Alcohol Consumption during Adulthood Does Not Impair Later Go/No-Go Reversal Learning in Male Rats

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Abstract: Reversal learning tasks are used to model flexible decision-making in laboratory animals, and exposure to drugs of abuse can cause long-term impairments in reversal learning. However, the long-term effects of alcohol on reversal learning have varied. We evaluated whether six weeks of voluntary alcohol consumption through chronic intermittent alcohol access (elevated by food restriction) in adult male rats would impair rats in a go/no-go reversal learning task when tested at an interval beyond acute withdrawal. In our go/no-go task, rats were reinforced for pressing one lever or withholding from pressing another lever, and the identities of the two levers were switched twice (once rats reached an accuracy criterion). We found no evidence that prior alcohol consumption altered discrimination or reversal learning in our task. This replicates previous patterns from our laboratory that higher alcohol consumption in food-restricted rats did not impair discrimination or reversal learning in a different go/no-go task and that alcohol consumption in free-fed adolescent/early adult rats did not impair go/no-go discrimination or reversal learning in the same task. It is unclear whether this represents an insensitivity of this task to alcohol exposure generally or whether an alcohol exposure procedure that leads to higher blood ethanol concentration (BEC) levels would impair learning. More research is needed to investigate these possibilities.

Keywords: alcohol; withdrawal; reversal learning; learning and memory

1. Introduction

Prior extensive alcohol use co-occurs with decision-making impairments and impulsivity, even after alcohol use is discontinued [1–4], but the cause of this relationship is unclear. Previous extensive alcohol consumption may cause increased impulsivity and impaired decision-making, impulsivity and impaired decision-making may precede and increase alcohol consumption, or the two may be caused by some other pre-existing factor [5–8]. Animal research can provide the ability to examine these potential causal relationships.

Prior alcohol consumption is also associated with differences in reversal learning abilities, even when reversal learning is tested after the cessation of alcohol exposure. In reversal learning tasks, one response earns a reinforcer and a different response is non-reinforced in a first phase. The reinforced and non-reinforced responses are then switched in a second phase. In some rodent models, prior alcohol exposure has impaired both the initial discrimination and the later reversal [9–12], whereas other experimental conditions found the original discrimination unimpaired and found a selective deficit in reversal learning [9,13–18]. However, many studies have instead found that prior alcohol exposure does not lead to a discrimination or a reversal learning impairment [19–25]. The prior literature on the long-term effects of alcohol exposure on reversal learning spans procedures from multiple laboratories, which differ in multiple experimental factors (e.g., type of reversal learning task, method and dose of alcohol exposure, age of alcohol exposure, alcohol-test interval), and does not fit a consistent pattern in which a single factor can explain all of the conflicting results. However, in a comparison using the same behavioral
procedures, intraperitoneal injections that led to higher blood ethanol concentrations (BECs) led to a reversal learning impairment, whereas intragastric injections that led to lower BECs did not impair reversal learning [19]. Likewise, the experiments using voluntary drinking as the exposure method that found reversal learning impairments tended to have a higher consumption because of the use of an alcohol solution as the sole source of water or because of the use of a solution of alcohol mixed with maltodextrin [10,17]. Conversely, studies that have failed to demonstrate reversal learning impairments (at least those not involving factors more closely associated with attentional processes) measured reversal learning after alcohol consumption in either unrestricted rats drinking unadulterated alcohol [21,25] or alcohol consumption in food-restricted rats [24]. Therefore, the level of alcohol intake and/or BECs may affect whether reversal learning impairment is observed. There is a more thorough discussion of these issues in the discussion section below.

