Published in final edited form as:

Behav Neurosci. 2011 December ; 125(6): 879–891. doi:10.1037/a0025922.

Effects of chronic intermittent ethanol exposure on orbitofrontal and medial prefrontal cortex-dependent behaviors in mice

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Abstract

In humans, stroke or trauma-induced damage to the orbitofrontal cortex (OFC) or medial prefrontal cortex (mPFC) results in impaired cognitive flexibility. Alcoholics also exhibit similar deficits in cognitive flexibility suggesting that the OFC and mPFC are susceptible to alcohol-induced dysfunction. The present experiments investigated this issue using an attention set-shifting assay in ethanol dependent adult male C57BL/6J mice. Ethanol dependence was induced by exposing mice to repeated cycles of chronic intermittent ethanol (CIE) vapor inhalation. Behavioral testing was conducted 72 hours or 10 days following CIE exposure to determine whether ethanol-induced changes in OFC-dependent (reversal learning) and mPFC-dependent (set-shifting) behaviors are long-lasting. During early ethanol abstinence (72 hrs), CIE mice showed reduced reversal learning performance as compared to controls. Reversal learning deficits were revealed as greater number of trials to criterion, more errors made and a greater difficulty in performing a reversal learning task relative to baseline performance. Furthermore, the magnitude of the impairment was greater during reversal of a simple discrimination rather than reversal of an intradimensional shift. Reversal learning deficits were no longer present when mice were tested 10 days after CIE exposure suggesting that ethanol-induced changes in OFC function can recover. Unexpectedly, performance on the set-shifting task was not impaired during abstinence from ethanol. These data suggest reversal learning, but not attention set-shifting, is transiently disrupted during short-term abstinence from CIE. Given that reversal learning requires an intact OFC, these findings support the idea that the OFC may be vulnerable to the cognitive impairing actions of ethanol.

Keywords

reversal learning; attention set-shifting; alcohol dependence; withdrawal; behavioral flexibility

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Introduction

Chronic consumption of alcohol is associated with various changes in cognition and behavior. These effects include increased anxiety and irritability (Rassovsky et al, 2004; Schuckit et al, 1990; Thevos et al, 1991), memory deficits (Ahveninen et al, 2000; Joyce and Robbins, 1993), and poor decision making skills (Kamarajan et al, 2010; Loeber et al, 2009). In particular, alcoholics show deficits in attentional set-shifting and reversal learning tasks that require areas of the prefrontal cortex (PFC) and orbitofrontal cortex (OFC), respectively. In PFC-dependent set-shifting tasks such as the Wisconsin Card Sorting Task (WCST) and the Trail making test (Bijl et al, 2005; Ratti et al, 2002), subjects are instructed to attend to only one perceptual dimension (e.g., color) of a compound stimulus (e.g., color + number) in order to earn a reward. Without warning, the rule of the task changes and subjects must learn that another perceptual dimension now signals reward (e.g., number). Risky (Fernie et al, 2010) and impulsive (Littlefield et al, 2010) decision making skills can predict the severity of alcohol misuse. However, alcoholics with “normal” decision-making skills have difficulty making appropriate decisions specifically during a period of alcohol abstinence. In set-shifting tasks, abstinent alcoholics are able to follow the initial rule and maintain an attention set as indicated by reaching criterion performance prior to the rule switch (Jenkins and Parsons, 1979). However, these individuals show a delay in shifting their attention to the new rule as indicated by an increase in number of errors committed (Goldman et al 1985; Jenkins and Parsons, 1979; Kish et al, 1980; Parsons, 1983; Rourke and Grant, 1999; Yohman et al, 1985). Abstinent alcoholics also perform poorly on tests of reversal learning: discrimination tasks that require the unlearning of previously predictable stimulus-outcome associations (Fortier et al, 2008; Fortier et al, 2009). This deficit is similar to that observed in individuals with lesions to the orbitofrontal cortex (OFC), suggesting that the OFC plays an essential role in mediating reversal learning (Hornak et al, 2004; Tsuchida et al, 2010). Poor performance on PFC- and OFC-dependent tasks suggests that chronic consumption of alcohol impairs PFC and OFC function and such impairment likely contributes to poor decision-making skills observed in alcoholics. Longitudinal studies suggest that these cognitive deficits are the result of long-term consumption of alcohol rather than a result of pre-existing neuropsychological disorders since deficits in task performance recover within 3 weeks to 2 years of abstinence (Kish et al, 1980; Parsons, 1983; Rourke and Grant, 1999; Squeglia et al, 2009; Yohman et al, 1985).

Set-shifting and reversal learning also have been examined in non-human primates (Roberts et al, 1994; Walton et al, 2010) and in rodents (Birrell and Brown, 2000; Bissonette et al, 2008) using visual, tactile, or olfactory cues to signal reward. Similar to humans, lesions or damage to the medial PFC (mPFC) in rodents compromises performance during set-shifting (Birrell and Brown, 2000; Bissonette et al, 2008) while lesioning OFC impairs reversal learning (Bissonette et al, 2008). Furthermore, acute exposure to ethanol disrupts reversal learning in primates (Jedema et al, 2010) and rodents (Brown et al, 2007; Thomas et al, 2004). Despite observations that alcoholics show cognitive inflexibility on PFC- and OFC-dependent tasks, there is a paucity of data regarding the effects of chronic ethanol exposure on mPFC- and OFC-dependent processes in the rodent. As these animal models involving ethanol exposure allow for detailed analysis of underlying mechanisms of such effects, we sought to determine if 1) set-shifting and reversal learning were impaired in a mouse model of chronic intermittent ethanol exposure (CIE) and 2) whether deficits in cognitive performance following CIE exposure resolve during a protracted (or extended) period of abstinence. Given that abstinent alcoholics persevere more on tasks requiring behavioral control and cognitive flexibility (Noel et al, 2007; Oscar-Berman et al, 2004) and our previous findings showing that acute ethanol disrupts mPFC- neuronal function (Tu et al, 2007; Weitlauf and Woodward, 2008), we predicted that CIE would disrupt both mPFC and OFC-mediated behaviors in the mouse.

Methods

Subjects

Sixty-five male C57BL/6J mice purchased from Jackson Laboratories at 6 weeks of age were used to test the effects of CIE on reversal learning and set-shifting. After arrival, mice were group-housed (4/cage) and allowed to acclimate to the colony room for at least two weeks. After acclimation, mice were brought to a laboratory testing room, singly housed, and maintained under a modified 12 hr light/dark cycle (lights on at 1400 hr). At all times, mice were maintained in a temperature and humidity controlled AAALAC-approved facility, with ad libitum access to water. Mice were food restricted to 85% of their free-feeding body weight by single daily feeding prior to the start of training and during discrimination training. Water was available at all times. In all respects, maintenance and treatment of mice were conducted within the guidelines for animal care as approved by the Medical University of South Carolina's Institutional Animal Care and Use Committee and the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No.: 80-23, revised 1996).

