilon=0.25$). All rats achieved stable 5-CSRTT performance within 27d of training, and no group differences were evident in any performance index during the final days of training (Table 2; column 1, Student t test, all indices, $p>0.05$). Similarly, no significant group differences were evident in any session employing standard task parameters following the 4th and 5th cycles of liquid diet exposure (summary in Table 2; accuracy, premature responses,

perseverative responses, omissions, correct response latency, feeder latency, group: $F_{1,21}<3.495$, NS for all parameters; group × time: $F_{4,84}<2.248$, NS for all parameters).

Effect of renewed EtOH consumption following prolonged abstinence on 5-CSRTT performance under conditions of enhanced cognitive load (varITI challenges)—EtOH-exposed rats were more impulsive than controls when challenged for the first time with a variable ITI session following 28d abstinence from liquid diet exposure (Figure 4; 2-way ANOVA, premature responses, group: $F_{1,21} = 4.445$, $p < 0.05$; ITI × group, $F_{3,63} = 1.996$, NS). There were no group differences in response accuracy, perseverative responses or omission errors (for all parameters, group: $F_{1,21} <3.061$, NS; ITI × group: $F_{3,63} < 2.087$, NS). To evaluate the effects of renewed EtOH exposure rats were given an additional 5d period of liquid diet (Cycle 4; 11.8 ± 0.39 g/kg/day EtOH, avg. BAL of 242 ± 15 mg%).

To test the effects of renewed EtOH exposure the animals were subsequently given an additional cycle of diet and tested in the varITI paradigm on the third abstinence day. 3-way ANOVA comparing the varITI sessions immediately prior to and after this 4th diet cycle revealed a significant effect of challenge test on premature responding (challenge test: $F_{1,21}=6.431$, $p<0.05$) but no group × challenge interaction (group: $F_{1,21} =2.829$, NS; challenge × group: $F_{1,21}=1.089$, NS; ITI: $F_{3,63}=52.503$, $p<0.0001$; ITI × group: $F_{3,63}= 2.142$, NS; challenge × ITI × group: $F_{3,63}<1$, NS). Simple effects ANOVA revealed no significant difference in premature responding by CON animals in these two sessions (challenge test, $F_{1,10}=1.024$, NS; challenge × ITI, $F_{3,30}=1.436$, NS) but demonstrated a significant reduction in premature responding by EtOH animals in the session following EtOH re-exposure (challenge test, $F_{1,11}=6.961$, $p<0.05$; challenge × ITI, $F_{3,33}=2.839$, $p=0.053$). There was no significant effect of diet re-exposure on other behavioral indices (3-way ANOVA, accuracy, perseverative responses, errors of omission, for all parameters: challenge, $F_{1,21}<1$, NS; challenge × group, $F_{1,21}<1$, NS; ITI: $F_{3,63}<1$, NS; ITI × group: $F_{3,63}= 2.291$, NS, except for perseverative responses (ITI: $F_{3,63}=28.044$, $p<0.0001$; ITI × group: $F_{3,63}= 1.427$, NS); for all parameters, challenge × group × ITI, $F_{3,63}<1.638$, NS; group: $F_{1,21} <2.316$, NS).

To evaluate possible re-emergence of impulsive behavior in EtOH-exposed rats an additional variable ITI challenge was given on abstinence day 34. To minimize adaptation to the challenge task this test employed a novel set of ITIs of longer duration than used in prior tests. As shown in Figure 4, rats in the EtOH group elicited significantly more premature (group, $F_{1,20}= 5.420$, $p < 0.05$; group × ITI, $F_{3,60}=2.270$, NS) and perseverative (group: $F_{1,20}= 5.625$, $p < 0.05$; group × ITI, $F_{3,60}= 3.299$ $p<0.05$) responses than did CON animals, indicating a re-emergence of impulsive behavior during protracted abstinence. No group differences were evident in any other index of task performance (accuracy: group, $F_{1,20}= 2.246$, NS; group × ITI, $F_{3,60} <1$; errors of omission, group, $F_{1,20}= 2.826$, NS; group × ITI, $F_{3,60} <1$). An additional diet cycle was given after this test to allow re-evaluation of the abatement of abstinence-related impulsive behavior by renewed EtOH exposure (Cycle 5; 7.7 ± 0.75 g/kg/day EtOH, BALs of 135 ± 7 mg%). On the 3rd abstinence day a varITI challenge was given employing a novel set of particularly long ITI durations (5, 11, 21 & 30 sec) to increase sensitivity for detecting impulsive action. Despite these more challenging

