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Research report

# Individual differences in conditioned fear are associated with levels of adolescent/early adult alcohol consumption and instrumental extinction

Alisa Pajser, Morgan Breen, Hayley Fisher, Charles L. Pickens*

*Department of Psychological Sciences, Kansas State University, Manhattan, KS, 66506, USA*

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ABSTRACT

Previous research has shown a relationship between alcohol exposure and conditioned fear, but the nature of this relationship remains unclear. We determined whether chronic intermittent access to alcohol during adolescence and early adulthood would alter or be associated with the level of conditioned fear to an auditory cue in male Long Evans rats. Rats received 6 weeks of chronic intermittent access to 20% alcohol or water from PND 26–66 and began behavioral testing 10 days later. We found no evidence that voluntary alcohol consumption altered fear. However, we found that rats that consumed more alcohol had lower fear, as measured with conditioned suppression of lever-pressing and conditioned freezing to an auditory cue. We have previously shown that higher levels of alcohol consumption are correlated with faster instrumental extinction learning. Therefore, we determined whether instrumental extinction would be directly associated with conditioned fear in rats never given alcohol access. As predicted, we found that rats that exhibited faster instrumental extinction also exhibited lower conditioned fear, as measured with conditioned suppression of lever-pressing and conditioned freezing. Our results suggest that at least part of the relationship between alcohol consumption levels and fear learning differences may be due to pre-existing individual differences. In addition, our finding that conditioned fear and instrumental extinction abilities (both separately associated with alcohol consumption levels) are associated with each other suggests that alcohol consumption levels may be a marker that can distinguish two separate phenotypes that encompass a wide variety of learning traits.

## 1. Introduction

There is a high degree of comorbidity between alcoholism and post-traumatic stress disorder (PTSD) [1,2], but the reason for this relationship is unclear. Individuals may drink to alleviate pre-existing fear/anxiety, alcohol consumption may alter the brain to increase the likelihood of PTSD development, or some pre-existing factor may affect both anxiety and alcohol consumption. Although it is likely that a combination of these factors may be involved in the alcohol-fear/anxiety relationship, most explanations of the relationship have focused on fear and anxiety symptoms leading to increased alcohol use for "self-medication" [3]. However, prospective analyses of symptom development have shown that pre-existing anxiety disorders (including PTSD) increase the probability of later development or worsening of alcohol abuse problems and pre-existing alcohol abuse problems increase the probability of later development of an anxiety disorder [4,5]. Unfortunately, experiments in humans (including prospective analyses) generally lack the experimental control needed to isolate the causal relationships between alcohol use and fear/anxiety. The control provided by animal models can provide sufficient experimental control to determine the direction of this relationship.

Investigations of relationships between alcohol exposure/consumption and conditioned fear in animal models could help to reveal the nature of alcohol-fear relationships in humans. Pavlovian fear conditioning, in which an initially neutral cue is paired with a foot-shock leading to acquisition of a fear response to the previously neutral cue, is widely used as a model of human fear and anxiety disorders [6,7]. Notably, individual differences in fear expression after conditioned fear training tend to be relatively stable, making this a good measure to study individual differences in fear reactivity [8]. Previous animal studies have focused more on determining effects of forced exposure to high doses of alcohol [9–15] than examining effects of lower levels of voluntary alcohol consumption on conditioned fear or possible correlations between motivation to consume alcohol and conditioned fear (but see [16]). As a result, the effect of lower levels of alcohol exposure on conditioned fear, and whether pre-existing differences in the motivation to consume alcohol would be associated with pre-existing differences in fear learning abilities, is unknown.

* Corresponding author at: Department of Psychological Sciences, 469 Bluemont Hall, Kansas State University, Manhattan, KS, 66506, USA.
*E-mail address:* pickens@ksu.edu (C.L. Pickens).

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We performed two experiments in the current report. In Experiment 1, we gave rats alcohol or water-alone access during adolescence/early adulthood (post-natal day [PND] 26–66). Our alcohol access method was the chronic intermittent access model (CIA) model, in which animals receive access to alcohol for 24-hour periods that alternate with alcohol-free periods [17]. We then determined whether alcohol consumption would affect conditioned fear (difference between alcohol and water groups) or the level of consumption would be associated with conditioned fear (no difference between alcohol and water groups, but high drinking alcohol rats differing from low drinking rats in their fear behavior). We found a relationship between the level of voluntary alcohol consumption and conditioned fear expression, with no difference between the alcohol and water groups, suggesting that pre-existing differences in the propensity to consume alcohol are associated with individual differences in fear learning abilities.