Testing Apparatus

The testing chamber for assessing performance in discrimination tasks was a rectangular box (30 × 20 × 20 cm) constructed of clear acrylic with a clear plastic lid. The chamber was divided into three separate compartments. Half of the chamber (15 × 20 × 20 cm) was the starting area in which mice were placed at the beginning of a trial. The remaining half of the chamber contained a piece of acrylic placed perpendicular to the shortest wall of the chamber to divide the front part of the apparatus into two separate choice areas (15 × 10 × 20 cm). The divider was used to deter mice from entering the alternate choice area after committing an error. During discrimination trials, a rodent food bowl (6 × 6 × 2 cm; PetSmart, Phoenix, AZ, USA) was placed on each side of the divider. Both bowls were filled with various digging materials and spices to provide specific textures and odors during the discrimination trials. A list of the different digging media and odors used are depicted in Table 1. Digging media were obtained from PetSmart (Phoenix, AZ, USA) or the Division of Laboratory Animal Research at the Medical University of South Carolina while all odors were obtained from Spice Place (Keedysville, MD, USA) or local grocery stores. In each discrimination trial, one of the bowls in the testing chamber was baited with a buried food reward (1/4th Honey Nut Cheerio Loop, General Mills, Minneapolis, MN). All trials were video taped using a mini digital camcorder (Insignia, Richfield, MN, USA).

Habituation

Mice were habituated to materials used in the discrimination trials by placing one of the rodent food bowls filled with a small sample of each of the digging medias and odors listed in Table 1. To prompt mice to dig in the bowl, 1/4th of a Honey Nut Cheerio Loop was placed on top of the bowl. Sample bowls were placed inside the mouse home cage and remained there overnight the day before training began.

General Testing Procedure

Discrimination tasks were presented in the same order for all mice and each type of discrimination was conducted on separate days. All discrimination trials were conducted in the same manner except for differences in the placement of the food reward and the type of cues presented (Table 2). Each trial began by placing a mouse in the start area of the testing chamber and allowing mice to make a choice between two cued bowls, one of which contained the buried food reward. The mouse was allowed to dig in one of the food bowls to find the bait. The first four trials were exploratory and allowed the mouse to dig in either of

the bowls. If the mouse dug in the unbaited bowl, the trial was recorded as an error but the mouse was still allowed to go to the other bowl, dig and obtain the bait. If the mouse dug in the baited bowl, the trial was recorded as a correct choice and was counted towards reaching criterion (i.e., 6 consecutive correct trials). Following the exploratory trials, mice were only allowed to dig in one bowl. If the mouse dug in the baited bowl first and obtained the food reward, the mouse was allowed to eat the bait for 10 sec, the trial was terminated and recorded as a correct choice. If the mouse dug in the unbaited bowl first, the mouse was quickly removed from the testing area and placed on top of the baited bowl for 10 sec. Mice were not allowed to dig in the baited bowl or obtain the bait at this time, and placement on top of the baited bowl was solely done to indicate an error was made. These trials were terminated and recorded as an error. At the end of each trial, mice were returned to the home cage to signify the end of the trial. Inter-trial intervals were 30 sec followed by the immediate start of the next trial. To keep the odor concentration constant between trials, extra odor was mixed into the digging medias after every few trials. Groups were counterbalanced for direction of set-shift by having some mice switch from odor to digging media discriminations while others switched from digging media to odor discriminations. Furthermore, no more than two consecutive trials had the same combination of exemplars. Switching the irrelevant exemplars verified that each mouse learned which dimension was irrelevant (e.g., neither alpha-dri or pet-bedding consistently signified the presence of reward when odor was the relevant dimension). Placement of the baited bowl in the testing apparatus was randomized to prevent a side bias. Trials continued until mice reached a criterion of 6 consecutive correct trials. Once mice achieved criterion performance, they were returned to their home cage and fed to maintain 85% of their body weight. For each discrimination task, the total number of trials required to reach criterion performance and the number of errors committed were recorded. The time required to obtain the bait during a given trial (latency) was also recorded to assess activity rates and motivation to find the reward.

Chronic Intermittent Ethanol Exposure

Ethanol was administered via the inhalation route using a well-established dependence model (Becker and Lopez, 2004; Griffin et al., 2009a; Griffin et al., 2009b; Lopez and Becker, 2005). Briefly, one group of mice (CIE group) received vapor exposure in inhalation chambers (16 hr/day for 4 consecutive days) while the remaining mice were similarly handled, but maintained in control (air) inhalation chambers. At the end of the 4th day, mice were given three days of abstinence before beginning the next cycle of CIE exposure. This pattern of CIE (or air) vapor exposure was repeated for a total of three consecutive weekly cycles. Ethanol (95%) was volatilized by passing air through a submerged airstone. Ethanol vapor was mixed with fresh air and delivered to Plexiglas inhalation chambers at a rate of 5 L/min to maintain consistent ethanol concentrations (17 – 21 mg/L air) in the chamber. This yielded blood ethanol concentrations (BEC) in the range of 150 – 250 mg/dl. Prior to entry into the ethanol chambers, CIE mice were administered ethanol (1.6 g/kg; 8% w/v) and the alcohol dehydrogenase inhibitor pyrazole (1 mmol/kg) by intraperitoneal injection (20 ml/kg body weight) to maintain stable intoxication. Control mice received injections of saline and pyrazole before being placed in air chambers. The housing conditions were identical to those in the colony room. Chamber ethanol concentrations were monitored daily and air flow was adjusted to maintain concentrations within the specified range. Additionally, blood samples were collected from all animals to monitor BECs during the course of inhalation exposure. Chamber ethanol concentrations and BEC levels were determined as previously described (Griffin et al., 2009a). All mice resumed discrimination training at 72 hr (Experiment 1 and 2) or 10 days (Experiment 2) following the final cycle of CIE (or air) exposure.