task parameters, no significant group differences were evident in any task parameter including premature and perseverative responding (accuracy, premature, perseverative and errors of omission parameters, group: $F_{1,21}$ <2.039, NS; group × ITI, $F_{3,63}$ <1, NS).

To further evaluate the persistence of abstinence-related increases in impulsive action an additional ITI challenge test was given following 7 weeks of abstinence. In contrast to the sustained increases in premature and perseverative responding evident after 3 – 4 weeks of abstinence (Figures 2 & 4), group differences in these measures were no longer present at 49d abstinence (there were also no group effects for any other measure; for all parameters, group: $F_{1,21}$ <1.348, *NS*; group × ITI, $F_{3,63}$ <1.012, NS). Although it would have been ideal to verify the re-emergence of increased impulsive action in the EtOH group at an intermediate period of abstinence from the 5th diet cycle, this was not pursued so as to retain the limit of two varITI challenges per abstinence period to minimize behavioral adaptation to the challenge test. Collectively, these findings indicate that renewed alcohol consumption results in an acute reversal of impulsive and perseverative behaviors, though these impairments re-emerge during protracted abstinence (at least 34d), and dissipate following long-term abstinence (by 49d).

DISCUSSION

The present results provide evidence that repeated cycles of alcohol intoxication and abstinence lead to impaired 5-CSRTT performance characterized by increased impulsive behavior. These findings corroborate and extend previous clinical observations of cognitive deficits in the domain of impulsivity and attention in detoxified alcoholics (Stavro et al., 2012).

We found that disruptions in impulsive action were not evident following a single 5d episode of EtOH consumption but developed following multiple cycles of intoxication and abstinence. Increased premature and perseverative responding were evident only during variable ITI challenge tests, were not present during acute abstinence (3d) but emerged within 7d of abstinence and persisted for at least 34d. These findings are consistent with a recent study by Walker and colleagues demonstrating increased impulsive action in EtOH-exposed mice during variable ITI tests conducted during 14d abstinence (Walker et al., 2011). The present findings extend the window of increased impulsivity to at least 34d, and demonstrate that renewed EtOH exposure temporarily alleviates deficient impulse control. Similar to the observations of Walker et al., the profile of results from Experiment 1 raise the possibility that group differences in impulsive action derive more from diminished adaptation of EtOH-exposed animals to repeated ITI challenges than from progressive increases in premature responding resulting from prior cycles of EtOH consumption (see Figure 2). However, data from Experiment 2 provide evidence that EtOH-exposed rats are more impulsive than controls at 28d abstinence even when presented with a varITI challenge for the first time, thus discounting an influence of adaptation to the challenge conditions. The loss of group differences in impulsive action following renewed EtOH consumption and re-emergence of impulsive action during subsequent abstinence (Figure 4) suggests this behavioral phenotype is temporally associated with prior alcohol exposure, rather than the history of behavioral testing. Lastly, we did observe significantly greater premature and

perseverative responding by EtOH-exposed animals during the first 2 days of 5-CSRTT acquisition (abstinence days 8 & 9), however group differences did not persist in subsequent sessions. All rats achieved similar levels of task performance by the end of 5-CSRTT training, and although the mechanisms involved in adaptation to changing stimulus durations (during acquisition) and varying ITIs (during challenge tests) are likely different, these data suggest that EtOH-exposed rats do not have a generalized learning impairment.