Our experiment represents an examination of one previous finding that alcohol access did not lead to an impairment in go/no-go reversal learning [21]. In go/no-go discrimination learning, one cue indicates that responding will be reinforced, whereas a second cue indicates that responding will not be reinforced. In our version of the task, pressing one lever led to a reinforced response, while withholding lever-pressing on another lever was reinforced in the first phase and the identities of the two levers were switched in a second phase. In our previous investigation of the effects of prior alcohol access on behavior in this task [21], we used a chronic intermittent access (CIA) to alcohol procedure [26,27] in which rats were given 24-h access to alcohol three times per week with 24- or 48-h alcohol-free periods between each alcohol access period. We gave free-fed male rats this exposure regimen during adolescence and early adulthood from post-natal days (PND) 26–66, and we found no alteration of discrimination or reversal learning when comparing the alcohol and water-only groups [21]. Instead, we found that, within the alcohol access group, the amount of alcohol consumed in weeks five and six (corresponding with early adulthood) was correlated with the number of commission errors (errors in which rats incorrectly pressed the lever that earned food only if the rats withheld responding) during reversal learning. This suggested that alcohol access does not alter reversal learning but that individual differences in the propensity to drink alcohol are associated with reversal learning behavior in this task. Here, we examined whether alcohol access during adulthood would alter reversal learning if the level of alcohol consumption was increased by food-restricting the rats, using a procedure that we have previously shown increases alcohol consumption above the levels seen in free-fed rats [28]. Although the food restriction was likely to increase consumption in ways unrelated to the motivation for the pharmacological effects of alcohol (meaning that correlations or the lack of correlations between alcohol consumption and reversal learning would be less meaningful), the higher alcohol intake was designed to increase the likelihood that alcohol consumption would alter behavior. However, our results, described below, indicate that there was no effect of prior alcohol access on discrimination or reversal learning.

2. Methods
2.1. Subjects
Male Long–Evans rats (n = 24) from Charles River Laboratories in Raleigh, NC, weighing 300–325 g upon arrival, were used for the experiments. Based on the estimated date of birth from Charles River, the rats received alcohol access approximately from PND81-121 and began discrimination training on approximately PND125. The rats were individually housed and maintained on a 12-h reverse light cycle with lights off at 7:30 am. The rats were housed in a humidity- and temperature-controlled room. Following an acclimation period, the rats were food-restricted to 85% of their initial free-feeding weights through once-daily feedings. The minimum daily food ration was 5 g of food chow. Once rats reached 85% of their initial free-feeding weight, their body weight target was increased by 1 g/day from this initial 85% target for the remainder of the experiment. Thus, rats gained 7 g of body weight each week. The daily food ration was adjusted each day to keep
the rats at their target weights. Our lab has previously found that food restriction using this procedure leads to high lever press rates while the rats gain weight over time. Water was freely available throughout the experiment. All of the experimental procedures and animal care procedures were in accordance with the guidance of the Institutional Animal Care and Use Committee of Kansas State University (IACUC approval #3420) as well as the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and federal law of the United States.

2.2. Behavioral Apparatus

Operant behavioral training was conducted in standard self-administration chambers purchased from Med Associates (St. Albans, VT, USA). The chambers contained two retractable levers on either side of the food cup (at approximately the same height). The chambers contained two white stimulus lights, with one light located above each lever. The back wall of the chambers (opposite from the food cup) contained a red houselight (on the top-center) and a speaker for delivering auditory stimuli (on the left side). The equipment was controlled by (and lever presses recorded by) a Dell Optiplex computer equipped with Med-PC for Windows.

2.3. Behavioral Procedure

Once the rats (n = 24) had stabilized on the food-restriction conditions, they received 6 weeks of chronic intermittent alcohol access. There were 2 groups (n = 12/group): the water group and the alcohol group. The water group received water in both bottles, whereas the alcohol group received alcohol in one of the 2 bottles, 3x/week. During the chronic intermittent alcohol access period, all rats had 2 bottles on their cages on all days, with at least one of the bottles containing tap water at all times (a permanent water bottle). The animals in the water group received no alcohol during the experiment and had tap water in both bottles for the duration of the access period. The alcohol group had 20% alcohol (v/v) in one of the bottles for a 24 h period every other day (3 days/week) and water in both bottles on other days. In these groups, the alcohol bottle was placed onto the rats’ cages on Sunday, Tuesday, and Thursday. In the alcohol group, the location of the alcohol bottle was randomized between left (L) and right (R), in the order RLLR-LRRL-RLLR-RLLR-LR, to avoid side preferences affecting consumption. The alcohol and water bottles were weighed and exchanged between 1:00 and 2:30 pm each afternoon. After 6 weeks of chronic intermittent alcohol access/exposure, all rats were given a single water bottle for the remainder of the experiment.