Experiment 1: Effects of CIE on reversal learning and set-shifting

To examine the effects of CIE exposure on reversal learning and set-shifting, mice were trained to complete a series of discrimination tasks. Each discrimination task differed on the perceptual dimension that signaled reward (i.e., odor vs. digging media) and on the specific type of odor and digging media used (e.g., garlic vs. cinnamon or gravel vs. confetti). The experimental design is depicted in Figure 1A. Ethanol-naïve mice, maintained at 85% ad libitum body weight, were first trained to perform 4 tasks including simple discrimination (SD), compound discrimination (CD), and two intradimensional shifts (ID1 and ID2). During the simple discrimination task, mice learned to associate the presence of a buried food reward (1/4th Honey Nut Cheerio Loop) in one of two cued bowls. For example, if odor was the relevant dimension, one bowl contained cloves/gravel while the other bowl contained smoked paprika/gravel. If digging media was the relevant dimension, one bowl contained cloves/gravel while the other bowl contained cloves/confetti. For compound discrimination, the same relevant dimension from simple discrimination was used, however, an irrelevant dimension (e.g., novel odor or digging media) was added making the bowls differ on both odor *and* digging medium (e.g., cloves/gravel vs. smoked paprika/confetti). The ability to shift attention within the same perceptual dimension was then assessed by changing the type of relevant cue used (i.e., cloves/smoked paprika changed to garlic/cinnamon). Each intradimensional shift was essentially performed in the same manner as a compound discrimination but had novel odors and digging medias. Following the second intradimensional shift (ID2), mice were divided into treatment groups (CIE or control) and matched according to their SD performance. During the treatment phase, mice were exposed to 3 consecutive weeks of CIE vapor (or air) inhalation. At 72 hr following the final CIE exposure, mice were re-tested for performance on an intradimensional shift (ID3 and ID4) in order to reorient mice to the task. The ability to reverse the association between odor and the presence of a food reward was measured during reversal learning (RevID4). The same odors and digging medias were used from the previous intradimensional shift but the food reward was buried in the previously unbaited bowl. Another intradimensional shift was performed (ID5) followed by an extradimensional shift (ED) that evaluated the ability to shift attention to another perceptual dimension in order to receive the food reward. Novel odors and digging medias were used for extradimensional shift but the food reward was paired with another perceptual dimension (e.g., switched from odor to digging media).

Experiment 2: Effects of CIE exposure and protracted abstinence on reversal learning

To examine the possibility that deficits in cognitive performance could reverse over a more extended period of abstinence, Experiment 1 was repeated but included three procedural changes. First, only reversal learning was tested since this task was the only one that showed a change in CIE treated animals. Specifically, reversal learning of a simple discrimination (rather than an intradimensional shift) was incorporated in this study design because we hypothesized that simplifying the task might reveal an even greater deficit in reversal learning for CIE exposed mice. Second, performance during reversal of a simple discrimination task was measured prior to and following the CIE exposure. This was conducted to verify reversal of a *simple* discrimination task could be established in mice prior to CIE exposure, and provides a measure for baseline performance for comparison to post-CIE exposure training. Third, mice were tested for the effects of CIE on reversal learning at two time-points during abstinence: 72 hours and 10 days. Briefly, mice were first trained to complete a SD and reversal learning discrimination task. Mice were then divided into treatment groups (CIE or control) and matched according to their SD performance. As in Experiment 1, mice were exposed to 3 consecutive weeks of CIE vapor (or air) inhalation during the treatment phase. To determine how long the effects of CIE exposure on reversal learning persist, each treatment group was further divided into two separate abstinent groups (between-subjects design). Half of the mice from each treatment group were tested at 72 hrs

following the last CIE (or air) exposure. The remaining mice from both treatment groups were tested 10 days following the last CIE (or air) exposure. To allow for similar intoxication levels between each abstinent group (72 hrs vs. 10 days), group assignments were additionally matched according to BECs. The experimental design for this study is depicted in Figure 1B. Simple discrimination and reversal learning tasks were performed in the same manner during baseline (SD1 and RevSD1) and post-treatment testing (SD2 and RevSD2), except different (novel) odors and digging media were used (Table 1).

Data Analysis

For Experiments 1 and 2, separate analyses of variance (ANOVA) were used to analyze the number of trials to reach criterion, the number of errors committed, and latency (seconds) to obtain the food reward for each experiment. For Experiment 1, separate two-way mixed factor ANOVA with Treatment as the between-subjects factor and Task as the repeated measure were used to analyze trials, errors and latency before and after ethanol exposure. As expected, there were no differences between CIE and control mice in performance prior to CIE treatment and thus, data were collapsed across treatment groups. For experiment 2, a three-way mixed factor ANOVA with Treatment and Time of testing as between-subject factors and Task as the repeated measure was used to analyze trials, errors and latency before and after ethanol exposure. Again, as expected, there were no group differences in performance prior to CIE treatment and this warranted collapsing pretreatment data across Treatment and Time groups. For all analyses, post-hoc comparisons were performed by isolating simple effects and by using Fisher's Least Significant Difference (LSD) test (significance level set at $p < 0.05$).

To compare performance between Experiments 1 and 2, all three measures (trials, errors, latency), were normalized to SD performance by using the overall SD mean obtained from all mice [(score/mean of SD)*100]. The overall SD mean included scores from both CIE and control mice given that there were no initial group differences in SD performance (see Results section for details). Separate group means were used for Experiments 1 and 2. Scores were normalized to SD since this was the first discrimination task mice were exposed to and thus provided a baseline measure of discrimination learning. Expressing scores as percent of SD also allowed the magnitude of CIE effects to be easily compared between Experiments 1 and 2.

Results

Experiment 1

Performance on Discrimination Tasks Prior to CIE Exposure—Prior to inhalation treatment, mice required between 10–12 trials to learn the discrimination tasks and committed 2–3 errors. Overall, there were no differences between the different discrimination tasks in the number of trials needed to reach criterion [Figure 2A Task: $F(3, 57) = 1.32, p > 0.05$] or the number of errors committed [Figure 2B Task: $F(3, 57) = 1.13, p > 0.05$]. The time required for mice to find the bait during each discrimination task varied between 33–111 sec. There was a significant main effect of Task for latency to obtain the reward [Figure 2C Task: $F(3, 57) = 7.36, p < 0.05$]. Latencies decreased to 36 and 33 sec during ID1 and ID2 respectively, suggesting mice became faster at obtaining the reward with each successive discrimination task. Following baseline measurement of discrimination learning, mice were divided into two separate treatment groups (CIE, control) and matched on their performance during SD. No statistical differences were noted between treatment groups at baseline [for trials: Treatment x Task $F(3, 54) = 0.37, p > 0.05$; for errors: Treatment x Task $F(3, 54) = 0.09, p > 0.05$; for latency Treatment x Task $F(3, 54) = 0.92, p > 0.05$; data not shown].