The effects of prior EtOH exposure on impulsive action are primarily evident during variable ITI "challenge" sessions in both rats (current study) and mice (Walker et al., 2011), consistent with functional imaging studies in which impaired neural activity in alcoholics is most apparent during performance of cognitively challenging tasks (Boettiger et al., 2007; Li et al., 2009). In the long ITI challenge tests, both EtOH-exposed and drug-naïve animals exhibited increased premature/perseverative responding and decreased response accuracy relative to sessions employing standard task parameters. Although group-related differences in premature responding were consistently evident during protracted abstinence, group differences in response accuracy (attentional capacity) were not commonly observed. Because attentional capacity and response inhibition in the 5-CSRTT are mediated through largely distinct neural mechanisms, the present findings may provide initial insight into the neural mechanisms that are disrupted by long-term EtOH exposure and withdrawal. Restraint of premature and perseverative responding in the 5-CSRTT relies on proper infralimbic and prelimbic cortical function (Chudasama et al., 2003). Increased PFC glutamate levels during alcohol abstinence (Hermann et al., 2012) could contribute to increased premature responding (Murphy et al., 2012) while disrupted cortical GABAergic signaling may contribute to rash impulsivity, poor self-control and impaired cognitive flexibility (Boy et al., 2011; Silveri et al., 2013). At present there is little information on the effects of chronic alcohol exposure and abstinence on cortical neurochemistry, and this represents an area that requires further study.

Substantial evidence suggests that cognitive impairments in alcohol-dependent patients are, at least partly, reversible with sufficient periods of abstinence (Fein et al., 2006b; Loeber et al., 2009). This recovery of function follows a distinct temporal profile. A recent meta-analysis of clinical data by Stavro and colleagues estimated that dysfunction in the inhibition/impulsivity cognitive domain in recovering alcoholics is more pronounced during intermediate periods of abstinence (up to one year) as compared with earlier periods of abstinence (up to 1 month) (Stavro et al., 2012). The present findings are consistent with this profile in demonstrating disturbances in inhibitory response control that emerge following several days of abstinence and that resolve over the course of long-term protracted abstinence.

The number of withdrawal episodes may be an important factor in recovery of cognitive function during abstinence. Alcohol-dependent patients with 2 or more detoxifications show decreased inhibitory control (Duka et al., 2003; Duka et al., 2011b) and decreased grey matter volume in ventromedial prefrontal cortex (Duka et al., 2011b), an area of the brain important for the control of impulsivity, compared to controls or patients with a single detox episode. In rats, repeated EtOH withdrawals disrupt fear conditioning (Stephens et al., 2001), negative patterning learning (Borlikova et al., 2006) and high response rates under

Fixed Interval schedules (Borlikova et al., 2006) reminiscent of increased premature responses on the 5-CSRTT. These behavioral disruptions may reflect neural adaptations resulting from kindling-like processes that are more pronounced following intermittent vs. continuous alcohol exposure and that ultimately increase allostatic load (Breese et al., 2011; Koob, 2003). The present observation of increased impulsive action following at least 3 cycles of prolonged intoxication and withdrawal provides initial evidence that repeated withdrawals influence impulsive behavior in rats in a manner that is consistent with clinical findings in alcoholics.