Three days following the final ethanol access period, the rats began magazine training. The rats underwent one 40-min magazine training session with one food pellet (catalogue #1811155, TestDiet, Richmond, IN, USA) delivered every 125 sec. This food pellet was the reinforcer for all phases of the experiment. The following day, the rats began go/no-go discrimination training in which rats were presented with one of the two levers available one at a time in alternating fashion. The rats were reinforced for pressing one lever (the go lever) and reinforced for withholding lever-pressing to the other lever (the no-go lever). Each trial began with a 2 s period where the cue-light above one of the levers was illuminated for 2 s. After this 2 s period, the lever below the light was extended while the light remained illuminated. This lever-light compound was available for 6 s or until the rat made a response on the lever, whichever came first. Once the rat responded or 6 s elapsed without a response, the cue terminated with the lever retracting and the cue-light above the lever extinguished. Correct responses (correct lever-presses on the go lever or correct withholding of responses to the no-go lever) were followed by the delivery of a food pellet and the immediate beginning of the inter-trial interval (ITI) in which the house light remained illuminated but there were no levers or lights available (ITI = 5–11 sec). Incorrect responses (incorrect lever-presses on the go lever (commission errors) or incorrect failures to press the go lever (omission errors)) were followed by a 1 s tone and a 15 s timeout, with the house light off during the timeout. The ITI began after the end of
the timeout so that errors delayed the beginning of the next trial by 15 s. The identity of
the reinforced go-response lever in the original discrimination phase was counterbalanced.
For half of the rats during discrimination, the left lever was the go lever and the right lever
was the no-go lever; for the other rats, the identities of the levers were the opposite. The
session ended either when the rat made 26 correct responses in a row, after the first error
occurring after 48 min, or after the first error occurring after at least 120 correct responses.
The rats were required to meet a discrimination criterion of 26 correct responses in a row
for 2 consecutive days before they began reversal learning.

Once the discrimination criterion was met, reversal training began, and the original
contingencies were reversed. During reversal one, the lever that was provided reinforce-
ment for lever-pressing (the go lever) became the lever that provided reinforcement for
withholding lever-pressing (the no-go lever) and vice-versa. Once the rats reached the rever-
sal criterion of 26 correct responses in a row for 3 consecutive days, the contingencies were
reversed back to the original contingencies for reversal two and the rats were then required
to meet the reversal criterion of 26 correct responses in a row for 3 consecutive days.

For the reversal learning sessions, rats were considered to fail" and discontinued from
training if they made more than 300 commission errors in either reversal. An analogous
criterion was also present for omission errors, but this was moot as no rat made more than
100 omission errors in either reversal. If a rat failed, their scores for that reversal were
300 commission errors and the total number of omission errors made up until that final
training session. If the rat failed in reversal one, then they were not trained in reversal two.
Instead, their reversal one score was also used to provide scores for reversal two.

2.4. Statistical Analysis

For the alcohol consumption phase, the alcohol consumed was calculated based on
the difference in bottle weight between the start and end of the 24-h access period, minus
2 g for spillage and evaporation, as in our previous publications [21,24,28–32]. This weight
of alcohol solution consumed was then multiplied by 0.162 for the weight of pure alcohol
in 1 g of a 20% (v/v) alcohol solution. Pilot research within our laboratory determined that
spillage and/or evaporation was ~2 g/day if bottles were placed on empty cages. The
daily alcohol consumption for the alcohol group rats was combined into weekly averages.
These weekly averages were analyzed with a within-subjects factor ANOVA with the factor
of week (the 6 weeks of alcohol access that contained 3 daily access periods).