Effects of CIE exposure on reversal learning and set-shifting—Sample sizes for Experiment 2 were as follows: CIE ($N = 11$) and control ($N = 9$) mice. Average BEC during the 3 weeks of CIE exposure was 237.02 ± 15.51 mg/dl. ID3 scores were excluded from the analyses because performance was quite variable given that mice were still reorienting to the task after 3 weeks off from training (trials: CIE 12.6 ± 1.3 , control 17.8 ± 3.4 ; errors: CIE 4.0 ± 1.0 , control 6.5 ± 2.0 ; latency: CIE 66.0 ± 11.3 , control 55.4 ± 13.5). However, it should be noted there were no differences in performance during ID3 between CIE and control mice on any of the measures ($p > 0.05$). ANOVA did not reveal a significant Treatment x Task interaction for number of trials required to achieve criterion performance [Figure 2D $F(3,54) = 1.23$, $p > 0.05$] or the number of errors committed [Figure 2E $F(3,54) = 1.11$, $p < 0.05$]. There was a significant Main effect of Task for trials [Figure 2D $F(3,54) = 7.81$, $p > 0.05$] and errors [Figure 2E $F(3,54) = 10.34$, $p < 0.05$]. For set-shifting, both CIE and control mice required more trials to reach criterion during ED than ID5 (ED > ID5) indicating set-shifting was more challenging than an intradimensional shift (Figure 2D–E; $p < 0.05$). Additionally, both CIE and control mice required more trials to reach criterion and made more errors during reversal learning than ID4 (RevID4 > ID4) indicating reversal learning was more challenging than an intradimensional shift (Figure 2D–E; $p < 0.05$). Although there were no statistically significant differences between treatment groups on reversal learning performance ($p > 0.05$), it should be noted that the number of trials needed to reach criterion performance during reversal learning marginally increased from 12 (controls) to 15 (CIE) trials. Similarly, the number of errors committed during reversal learning slightly increased from 5 (control) to 7 (CIE) errors ($p > 0.05$). Furthermore, no other tasks (ID, ED) showed any indication of a change in performance between control and CIE mice suggesting CIE-induced cognitive deficits would be selective to reversal learning tasks. There was no significant interaction of Treatment x Task for latency to obtain the bait [Figure 2F $F(3,54) = 1.26$, $p > 0.05$], but there was a significant main effect of Task [$F(3,54) = 18.59$, $p < 0.05$]. These data indicate that mice were faster at finding the bait with successive discrimination tasks regardless of their treatment history. There was also a significant Main Effect of Treatment [Figure 2F $F(1,54) = 5.11$, $p < 0.05$] with CIE mice finding the bait a bit faster than control mice. Counterbalancing the direction of the attention shift (odor to medium vs. medium to odor) did not impact findings or interact with other variables for any of the measures obtained [Treatment x Shift Direction x Task: trials, $F(3, 48) = 0.33$, $p > 0.05$; errors, $F(3, 48) = 0.33$, $p > 0.05$; latency, $F(3, 48) = 0.58$, $p > 0.05$].

Experiment 2

Performance on Simple Discrimination and Reversal Learning Prior to CIE Exposure—Prior to inhalation treatment, mice learned the simple discrimination (SD1) in 7–8 trials and committed an average of 1 error. During reversal learning (RevSD1), the number of trials required to reach criterion performance increased to 16 trials [Table 3; $t(88) = -8.11$, $p < 0.05$]. Further, the number of errors committed increased to 6 errors [Table 3; $t(88) = -10.17$, $p < 0.05$], indicating reversal learning was more challenging than a simple discrimination. There was a significant main effect of Task for latency to obtain the food reward indicating that latencies decreased from 32 to 24 sec in the SD1 and RevSD1 tasks, respectively [Table 3; $t(88) = 2.01$, $p < 0.05$]. Following this initial measurement of reversal learning in naïve animals, mice were divided into two separate treatment groups (CIE, control) and matched on their performance during SD. Each treatment group was further divided into two separate time groups (72 hrs, 10 days). No statistical differences were noted between treatment groups at baseline [for trials: Treatment x Time x Task $F(1, 41) = 0.08$, $p > 0.05$; for errors: Treatment x Time x Task $F(1, 41) = 0.45$, $p > 0.05$; for latency Treatment x Time x Task $F(1, 41) = 0.57$, $p > 0.05$; data not shown].

Effects of CIE exposure on simple discrimination and reversal learning—

Sample sizes for Experiment 2 were as follows: 72 hrs CIE $n = 10$, control $n = 9$, 10 days CIE $n = 16$, control $n = 10$. Average BECs during the 3 weeks of CIE exposure were 196.55 ± 5.60 and 182.45 ± 5.67 mg/dl for the 72 hr and 10 day abstinent groups, respectively. Overall, reversal learning was impaired in CIE mice compared to controls in a time-dependent manner. ANOVA revealed a significant Treatment $\times$ Time $\times$ Task interaction for number of trials required to achieve criterion performance (Figure 3A, D) [$F(1,41) = 5.47$, $p < 0.05$] and for the number of errors committed [Figure 3B, E; Treatment $\times$ Time $\times$ Task: $F(1,41) = 8.98$, $p < 0.05$]. For the 72 hr abstinent group, there were no differences between CIE and control groups during simple discrimination (SD2) learning in the number of trials needed to reach criterion performance (Figure 3A; $p > 0.05$) or the number of errors made (Figure 3B; $p > 0.05$). Similar to Experiment 1, both CIE and control mice required more trials to reach criterion (Figure 3A; $p < 0.05$) and made more errors (Figure 3B; $p < 0.05$) during reversal learning than simple discrimination indicating reversal learning was more challenging than a simple discrimination. However, in this experiment, CIE mice required more trials to reach criterion performance (Figure 3A; $p < 0.05$) and made more errors (Figure 3B; $p < 0.05$) in the reversal learning task than control mice. For the 10 day abstinence group, there were no statistically significant differences between CIE and control mice in the number of trials needed to reach criterion performance (Figure 3D; $p > 0.05$) or in the number of errors committed (Figure 3E; $p > 0.05$). Although the Treatment $\times$ Task interaction in the 10 day abstinence group was not significant, it was evident that control mice in the 10 day abstinence group committed more errors during the reversal learning task than during simple discrimination again indicating that the reversal learning task was challenging (Figure 3E; $p < 0.05$). The interaction terms for latency to obtain the food reward did not significantly differ for Treatment, Task and Time factors [Treatment $\times$ Time $\times$ Task: $F(1,41) = 0.77$, $p > 0.05$]. However, there was a trend for a significant Main effect of Task [$F(1,41) = 3.70$, $p = 0.061$] indicating longer latency during SD2 compared to RevSD2 in all groups (Figure 3C, F). Deficits in reversal learning seen in CIE mice at 72 hr were no longer evident at 10 days following CIE exposure as there was no impairment in the ability to learn simple discrimination or reversal learning tasks.