In Experiment 2, we followed up on this alcohol-fear association and its meaning for our previous finding (in [18]) that alcohol consumption was also associated with the speed of instrumental extinction learning. One possible follow-up experiment would be to determine whether the alcohol consumption-fear conditioning association would be seen if the fear conditioning was assessed first and alcohol consumption was assessed second. Two possible designs for this would be to assess fear conditioning in pre-adolescence so we could keep the age range of alcohol consumption the same, or assess alcohol consumption entirely in adulthood so we could keep the age range for fear conditioning the same. However, as discussed more fully in the Discussion section, there are several reasons to suspect that switching the order of the alcohol consumption and fear conditioning phases could lead to prior stress altering alcohol consumption patterns or that developmental effects could alter the patterns of fear conditioning or alcohol consumption in these designs. Previous research from our lab has also shown that voluntary alcohol consumption is correlated with the rate of instrumental extinction and reversal learning errors in a go/no-go task (with stronger correlations with commission errors [pressing the no-go lever] and maintenance/discontinuation errors [inability to maintain the reversal for multiple days in a row after meeting criterion once]) [18]. For this reason, we performed a different follow-up experiment to Experiment 1. In particular, we determined whether this alcohol consumption-conditioned fear association is part of a larger constellation of behavioral traits that are *all* associated with one another, with a *high alcohol (drinking)-low fear-fast instrumental extinction-low discontinuation errors in reversal* phenotype (which we abbreviate HALF-FIELDER) and a phenotype with none of these traits (which we abbreviate non-HALF-FIELDER). If so, this would broaden our findings beyond an alcohol-centric view of these traits, demonstrate that individual differences in these traits can be found even in rats without alcohol exposure, and show that our alcohol findings have broader significance for individual differences in the general phenotype of behavioral traits. In Experiment 2, we investigated whether instrumental extinction is associated with conditioned fear. We expected that low conditioned fear and fast instrumental extinction (both associated with high drinking in previous studies) would be associated with one another.

## 2. Methods

### 2.1. Subjects

Male naïve Long Evans rats (n = 36) from Charles River Laboratories (Kingston, NY and Raleigh, NC), PND 21 upon arrival in the facility, were used for Experiment 1. All animals were individually housed and maintained on a 12-hour reverse light-dark cycle with lights off at 07:30 am in a temperature and humidity controlled room. The rats were given 5 days to acclimate to the facility, and then received CIA starting on PND 26. Water and food were available ad libitum during this 6-week period. Three days after the final alcohol access period, body weights were recorded and rats were food-restricted and subsequently allowed to grow 1.5 g/day from this initial weight. Water was available ad libitum throughout the period of food restriction. In Experiment 2, adult male Long Evans rats (n = 12), 175–200 g on arrival in the facility from Charles River Laboratories (Kingston, NY), were used. After 23 days in the facility, they were food restricted and allowed to grow 1.5 g/day from their initial weights. Training took place during the dark cycle and rats were weighed and fed after the daily sessions. All procedures and animal care were in accordance with the Kansas State University Institutional Animal Care and Use Committee guidelines, the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and United States federal law.

### 2.2. Apparatus

Experiments were conducted in 12 operant chambers (Med Associates, St Albans, VT). Each chamber had two retractable levers 9 cm above the floor, but only one lever ("active", retractable lever) was extended into the chamber during behavioral sessions. The right lever was the active lever during the fear conditioning phases of the experiments, and the left lever was the active lever during the instrumental extinction phase of Experiment 2. Responding on the active lever activated the pellet dispenser, delivering 45-mg precision pellets (#1811155, 5% fat, 66% carbohydrate, 20.3% protein; TestDiet, Richmond, IN). A red house-light was located in the center at the top of the back wall of the chambers. A tone generator that delivered a 2900 Hz tone (20 dB above background) was located directly to the right of the house-light. The chambers had grid floors connected to electric shock generators that were capable of delivering a 0.5 mA scrambled foot-shock. A camera was mounted above each chamber. These cameras digitally recorded the behavior of the rats on a computer system that allowed later playback so that their behavior could be scored.

### 2.3. Alcohol access

Rats received 6 weeks of alcohol (using CIA) or water-only access beginning in adolescence and extending into early adulthood (PND 26–66). A two-bottle choice procedure was used in which all rats had access to water in at least one bottle at all times. Twenty-five of the rats in Experiment 1 (the Alcohol group) received 24-h access to 20% (v/v) ethanol mixed with tap water 3X per week in one bottle, while the other bottle contained tap water. Animals in the Alcohol group received alcohol access starting on Sunday, Tuesday and Thursday, with the other days being water-only days in which both bottles contained water. The Water group (n = 11) received two water bottles during the six weeks. Bottles were weighed and changed (for alcohol groups) starting at 1 p.m. and ending by 2 p.m. every day except Saturday, and placement was counterbalanced to control for any side preference.

### 2.4. Behavioral training in fear experiments

Behavioral training was largely the same as in previous studies [19,20]. Rats were given a 40–60-min food-cup training session, with pellet deliveries every 125 s. The following day, the rats received 2 sessions on a fixed-interval-1 (FI-1) reinforcement schedule for lever-presses on the right lever (lever-presses could earn a pellet each sec) 2–4 h apart. These sessions ended when rats received 50 pellets (with a limit of 1 h). The rats were then given one 90-min session in which pellets were earned on a variable-interval-30 (VI-30) reinforcement schedule (pellet availability for lever-presses ranging from 1 to 59 s), and 2 daily 90-min sessions on a VI-60 schedule (pellet availability ranging from 1 to 119 s). Rats were maintained on the VI-60 schedule for the remainder of the behavioral training.

During the fear conditioning session, animals earned pellets on a VI-60 schedule. Sessions began with the extension of the active lever and illumination of a red house-light. On this day, they received 10 30-