The operant behavioral measures used were the number of commission errors (in-
correct lever presses on the no-go lever) and omission errors (incorrect failures to press
the go lever) made in each phase before meeting the criterion to pass the discrimination
or reversal learning phases. Data from the discrimination phase represented the number
of errors of each error type made by the rat before the rat made 26 correct responses in a
row for 2 consecutive days. These data were analyzed with a mixed-factor ANOVA with
the between-subjects factor of exposure group (alcohol and water) and the within-subjects
factor of the error type (omission and commission errors). The data from the reversal
learning phases represented the number of errors of each error type committed before the
rat made 26 correct responses in a row for 3 consecutive days. These data were analyzed
with a mixed-factor ANOVA with the between-subjects factor of exposure group (alcohol
and water) and the within-subjects factors of reversal test (reversal one and reversal two)
and error type (omission and commission errors). Reversal one represented a reversal
away from the discrimination contingencies and reversal two represented a reversal back
to the original discrimination contingencies. One rat in the water group failed to complete
the first reversal before making 300 commission errors. This rat was given a commission
error score of 300 and an omission error score of 84 (the total number of omission errors
made up to the session where the rats met the 300-commission error limit) for both reversal
one and reversal two. Inclusion or exclusion of this rat from the analyses of all phases
(discrimination and reversal) did not affect the pattern of significant effects found.
All data analysis was conducted using Statistica 5.1 (StatSoft, Tulsa, OK, USA). If the main effects or interactions of the exposure group had been significant \( (p < 0.05) \), post-hoc analyses using Tukey Honestly Significant Difference tests would have been run. However, there were no significant main effects or interactions of these factors.

### 3. Results

#### 3.1. Alcohol Consumption

During the alcohol consumption phase, rats in the alcohol group increased their alcohol consumption from \( 5 \, \text{g/kg} \) in the first week to \( \sim 8.5-10 \, \text{g/kg} \) in weeks 2–5 (Figure 1A). The within-subjects ANOVA with the factor of week (the six alcohol access weeks) found a significant effect of that factor \( (F_{(5,55)}=4.3, \ p < 0.01) \). Post-hoc analyses revealed that alcohol consumption in week 1 was significantly lower than consumption in weeks 2–5.

![Figure 1. Experimental data. (A) Alcohol consumption (g/kg/24 h access period, mean+SEM) across the six-week alcohol access period. (B) Errors to criterion (mean+SEM) for discrimination session (disc), reversal 1 (rev 1), and reversal 2 (rev 2) sessions. Errors were divided into commission (C) or omission (O) errors. White symbols/bars represent the water group \((n=12)\) and gray symbols/bars represent the alcohol group \((n=12)\).](image)

#### 3.2. Discrimination Learning

During the discrimination phase, the number of commission or omission errors was unaffected by prior alcohol consumption (Figure 1B, left). A mixed-factor ANOVA with the between-subjects factor of the exposure group (alcohol and water) and the within-subjects factor of the error type (omission and commission errors) revealed a main effect of the error type factor \( (F_{(1,22)} = 10.2, \ p < 0.01) \). There was no significant main effect or interaction of the exposure group factor (all \( F_s < 1 \)).

#### 3.3. Reversal Learning

During the reversal learning phases, the number of commission or omission errors was unaffected by prior alcohol consumption (Figure 1B, right). A mixed-factor ANOVA with the between-subjects factor of the exposure group (alcohol and water) and the within-subjects factor of the reversal test (reversal one and reversal two) and the error type (omission and commission errors) revealed a main effect of error type \( (F_{(1,22)} = 76.3, \ p < 0.01) \). No other interactions or main effects were significant (all \( p_s > 0.05 \)).

#### 3.4. Alcohol-Operant Behavior Correlations

Finally, we examined whether there were any potential dose related effects of alcohol consumption on behavior during the reversal learning sessions. We determined whether alcohol consumption across the six weeks or alcohol consumption in the last two weeks of alcohol access were correlated with the number of omission or commission errors during discrimination or reversal learning. We found no correlations between the quantity of alcohol consumed across the six weeks or during the last two weeks with omission or commission errors during discrimination or reversal learning (all \( p_s > 0.10 \)) (Figure 2).
3.5. Discussion