Comparison of reversal learning protocols

Although both experiments indicated an increase in the number of trials needed to reach criterion performance during reversal learning and the number of errors committed in CIE mice, only Experiment 2 revealed statistically significant deficits in reversal learning performance. These data indicate that the procedures used in Experiment 2 were more adept at revealing the cognitive impairing effects of CIE in reversal learning. Therefore it was our aim to identify what factors (experimental, procedural) contributed to a more sensitive assay in Experiment 2. Given the different protocols used, scores from the above experiments were normalized to SD performance so that discrimination performances in Experiments 1 and 2 could be directly compared (see Data Analysis section above for details). Normalized scores (Table 4) revealed significant interactions in both Experiments 1 and 2 for number of trials required to achieve criterion performance [Experiment 1: $F(3,54) = 3.01$, $p < 0.05$; Experiment 2: $F(1,41) = 5.44$, $p < 0.05$] and the number of errors committed [Experiment 1: $F(3,54) = 4.24$, $p < 0.05$; Experiment 2: $F(1,41) = 9.08$, $p < 0.05$]. Controlling for changes in performance rates relative to SD (baseline) performance aided in revealing deficits in reversal learning for both experiments (RevID4 and RevSD2) with CIE mice requiring more trials (Figure 4A) and making more errors (Figure 4B) during reversal learning than control mice. However, comparing the two reversal learning tasks revealed that the magnitude of the performance deficit in Experiment 2 (RevSD2) was greater than that observed in Experiment 1 (RevID4). Specifically, CIE mice in RevSD2 need more trials (222%) and made more errors (653%) relative to SD performance than CIE mice in RevID4 (152%,

265%). For both experiments, the interaction terms for latency to obtain the food reward relative to SD (baseline) performance did not significantly differ [Experiment 1: $F(3,54)=1.26$, $p>0.05$; Experiment 2: $F(1,41)=0.24$, $p>0.05$]. Together these data suggest that the reversal of a simple discrimination in Experiment 2 was more sensitive to revealing the cognitive impairing effects of CIE exposure than the reversal of an intradimensional shift in Experiment 1.

Discussion

The present study utilized an established mouse model of alcohol dependence (CIE) to address the following questions: 1) are set-shifting and reversal learning impaired following chronic exposure to ethanol and 2) do CIE-induced deficits in task performance persist during prolonged abstinence. Somewhat unexpectedly, we observed that performance on a mPFC-dependent behavior (extradimensional set-shift) was not impaired 72 hrs following CIE exposure. Although CIE mice made fewer errors than controls during ED when compared to SD, this level of performance (3 errors) was more similar to that observed during baseline discrimination tasks and is not likely a true facilitation of set-shifting performance. In contrast, CIE exposure did significantly impair performance of a reversal learning task when tested at 72 hr following CIE, but not at 10 days abstinence. Deficits in reversal learning 72 hr following CIE were revealed as greater number of trials needed to reach criterion, more errors made and a greater difficulty in performing a reversal learning task relative to baseline discrimination performance. Furthermore, the magnitude of the impairment was greater when mice were required to reverse a simple discrimination rather than reversing an intradimensional shift. These data suggest that reversal learning, but not attention set-shifting, is transiently disrupted during short-term abstinence from ethanol. Given that reversal learning requires an intact OFC, these findings suggest that the OFC may be particularly sensitive to the cognitive impairing actions of CIE exposure.

CIE-induced cognitive impairment during reversal learning is dependent on the type of reversal learning task

Although both RevID4 and RevSD2 tasks indicated an increase in the number of trials needed to reach criterion performance during reversal learning and the number of errors committed in CIE mice, only Experiment 2 revealed statistically significant deficits in reversal learning (raw scores). These data indicated that Experiment 2 procedures (RevSD2) were more adept at revealing the cognitive impairing effects of CIE in reversal learning. To verify this, scores were normalized according to SD (baseline) performance and compared between the two experiments. These secondary analyses indicated both types of reversal learning assays (RevID4 and RevSD2) revealed deficits in reversal learning for CIE mice. However, the magnitude of the impairment was much greater for reversal of a simple discrimination relative to reversal of an intradimensional shift.

One factor that may underlie the greater magnitude of impairment in CIE mice during RevSD2 performance is task complexity. It is quite possible that CIE-induced reversal learning deficits are revealed in simple rather than more challenging tasks. Simple discrimination tasks require the subject to pay attention to only one perceptual dimension (i.e., odor). An intradimensional shift may be more difficult for mice in general to complete given that this task incorporates two perceptual dimensions (i.e., odor, bedding), forcing the subject to decide which dimension is relevant. However, it is unlikely that the difficulty of the task itself is the factor leading to greater reversal learning deficits in RevSD2 given that no statistical differences were found between SD and any ID. Another procedural difference between the two experiments is that Experiment 2 (RevSD) incorporated a baseline measure of reversal learning prior to CIE exposure and could also be a factor leading to greater CIE-induced deficits in RevSD2. Others have shown that learning deficits were eliminated with

repeated exposures to a reversal learning task (Black et al, 2006; Bondi et al, 2008). Therefore it is unlikely that adding a baseline measure of reversal learning allowed RevSD2 to be a more sensitive measure of CIE-induced cognitive deficits. The greater magnitude of cognitive impairments in RevSD2 may most likely be a result of mice in Experiment 2 having less discrimination training in general. Mice in Experiment 1 had to learn and complete six separate discrimination tasks (SD, CD, ID1-4) prior to RevID4 while mice in Experiment 2 had half as much prior discrimination training (SD1, RevSD1, SD2). Therefore less exposure to discrimination training, as seen in Experiment 2 (RevSD), may render mice more vulnerable to factors that disrupt learning. Results from another study indicated that the number of discriminations presented prior to reversal learning and ED tasks can influence the outcome; however, these reports suggest that more training reveals greater cognitive deficits in lesioned mice (Bissonette et al, 2008).

Although previously published papers showing reversal learning deficits used raw data (i.e., trials to criterion and errors) for analysis, most of these reports involved lesioned rodents (Birrell and Brown, 2000; Bissonette et al, 2008) or the acute effects of a pharmacological agent (Kos et al, 2011) making it unnecessary to track changes in performance relative to baseline given that all of the behavioral training was conducted within a few days. In contrast, the present experiments incorporated alcohol abstinence as an experimental variable and broke discrimination training into before and after treatment sessions. Although the physical signs of alcohol withdrawal have largely dissipated by 72 hrs, we do not know how quickly the cognitive effects of CIE change overtime thereby making percent change from SD (baseline) performance a more appropriate measure for testing the effects of CIE-induced impairments during alcohol abstinence. Taken together, the experimental protocol utilized in Experiment 2 (RevSD) is optimal for revealing deficits in reversal learning in CIE mice. Future investigations of the effects of chronic exposure to pharmacological agents on reversal learning should be tested in this manner.