In summary, the present results provide evidence that repeated cycles of intermittent ethanol intoxication result in the emergence of increased impulsivity as indexed by premature and perseverative responding in the rat 5-CSRTT. The onset of this impulsivity occurs following several days of abstinence and persists for at least 4 weeks, though renewed alcohol consumption appears to “reset” the system for subsequent re-emergence of increased impulsivity. This profile of impaired inhibitory control aligns with clinical descriptions of impaired cognitive function in alcohol-dependent patients during protracted abstinence, supporting the use of this rodent model as a platform for characterizing the neurobiological mechanisms contributing to alcohol-induced disruptions in impulse control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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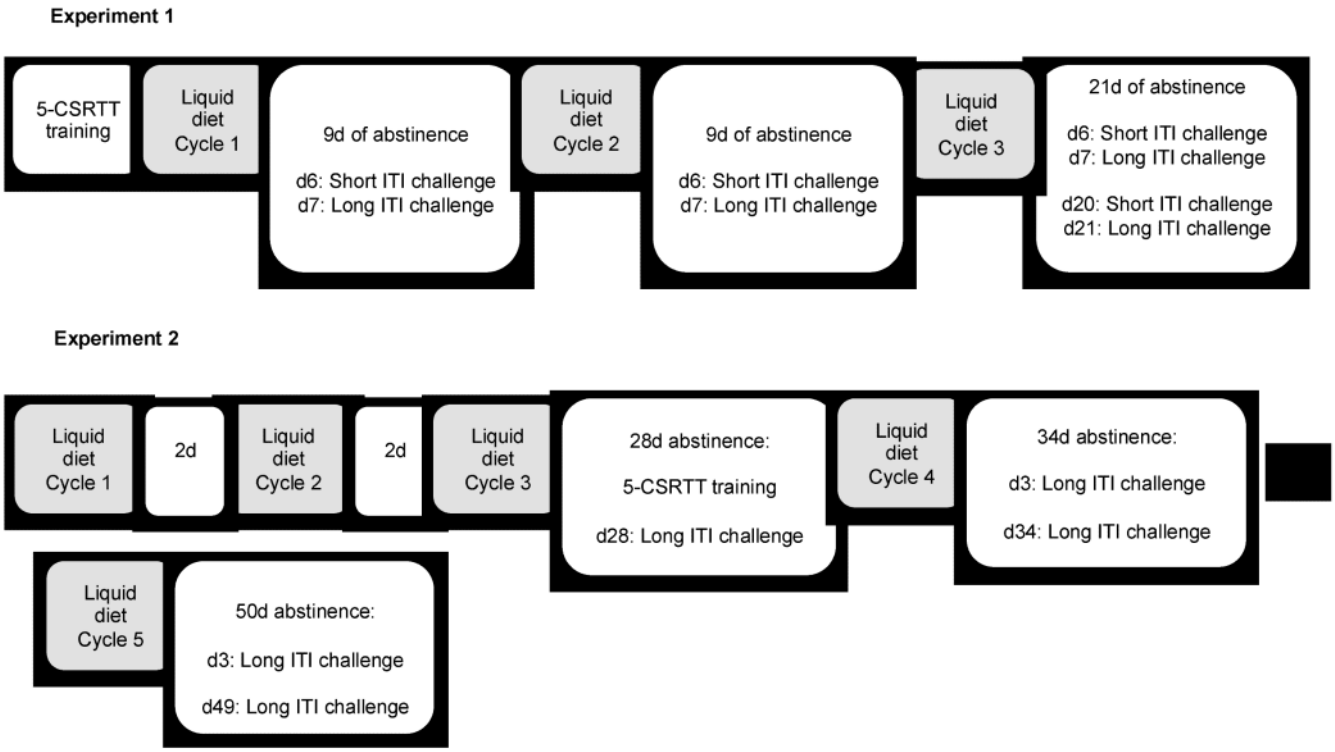

Figure 1. Experimental outline

Pictorial depiction of the design for Experiments 1 & 2. Each experiment employed 14d cycles of alcohol exposure comprised of 5d maintenance on liquid diet followed by 9d abstinence during which 5-CSRTT evaluations were performed. Animals in the EtOH group received liquid diet supplemented with 10% EtOH while the CON group received an equicaloric diet without EtOH. Shaded boxes represent times when animals were fed the liquid diet, white boxes represent days of abstinence. See text for further details.