We found no effect of prior alcohol consumption during adulthood (with elevated levels due to food restriction) on go/no-go discrimination or reversal learning in our task. The failure of alcohol access to alter go/no-go discrimination learning replicates patterns observed previously in our lab [21,32], (but see [33,34] for more mixed results in other procedures in other laboratories). However, we will focus our discussion below on our finding that alcohol access did not alter reversal learning. Notably, we also found no correlation between the amount of alcohol the rats consumed and their discrimination or reversal learning behavior. This suggests that the normal range of variation in voluntary alcohol drinking under chronic intermittent access may not reach levels sufficient to cause reversal learning deficits even at the higher individual levels of drinking. This also represents a different pattern from our previous investigation of the relationship between alcohol access and go/no-go reversal learning using this task, in which alcohol consumption levels in the last two weeks of consumption correlated with the commission errors during reversal [21].

Figure 2. Correlations between alcohol consumption and discrimination/reversal learning errors. The top row represents errors during the discrimination phase, the middle row represents errors during the first reversal phase, and the bottom row represents error during the second reversal phase.
However, as the rats were food-restricted in the current experiment, their consumption may have been motivated by the caloric value of the alcohol rather than the pharmacological effects of alcohol, and our measure of alcohol consumption may not have been analogous to the consumption measure in the previous paper.

Our failure to observe an effect in go/no-go reversal learning adds to the mixed literature on the effects of prior alcohol exposure on reversal learning tasks, with some experiments finding alcohol-induced impairments [9,11–19,35,36] and other experiments finding no impairment (with some experiments even finding an improvement [19–25]). Differences in the method of alcohol exposure, the dose of alcohol given (and present in the blood), or in the specifics of the reversal task may be responsible for the differing results in the literature and in our current experiment.

It is possible that alcohol access in the current experiment failed to meet a threshold of BEC levels required for reversal learning impairments to be observed. When we previously measured BECs after alcohol access in food-restricted adult rats, we found that an average consumption of 9–11 g/kg/24 h led to average BECs of 85–90 mg/dL (measured 30 min into the access period) [32]. As average consumption in the last two weeks of alcohol access across the alcohol group in the current experiment was ~9.2 g/kg/24 h, we expect that the BECs of the rats in the current experiment was near this 85–90 mg/dL. Estimated BECs of ~80 mg/dL altered conditioned fear after a withdrawal period if given prior to fear conditioning acquisition [37,38], which suggests that our alcohol access procedure induced a pharmacological effect. However, our BECs were likely below the 150–500 mg/dL BECs estimated/shown to result from alcohol manipulations that led to reversal learning impairments in previous experiments [9,13–16,19,36]. For example, within the same reversal learning task and laboratory, intraperitoneal injections (peak BEC = ~500 mg/dL) impaired later reversal learning, whereas intragastric injections (peak BEC = ~200 mg/dL) did not impair later reversal learning [19], suggesting the dose- and BEC-dependence of alcohol’s long-term effects on reversal learning. However, see [24] for discussion of patterns found in [9,14,20,22,23] with comparisons in which a higher dose/BEC and/or longer exposure led to no impairment and a lower dose/BEC and/or shorter exposure led to an impairment. In previous research that examined the long-term effects of voluntary alcohol consumption on later reversal learning, experiments that found reversal learning impairments tended to have higher consumption because of the use of an alcohol solution as the sole source of water or because of the use of a solution of alcohol mixed with maltodextrin [10,17]. Conversely, studies have failed to demonstrate reversal learning impairments (at least those not involving factors more closely associated with attentional processes) after alcohol consumption in either unrestricted rats drinking unadulterated alcohol [21,25] or alcohol consumption in food-restricted rats (24 and the current study). Future research is needed to investigate whether the presence or type of manipulations to increase alcohol consumption is systematically associated with the presence or absence of long-term reversal learning impairments.

Different tasks used to assess reversal learning could also lead to some of the discrepancy in the pattern of effects of alcohol access/exposure on reversal learning, as many versions of the reversal learning task have been used to assess the long-term effects of alcohol exposure on reversal learning [9,13–16,19–25,35,36]. The different task versions used could explain some of the discrepancy in the literature, but this cannot be the only source of variability, as reversal learning using the Barnes maze and bowl foraging tasks is either impaired [9,19,35,36] or unimpaired [19,23] by different prior alcohol exposure regimens.