Lack of evidence for impaired set-shifting during early abstinence from ethanol

CIE did not impair performance in set-shifting. These findings are somewhat unexpected given that set-shifting has been reported to be impaired in alcohol-dependent humans (Kish et al, 1980; Parsons, 1983; Rourke and Grant, 1999; Yohman et al, 1985). One explanation for these findings may be that although the ID/ED set-shifting task is a valid measure of set-shifting for primates/rodents (Birrell and Brown, 2000; Bissonette et al, 2008; Clarke et al, 2005), it may not be as sensitive as those used to test for cognitive impairments in alcoholics (e.g., WCST, Trails B). One major difference between the WCST and ID/ED tasks is that WCST incorporates a response conflict. In the WCST, the same cues are used for all rule changes (e.g., color, suit, number). A response conflict arises when the subject must choose between the previously correct and newly correct cue. In contrast, the ID/ED task incorporates novel cues at each ID and ED task and thereby eliminates the response conflict. A lack of response conflict may be the reason the present ED experiment did not reveal deficits in set-shifting for CIE mice. On the other hand, reversal learning in the present study did include a response conflict as the same cues were used in the prior task (SD or ID4). Therefore, deficits in reversal learning for CIE mice in the present study may have been exacerbated by response conflict.

Importantly, other pharmacological agents that block NMDA receptor function such as PCP (Broberg et al, 2008) and ketamine (Kos et al, 2011) impair performance on ID/ED set-shifting tasks. Therefore it is unlikely that the set-shifting assay used in the present study was not sensitive enough to reveal pharmacological induced impairments in set-shifting. Another possibility for the lack of impairment in set-shifting observed in CIE mice in the present study may be related to the degree of ethanol dependence achieved during the CIE treatment. Several studies have recently reported that deficits in set-shifting in alcohol-

dependent humans are only evident in treatment-seeking alcoholics, as non-treatment seeking alcoholics perform similarly to non-alcoholic controls on tests of cognitive flexibility (Fein et al, 2006; Smith and Fein, 2010). This suggests that deficits in set-shifting may only be evident in more severely dependent alcoholics or in animals that have a history of more robust and extensive chronic ethanol exposure. These effects could also reflect underlying differences in genetic and dietary factors between treatment-seeking and non treatment-seeking individuals. Clearly, further work is needed to more fully characterize the time and dose-dependent effects of CIE exposure on set-shifting behavior in rodents.

Impaired reversal learning in CIE mice is reversible

Results of the present study show that, in contrast to its effects on set-shifting, CIE exposure induced deficits in reversal learning that are similar to that reported for human alcoholics (Fortier et al, 2008; Fortier et al, 2009), primates (Jedema et al, 2010) and rodents (Brown et al, 2007; Oboernier et al, 2002; Ripley et al, 2003; Thomas et al, 2004; Wainwright et al, 1990). That is, performance during reversal learning was significantly impaired following short-term abstinence from alcohol exposure, as CIE mice required more trials, made more errors and performed worse relative to simple discrimination (baseline) than controls. Impaired performance in the reversal learning task in CIE mice is likely not due to differences in locomotor activity or motivation to obtain the food reward since there was no significant difference between CIE mice and controls in latencies to obtain the reward. Rather, the selective nature of the apparent learning deficit may reflect compromised neurophysiological and behavioral consequences of CIE exposure.

As mentioned above, results from the present study show that deficits in reversal learning in CIE treated mice were not permanent. While performance during reversal learning was clearly impaired at 72 hr post-CIE, this difference was not observed when mice were tested 10 days abstinent. These results are qualitatively similar to those observed in humans, where abstinent alcoholics initially perform poorly on tasks requiring behavioral inhibition and cognitive flexibility, but then recover within 3 weeks to 2 years abstinence (Kish et al, 1980; Parsons, 1983; Rourke and Grant, 1999; Yohman et al, 1985). It should be noted that these clinical studies used measures of set-shifting and decision-making to draw conclusions about alcohol's effects on cognition. While tasks that measure the ability to shift attention also require the ability to inhibit responses to the incorrect choice, set-shifting tasks do not directly measure reversal learning. Reports of deficits in reversal learning for alcoholics have only measured task performance during short-term alcohol abstinence (Fortier et al, 2008; Fortier et al, 2009). More clinical studies are needed to determine how long deficits in reversal learning persist in abstinent alcoholics.

Ethanol's effect on OFC- and medial PFC-dependent behaviors

Others have shown that primates and patients with OFC lesions perform more poorly than those with an intact OFC on reversal learning tasks. Despite these effects, lesioned subjects were still flexible and able to shift their attention to a new rule (Tsuchida et al, 2010; Walton et al, 2010). Interestingly, the present experiments in mice also showed impaired reversal learning and a modest facilitation of set-shifting following alcohol exposure. Given that reversal learning and set-shifting are mediated in part by two separate sub-regions of the PFC (OFC vs. mPFC), these data suggest the OFC and mPFC may respond to alcohol exposure differently. Interestingly, neurons in the OFC and mPFC also demonstrate contrasting morphological and physiological effects following various insults. For example, chronic stress increased apical dendritic arborization in OFC neurons but decreased it in the anterior cingulate portion of the mPFC (Liston et al, 2006) while amphetamine had the opposite effect (Kolb et al, 2004; Robinson and Kolb, 2004). Furthermore, findings from preliminary studies show that acute ethanol decreased the frequency of current-evoked spike

firing in OFC brain slices (Badanich et al, 2010) while previously published data report no effect of ethanol on this measure in the mPFC (Weitlauf and Woodward, 2008). Together these data support the idea that anatomical and functional differences between OFC and mPFC areas underlie the differential responses these regions often show in response to normal and pathological conditions (Kolb et al, 2004; Moghaddam and Homayoun, 2008).