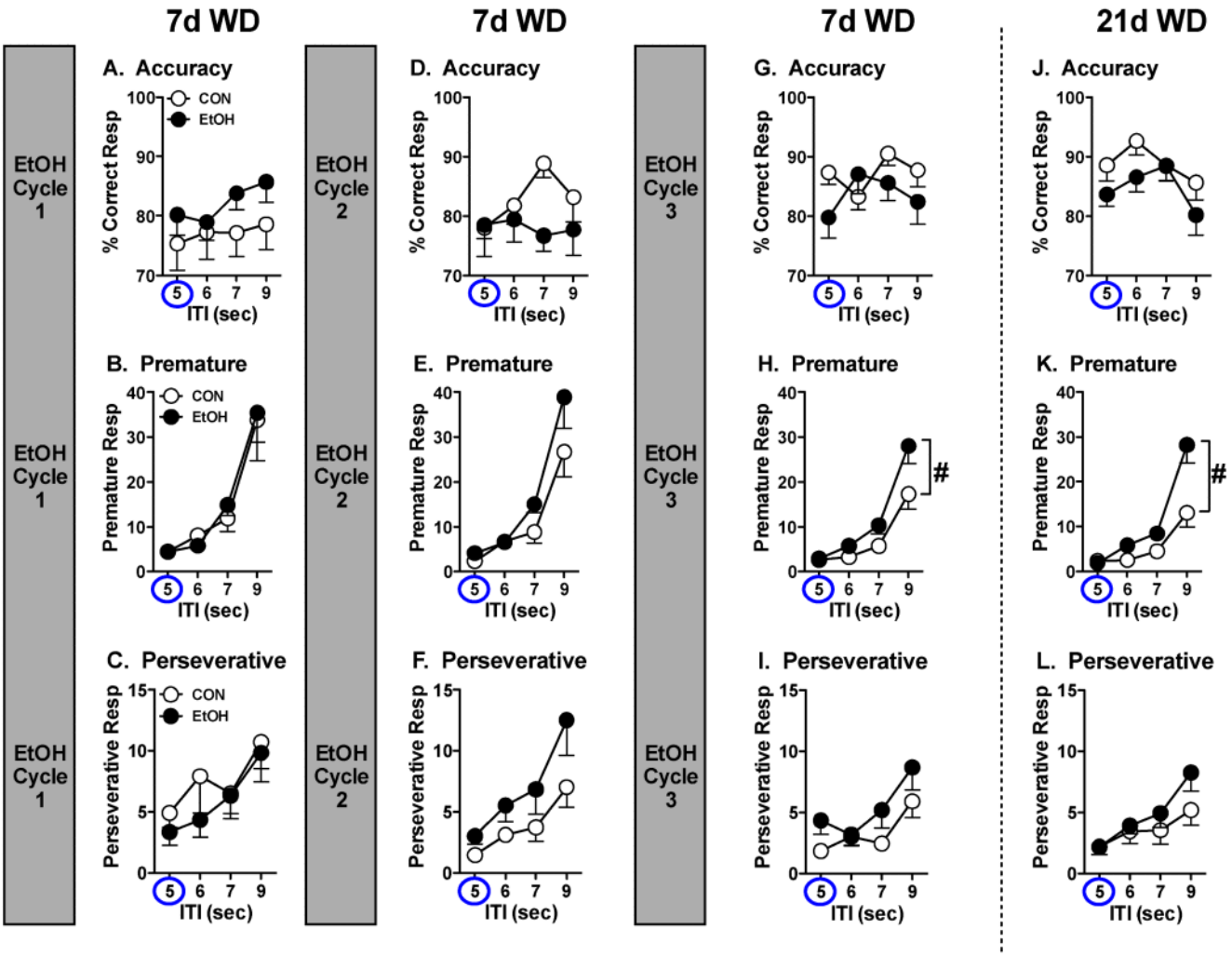

Figure 2. Experiment 1

Effects of repeating cycles of EtOH exposure and abstinence on impulsive action and attentional capacity indexed during within-session variable long ITI 5-CSRTT challenge tests. Data shown are from animals maintained on either EtOH (n = 12) or control (n = 11) liquid diet tested at 7 days of abstinence (7d WD) from 3 cycles of liquid diet exposure. No significant group differences in performance were evident following the first or second liquid diet cycle (panels **A – F**). However, following the third cycle of diet exposure EtOH-exposed rats elicited significantly more premature (**H**) responses than controls, with no group differences in response accuracy (**G**). Significant increases in premature responding were still evident in EtOH-exposed rats 21d after the final liquid diet exposure (**K**) though no group differences in perseverative responding were present (**L**). Brackets and "#" denote significant overall group effects (3-way ANOVA) and asterisks over specific ITI data points denote group differences based on simple effects ANOVA. *, # $p < 0.05$; **, ## $p < 0.01$. The 5 sec training ITI is denoted by a circle.

