Neuropathology of comorbid anxiety disorders and alcohol dependence
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INTRODUCTION
Anxiety disorders commonly occur along with other mental or physical illnesses, including alcohol use disorders (AUDs). Previous research has shown that a dysregulation of certain neuronal plasticity-related events in the prefrontal cortex (PFC) is implicated in anxiety disorders and alcohol dependence. However, the exact role this dysregulation plays in the comorbidity of these disorders is not well understood. The experiments conducted were part of a larger study aimed at understanding the neuropathological characteristics present when anxiety disorders and AUDs coexist. We examined anxiety-like behaviors of rats bred to consume large or small quantities ethanol before and after exposure to a stressful stimulus. All animals were then euthanized so that the brains could be examined for activation of plasticity-related genes in the PFC. Significant differences in alcohol consumption and anxiety-like behaviors were observed between alcohol-prefering and alcohol-non-prefering rats. In addition, we present preliminary results of brain activity. These findings are discussed in relation to drinking alcohol as a means to reduce anxiety.

METHODS

Subjects: Adult male alcohol-prefering (P) and non-alcohol-prefering (NP) rats were housed in a temperature-controlled facility under a 12 h light/dark cycle, with free access to food and water. All procedures were pre-approved by the Georgia Regents University Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Alcohol Administration: Some rats received alcohol via a 2-bottle choice intermittent-access drinking paradigm. For three days a week, one bottle of 20% ethanol solution was presented alongside an adjacent bottle containing water only, location randomized. This paradigm was administered for two weeks before administering footshock. Figure 1 lists the experimental conditions and shows a timeline of the study.

Footshock Procedure: Footshock consisted of 3 phases: acquisition, extinction, and post extinction. Acquisition = 4 CS-US pairings; CS = pure tone (30 sec, 80 dB, 5 kHz); US = footshock (0.8 mA, 1 sec) coterminating with CS. Extinction = 22 massed presentations of CS. Post extinction was identical to the extinction schedule, and delivered 3 days later.

Elevated Plus Maze (EPM): Was used to measured anxiety-like behavior after the first extinction session (day 19). See Figure 4 for results.

Statistical Analyses: Independent samples t-Tests were used to determine if there was a difference in freezing time between P and NP rats post-shock in both extinction sessions. Paired samples t-Tests were performed to investigate within group change between the two trials. A two-way ANOVA was conducted to determine statistical significance between P and NP rat performance on the EPM.

RESULTS

Figure 1. A. List of experimental conditions tested in this study. Both P and NP rats were tested in each condition. B. Timeline of experimental procedures.

Figure 2. Water and Alcohol consumption rates for P rats and NP rats.

Figure 3. NP rats spent more time freezing to the tone that was previously paired with shock (M = 46.7, SD = 34.6) compared to P rats (M = 81.4, SD = 22.5) in the first extinction trial. NP rats in the shock no alcohol condition continued to exhibit more freezing in the second trial (M = 75.0, SD = 21.2) than did P rats of the same condition (M = 24.9, SD = 34.3). However, NP and P rats in the shock + EtOH condition spent similar amounts of time freezing (M = 47.0, SD = 40.0 and M = 46.6, SD = 36.72 respectively) during the post-extinction trial.

Figure 4. NP rats spent significantly more time in the open arms than P rats (M = 108.27, SD = 21.67) and (M = 68.90, SD = 19.17) respectively. A two way ANOVA showed a significant main effect for breed of rat, F (1, 46) = 5.264, p = 0.03.

Figure 5. A. P rat- Control, B. P rat- Shock + EtOH, C. Percentage of neurons with H1a RNA Foc, which were induced by the post extinction session.

Figure 6. Homer1a (H1a) is an immediate early gene tied to plasticity-related events such as memory for a specific ‘trauma’ event. Below are images showing H1a RNA Foci, which were induced by the post extinction session.

CONCLUSIONS

- Consumption: We did not observe a significant alteration in alcohol or water consumption for NP or P rats after shock. This lack of significance was not consistent with our hypothesis. Possible explanations are:
  - Possible delay in post shock consumption
  - Shock strength not strong enough to elicit stress-induced alcohol consumption in home cage.
- Extinction & Post Extinction: Alcohol consumption in NP and P rats did not impact consolidation of fear memory, nor fear expression during the extinction test. However, alcohol impaired acquisition of fear extinction only in P rats. Because P rats had lower freezing overall, they were either impaired in encoding of the fear event, or switched to escape behaviors. Ongoing analyses will dissociate between the two.
- EPM: After the first extinction session, P rats spent less time in the open arms in the EPM than control NP rats. Interestingly, shock P rats behaved similarly to shock + EtOH or control P rats. This is consistent with the hypothesis that P rat behaved in a more ‘impausile’ manner and possibly showed maladaptive responses to shock. This change in behavior was reversed by alcohol.

Analysis is ongoing, however, current data suggests that alcohol may lead to an impairment of fear extinction in P rats, but not NP rats. Consistent with that, alcohol also impairs IEG expression in the prefrontal cortex only in P rats. Further investigations, including outbred rats, will shed more light on how alcohol is affecting memory formation in P and NP rats.

REFERENCES


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