Although CIE did not impair mPFC-dependent behaviors (i.e., set-shifting) measured in the present study, these data should not be interpreted to mean that ethanol has no effect on mPFC neuron function. Previous studies from this lab demonstrated that acute ethanol markedly decreased the duration and amplitude of network-dependent up-states in the mPFC (Tu et al, 2007; Woodward and Pava, 2009) and selectively reduced NMDA-, but not AMPA- or GABA-mediated synaptic currents in deep layer mPFC neurons (Weitlauf and Woodward, 2008). These data when taken together with the results of the present study suggest that while alcohol can disrupt normal mPFC neuron activity, adaptive responses may restore function and allow for adequate performance on certain cognitive behavioral tasks. Interestingly, a recent report using mice has shown that performance in set-shifting, but not reversal learning, is differentially altered by NMDA receptor antagonists. In that study, the non-selective NMDA channel blocker ketamine impaired set-shifting but not reversal learning and this deficit was reversed by the NMDA NR2B-subtype specific antagonist Ro 25-6981 (Kos et al, 2011). Ro 25-6981 alone improved performance on the EDS portion of the test and similar compounds have been shown to enhance response speed and accuracy on tasks requiring cognitive flexibility (Gilmour et al, 2009; Higgins et al, 2005). Given that chronic ethanol exposure is known to alter expression of NR2B and other NMDA subunits in various brain regions (Kalluri et al, 1998; Qiang et al, 2007; Roberto et al, 2006; Wang et al, 2010) it is possible that the enhanced performance of CIE mice during set-shifting reflects changes in NMDA receptor function. Future research in this lab is focused on determining how CIE affects NMDA receptor expression and function in mPFC and OFC neurons.

Ethanol dependence and the OFC

The disruption in reversal learning observed in CIE mice suggests that OFC function may have been affected. Lesions to the OFC in non-alcoholic patients (Hornak et al, 2004; Tsuchida et al, 2010) or rodents (Bissonette et al, 2008; McAlonan and Brown, 2003) are clearly associated with impaired performance in reversal learning tasks. Alcoholics also have some characteristics in common with patients that have OFC damage. Both alcoholics and patients with OFC damage exhibit deficits in behavioral inhibition (Verdejo-Garcia et al 2006) and have a tendency to perseverate (Hill et al, 2009; Tanabe et al 2009). With longer exposures, alcoholics also show less densely packed neurons/glia and less grey/white matter in the OFC than controls, an effect that is highly associated with perseverative and impulsive behavior (Hill et al, 2009; Miguel-Hidalgo et al, 2006; Tanabe et al 2009). Excessive alcohol exposure also alters neurophysiological function. For example, baseline brain metabolism in the OFC as measured by PET and 2-deoxy-2[18F]fluoro-D-glucose was lower in alcohol dependent individuals than controls, an effect that was more pronounced during earlier withdrawal periods (8–11 weeks; Catafau et al, 1999; Volkow et al, 1997; Volkow and Fowler, 2000). However, other studies have found differences in brain metabolism to be localized to the mediodorsal and dorsolateral PFC and not in the OFC (Dao-Castellana et al, 1998). It is not known whether neurotoxic damage to the OFC is required to produce deficits in OFC-related behaviors or if cognitive deficits following chronic alcohol exposure may precede signs of cell loss. The recovery of reversal learning performance after 10 days withdrawal in the CIE mice suggests that the 3-week ethanol vapor exposure regimen did not produce permanent cell loss in the OFC, although this needs to be verified.

It has been suggested that an impaired ability to unlearn familiar stimulus-outcome situations may be a critical factor that contributes to continued alcohol abuse/misuse (Fortier et al, 2008). Alcohol-dependent individuals with cognitive deficits may have greater difficulty abstaining from alcohol given their increased tendency to perseverate and their general lack of behavioral inhibition/control (Noel et al, 2007; Oscar-Berman et al, 2004). Cognitive deficits may also contribute to compulsive and heavy alcohol drinking by disrupting the ability to restrain from consuming alcohol when presented with alcohol-related cues or under stressful conditions (Volkow et al, 1997). Nonetheless, data from the present study indicate that at least in the CIE mouse model, deficits in reversal learning are not long lasting. These data suggest that OFC-dependent cognitive deficits that arise as a result of heavy drinking may dissipate with abstinence and may provide an opportunity for interventions that can help control compulsive drinking behavior. Understanding the extent and specificity of cognitive impairment in alcohol-dependent individuals may possibly lead to better treatments for alcohol-induced neurophysiological dysfunction in the PFC.

Conclusions

In summary, reversal learning, but not set-shifting, was impaired during short-term abstinence from CIE. Furthermore, the magnitude of the impairment was greater when mice were required to reverse a simple discrimination rather than reversing an intradimensional shift. Impaired performance on reversal learning was not permanent as performance recovered during a longer ethanol abstinence period. Given that the OFC is involved in mediating reversal learning, the results of this study suggest that CIE may alter OFC function. Ongoing studies are focused on determining what effects CIE has on the activity and signaling functions of the OFC. Elucidating the impact of ethanol on OFC function and cognitive flexibility is critical for developing a better understanding of the behavioral consequences of chronic alcohol drinking.

Acknowledgments

The authors would like to thank Dr. David S. Tait and Dr. Marcelo F. Lopez for helpful discussions during the preparation of this manuscript. This work was supported by NIAAA grants F32 AA019610 (KAB) and P50 AA010761 (JJW, HCB).

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Figure 1. Experimental design for the reversal learning and set-shifting experiments

A) Experiment 1 design B) Experiment 2 design. In this and all subsequent Figures, abbreviations denote type of task. SD = simple discrimination, CD = compound discrimination, ID = intradimensional shift, Rev = reversal learning, ED = extradimensional shift.

Figure 2. Reversal learning and set-shifting in CIE mice

Number of trials required to reach criterion (A, D), errors committed (B, E) and latency to obtain the bait (C, F) were measured on discrimination trials before CIE exposure (left panels) and 72 hrs following the last CIE exposure (right panels). Data in the left panels are collapsed across Treatment groups (N = 20/bar). Data in the right panels represent CIE (N = 11) and control (N = 9) mice. All bars represent mean $\pm$ SEM. # = differs from SD or previous ID.

Figure 3. Reversal learning is impaired during early but not protracted ethanol abstinence
 Number of trials required to reach criterion (A, D), errors committed (B, E) and latency to obtain the bait (C, F) were measured on discrimination trials 72 hrs (left panels) and 10 days (right panels) following the last CIE exposure (72 hrs CIE $n = 10$, control $n = 9$, 10 days CIE $n = 16$, control $n = 10$). All bars represent mean $\pm$ SEM. * = differs from control; # = differs from SD2.

Figure 4. CIE-induced cognitive impairment during reversal learning is dependent on the type of reversal learning task utilized

Percent change from SD (baseline) for the number of trials required to reach criterion (A), errors committed (B) and latency to obtain the bait (C) were compared between Experiment 1 (RevID4) and Experiment 2 (RevSD) protocols. All mice were tested at 72 hours following the last CIE exposure. Scores were taken from Table 4. All bars represent mean $\pm$ SEM. * = differs from control.