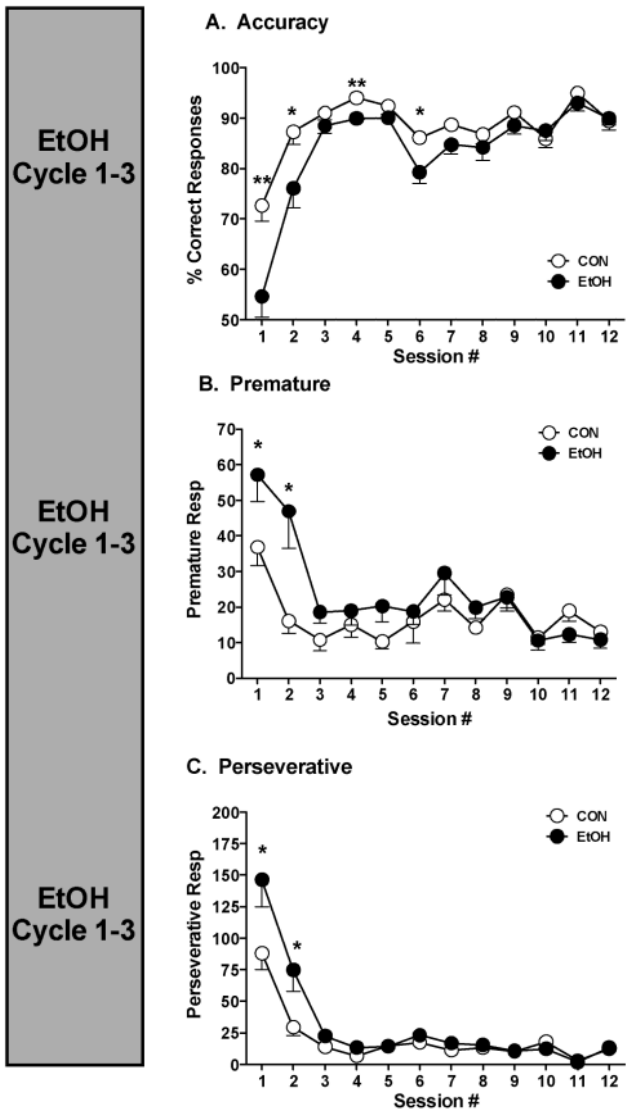

Figure 3. Experiment 2

The effect of prior EtOH exposure on the acquisition of the 5-CSRTT. Behavioral training began 8d following 3 cycles of liquid diet exposure (EtOH: n = 12,; CON:, n = 11), and significant group differences in behavior were evident only during the initial days of training with the full 5-CSRTT task. During this time EtOH-exposed rats exhibited diminished response accuracy (A; $p < 0.01$) and increased levels of premature (B) and perseverative responding (B, C; $p < 0.05$ for each). However, subsequent to these initial sessions no significant group differences in any task parameter were evident for the remainder of training. Note: as part of the training procedure the stimulus duration (SD) was changed across sessions as follows: session 1-3 = 32 sec; Session 4 = 16 sec; Session 5 = 8 sec; Session 6 = 4 sec; Session 7-12 = 2 sec. Sessions in the figure denote days of training with the full 5-CSRTT task, after the successful completion of preliminary phase training (no group differences at early stage training, data not shown). The SD was changed from one

session to the next if the rats had >90% accuracy and <20 omissions. * $p<0.05$ vs CON, ** $p<0.01$ vs CON