Another possible reason for the lack of an alcohol effect may be the interval between the end of alcohol access and the reversal testing. Badanich et al. [16] found deficits in reversal learning using a reversal learning procedure that was completed in a single day four to five days after the end of alcohol exposure, but no deficit if the mice were tested for reversal learning 11 days after the end of alcohol exposure. Here, the rats began reversal learning five to eight days after the end of alcohol access, depending on how quickly the rats completed reversal training. Thus, our test of reversal learning may have occurred in a
time interval after the effects of alcohol had faded. However, Kroener et al. [22] and our previous data [24] found no effect of alcohol access or forced exposure on reversal learning when reversal learning was tested four to five days after the end of alcohol exposure, so it seems unlikely that the interval between the end of alcohol exposure and the reversal test is the crucial factor in whether alcohol causes reversal learning deficits.

The current experiment represents the second demonstration that reversal learning in our go/no-go task is not impaired after six weeks of CIA to alcohol and the second demonstration that six weeks of CIA to alcohol in food-restricted adult rats (with consumption elevated from that seen in free-fed rats) does not impair learning in a different variant of the go/no-go reversal learning task. In our previous demonstration with this go/no-go reversal learning task, we found that six weeks of chronic intermittent access from PND26-66 in free-fed rats did not impair reversal learning [21]. However, previous pilot work using the same age range and methods of providing alcohol access led to average BECs of 6.2 ± 1.3, 11.4 ± 0.7, and 14.3 ± 1.0 across weeks 2, 4, and 6, respectively [29]. As a result, we chose to examine whether access during adulthood, with food restriction conditions we have used previously to boost alcohol consumption levels [28], might lead to impaired reversal learning in the current experiment. However, in line with other work in our lab, adult alcohol consumption under food restriction conditions did not lead to greater long-term behavior effects than voluntary access in free-fed adolescent/early adult rats. In fact, our previous research found that the voluntary access in free-fed adolescent/early adult rats had a greater effect on omission contingency learning [28]. Likewise, in an experiment from our laboratory using the same alcohol access procedure followed by testing in a different version of the go/no-go reversal learning task that allowed for multiple responses per trial, we previously found that there was no effect of prior alcohol access on reversal learning [24]. However, it is difficult to determine whether this pattern represents a common insensitivity of go/no-go reversal learning tasks (see also [34]) or is specific to our particular task variants and whether other go/no-go reversal learning tasks would be impaired by one or both of our alcohol access regimens. One alternative possibility is that our failures to find impairments after alcohol access result from the relatively low BECs that likely result from our alcohol access regimens (with BECs likely below 100 mg/dL in all cases). As such, our results do not invalidate earlier demonstrations of reversal learning impairments using different reversal learning task variants and/or passive exposure methods resulting in higher BECs. Additionally, the current experiment only examined the effects of alcohol consumption on later reversal learning in males, which represents a limitation of the current study. Female rats often consume greater amounts of alcohol during adulthood than male rats [25,39,40]. In addition, there is some evidence for females exhibiting greater behavioral alterations during reversal learning than males after prior alcohol exposure (although these alterations were in attentional aspects of behavior rather than in the motivational aspects of reversal learning behavior) [25]. Future research is needed to determine if the same behavioral pattern would be observed in female rats with prior alcohol access.

4. Conclusions

In conclusion, we found no effect of voluntary drinking in food-restricted rats on go/no-go discrimination or reversal learning. This replicates previous patterns from our laboratory that higher alcohol consumption in food-restricted rats did not impair discrimination or reversal learning in a different go/no-go task and that alcohol consumption in free-fed adolescent/early adult rats did not impair go/no-go discrimination or reversal learning in the same task. It is unclear whether this represents an insensitivity of this task to alcohol exposure generally or whether an alcohol exposure procedure that leads to higher BEC levels would impair learning. Additional research is required to investigate these possibilities.

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