Table 1**Exemplar Combinations**

Odor pairs	Medium Pairs
Experiment 1	
rosemary vs. tumeric	forest bedding vs. bark chips
smoked paprika vs. cloves	gravel vs. confetti
onion vs. nutmeg	aspen shavings vs. crushed coral
BBQ vs. thyme	stones vs. hydroballs
fennel vs. cumin	snake bedding vs. kay-kob
dill weed vs. ginger	wood pellets vs. paper shavings
garlic vs. cinnamon	Carefresh pet bedding vs. alpha-dri
Experiment 2	
garlic vs. cinnamon	cat litter
onion vs. thyme	alpha-dri

Exemplar Combinations. Stimuli for discrimination tasks consisted of pairs of odors and digging media.

Table 2

Order of Discrimination Tasks

Dimension		Exemplar Combinations		
Task	Relevant	Irrelevant	Correct	Incorrect
Experiment 1				
SD	odor	medium	O1 , M1	O2, M1
CD	odor	medium	O1 , M1 or M2	O2, M1 or M2
ID1	odor	medium	O3 , M3 or M4	O4, M3 or M4
ID2	odor	medium	O5 , M5 or M6	O6, M5 or M6
ID3	odor	medium	O7 , M7 or M8	O8, M7 or M8
ID4	odor	medium	O9 , M9 or M10	O10, M9 or M10
RevID4	odor	medium	O10 , M9 or M10	O9, M9 or M10
ID5	odor	medium	O11 , M11 or M12	O12, M11 or M12
ED	medium	odor	M13 , O13 or O14	M14, O13 or O14
Experiment 2				
SD1	odor		O1 , M1	O2, M1
RevSD1	odor		O2 , M1	O1, M1
SD2	odor		O3 , M2	O4, M2
RevSD2	odor		O4 , M2	O3, M2

Order of discrimination tasks for all mice. Some mice switched from odor to medium while others switched from medium to odor. Words in bold denote the positive stimulus (i.e., the baited bowl). O = odor; M = medium; SD = simple discrimination; CD = compound discrimination; ID = intradimensional shift; Rev = reversal learning; ED = extradimensional shift

Table 3

Performance on simple discrimination and reversal learning prior to CIE exposure

Task	TRIALS	ERRORS	LATENCY
SD1	7.7 ± 0.4	0.7 ± 0.1	31.6 ± 3.1
RevSD1	15.8 ± 0.9 [#]	6.5 ± 0.5 [#]	23.6 ± 2.3 [#]

Performance on simple discrimination and reversal learning prior to CIE exposure. Raw data for number of trials required to reach criterion, errors committed, and latency (sec) to obtain the bait. Each score represents mean ± SEM.

[#] differs from SD1.

Table 4

Percent change from SD (baseline) for trials, errors, and latency prior to and after CIE exposure

TIME	TASK	TRIALS		ERRORS		LATENCY	
		control	CIE	control	CIE	control	CIE
Exp.1	SD	84.7 ± 9.4	104.7 ± 9.9	67.3 ± 24.1	103.2 ± 26.4	144.0 ± 27.8	84.2 ± 27.6
	CD	79.2 ± 14.2	115.2 ± 17.0	43.4 ± 28.9	126.4 ± 47.7	124.8 ± 33.1	66.0 ± 20.0
	ID1	121.0 ± 23.6	124.2 ± 22.4	120.7 ± 45.7	162.0 ± 43.9	37.0 ± 8.1	36.7 ± 11.5
	ID2	100.1 ± 10.7	131.4 ± 24.3	111.1 ± 28.1	185.7 ± 73.7	51.2 ± 19.42	18.2 ± 3.9
	ID4	93.1 ± 6.6	83.7 ± 8.3	62.5 ± 12.7	59.2 ± 19.6	66.3 ± 14.9	45.0 ± 3.8
	RevID4	114.0 ± 9.0	152.7 ± 11.6 ^{##}	171.9 ± 28.2 [#]	264.6 ± 35.3 ^{##}	31.4 ± 5.4	16.3 ± 2.9
	ID5	89.6 ± 9.4	69.6 ± 4.2	62.8 ± 17.9	31.6 ± 11.8	25.4 ± 5.0	19.0 ± 3.0
	ED	126.4 ± 10.2 [#]	112.9 ± 15.6 [#]	156.3 ± 32.3 [#]	82.3 ± 13.4 ^{##}	14.4 ± 3.1	14.6 ± 3.9
Exp. 2		control	CIE	control	CIE	control	CIE
72 hrs	SD1	104.6 ± 12.4	103.8 ± 10.8	93.5 ± 47.8	105.3 ± 38.4	84.6 ± 14.8	53.9 ± 8.8
	RevSD1	212.3 ± 45.6	176.9 ± 13.7	888.9 ± 291.5	663.2 ± 133.2	63.6 ± 11.4	34.4 ± 5.0
10 days	SD1	103.8 ± 11.3	96.1 ± 7.2	126.3 ± 46.5	92.1 ± 35.7	105.9 ± 29.8	119.3 ± 14.4
	RevSD1	210.3 ± 14.3	210.4 ± 17.8	768.4 ± 87.4	750.0 ± 104.8	98.8 ± 19.4	76.5 ± 10.2
72 hrs	SD2	85.4 ± 4.7	103.8 ± 8.6	49.3 ± 26.3	94.7 ± 29.1	54.5 ± 10.8	30.0 ± 3.8
	RevSD2	125.9 ± 16.8 [#]	221.6 ± 21.5 ^{##}	261.1 ± 87.1 [#]	653.6 ± 71.7 ^{##}	44.4 ± 12.7	35.8 ± 8.9
10 days	SD2	95.1 ± 10.7	127.3 ± 13.6	54.7 ± 32.7	178.0 ± 44.8	54.3 ± 8.4	70.4 ± 10.1
	RevSD2	126.9 ± 13.5	132.3 ± 15.4	273.6 ± 78.7	243.4 ± 59.0	40.2 ± 8.0	50.7 ± 8.9

Percent change from SD (baseline) for trials, errors, and latency prior to and after CIE exposure. To compare performance between Experiments 1 and 2, all three measures (trials, errors, latency), were normalized to SD performance by using the overall SD mean obtained from all mice [(score/mean of SD)*100]. Each score represents mean ± SEM. These scores were calculated from raw scores presented in Figures 2–3.

* = differs from control;

differs from previous task (e.g., ID or SD).