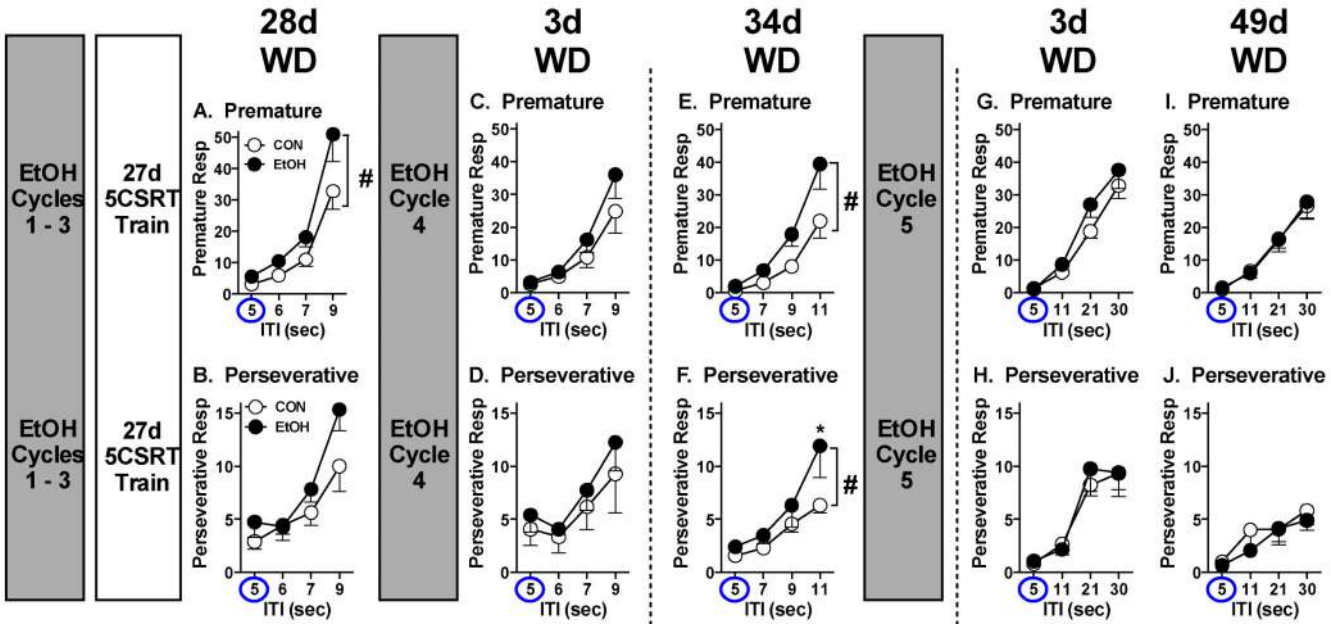

Figure 4. Experiment 2

The effect of repeated cycles of re-exposure to liquid diet and subsequent abstinence on impulsive action and attentional capacity indexed during variable long ITI challenge tests. Following three cycles of liquid diet exposure and 27d of 5-CSRTT training, EtOH rats (n = 12) made more premature (panel **A**) than did CON rats (n = 11) during a variable ITI challenge session presented on abstinence day 28 (28d WD). The deficits in impulsive and perseverative responding were alleviated by an additional cycle of EtOH diet (**C**, and **D** respectively). These deficits in inhibitory control re-emerged during subsequent abstinence and were evident when the rats were tested with a novel set of ITIs (5,7,9,11 sec) during a varITI challenge session given after 34d abstinence (**E, F**). There were no group differences in inhibitory control 3d after an additional liquid diet cycle (**G**, **H**). Group differences in premature and perseverative responding resolved after extended (49d) abstinence (**I**, **J**). Brackets and "#" denote significant overall group effects (2-way ANOVA) and asterisks over specific ITI data points denote group differences based on simple effects ANOVA. *,# $p < 0.05$; **,## $p < 0.01$. The 5 sec training ITI is denoted by a circle.

Table 1

Effect of 3 cycles of liquid diet exposure and subsequent abstinence on 5-CSRTT performance during sessions with standard task parameters in Experiment 1.

Measure	Group	Pre-Diet Baseline Performance	1st Diet Cycle: Abstinence days 3 - 5	2nd Diet Cycle: Abstinence days 3 - 5	3rd Diet Cycle: Abstinence days 3 - 5	3rd Diet Cycle: Abstinence days 10 -12	3rd Diet cycle: Abstinence days 17 -19
Accuracy	CON	91.77±0.34	92.67±1.18	94.45±0.89	93.62±1.16	95.77±0.60	95.56±1.00
	EtOH	89.80±0.29	90.68±1.03	91.88±1.05	92.26±1.13	94.43±0.78	93.67±0.911
Premature responses	CON	8.19±1.34	5.82±1.05	4.18±0.99	5.79±0.65	3.85±0.75	4.33±1.21
	EtOH	13.81±3.42	10.11±2.79	9.89±2.67	6.67±1.83	6.47±1.64	6.97±2.01
Perseverative responses	CON	7.85±1.48	8.09±1.38	4.06±0.75	6.94±1.40	4.85±1.42	7.36±2.27
	EtOH	12.83±3.02	7.78±2.02	9.94±3.49	7.00±1.76	6.42±2.02	6.28±2.47
Omissions	CON	13.00±0.78	17.64±2.34	13.15±1.85	14.85±1.82	13.79±1.75	14.06±2.54
	EtOH	14.58±2.24	20.00±3.64	10.64±1.50	9.00±1.21	7.58±1.19**	6.50±1.01**
Latency to Correct Response (sec)	CON	0.92±0.00	0.94±0.02	0.90±0.00	0.86±0.00	0.88±0.00	0.86±0.00
	EtOH	0.92±0.00	0.98±0.01	0.90±0.02	0.84±0.00	0.82±0.00	0.80±0.00
Feeder Latency (sec)	CON	1.36±0.11	1.39±0.12	1.55±0.16	1.49±0.16	1.72±0.24	1.68±0.20
	EtOH	1.28±0.10	1.59±0.15	1.70±0.17	1.44±0.10	1.46±0.11	1.44±0.10

** denotes $p < 0.01$ vs CON

Table 2

Effect of renewed EtOH consumption and subsequent abstinence on 5-CSRTT performance in sessions with standard task parameters during Experiment 2.

Measure	Group	3rd Diet Cycle: Abstinence days 25 - 27	4th Diet Cycle: Abstinence days 4 - 6	4th Diet Cycle: Abstinence days 25 - 27	5th Diet Cycle: Abstinence Days 4 - 6	5th Diet Cycle: Abstinence days 46-48
Accuracy	CON	90.61±1.13	94.98±1.02	94.58±0.86	96.03±0.06	94.70±1.06
	EtOH	90.36±1.46	94.00±1.17	93.47±1.95	95.38±1.30	92.64±1.21
Premature responses	CON	10.35±1.22	4.48±0.68	5.02±0.79	3.20±0.54	5.62±0.97
	EtOH	12.82±1.79	5.17±1.18	6.92±1.99	4.40±1.33	8.07±1.36
Perseverative responses	CON	10.67±1.65	7.05±1.36	4.71±1.13	4.40±0.90	5.67±1.09
	EtOH	10.97±1.86	7.82±1.73	4.60±1.69	3.40±0.87	5.33±0.72
Omissions	CON	11.95±1.81	10.38±1.60	10.23±1.95	14.49±1.71	21.02±3.97
	EtOH	9.42±1.16	11.25±2.01	7.64±0.94	12.55±1.34	14.20±1.61
Latency to Correct Response (sec)	CON	0.93±0.03	0.93±0.03	0.88±0.03	0.95±0.02	0.94±0.03
	EtOH	0.98±0.03	1.02±0.04	0.93±0.03	1.03±0.04	0.97±0.03
Feeder Latency (sec)	CON	1.49±0.09	1.84±0.17	2.30±0.19	1.75±0.18	1.75±0.18
	EtOH	1.54±0.18	1.85±0.25	2.01±0.19	1.79±0.24	1.79±0.24