Benztropine, an FKBP5 Inhibitor, Reduces Ethanol Drinking and Stress-Associated Phenotypes in a Rat Model of Comorbid Post-Traumatic Stress and Alcohol Use Disorder

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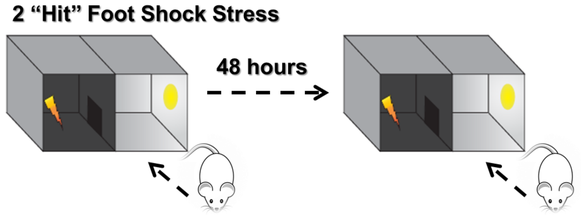
INTRODUCTION

Alcohol use disorder (AUD) and post-traumatic stress disorder (PTSD) are highly comorbid, and their co-occurrence worsens negative health outcomes. To date, effective therapeutic interventions to treat PTSD/AUD comorbid disorders continue to lack. Benztropine mesylate (identified for its anti-muscarinic, anti-histaminergic and dopamine-augmenting actions) has been shown as a promising therapeutic agent in ameliorating stress disorders. Evidence suggest that FK506-binding protein 51 (FKBP5) is implicated in PTSD/AUD patients, and benztropine recently was found to inhibit FKBP5, a novel target in potentially ameliorating PTSD/AUD comorbid disorders. Here, we examined the ability of benztropine to reduce post-traumatic ethanol drinking, sleep disturbances, hyperarousal, fear generalization, and irritability-like behavior. We used a translationally-relevant model of comorbid PTSD/AUD as described in Steinman et al (2020) to examine the effects of benztropine in male and female rats.

MATERIALS AND METHODS

Animals and Shock Stress Procedures: Male and female Wistar rats (*n*=8-10 per group) were used for this study. All rats were housed in temperature- and humidity- controlled vivarium on a 12 hr light/dark cycle with food and water *ad libitum*. We utilized a "2 hit" shock avoidance model whereby male and female rats received a single foot shock on two occurrences separated by 48-hrs on the dark compartment side of a shuttle box.



2 Bottle Choice (2BC) Ethanol Drinking: 2 weeks after the first foot shock, rats received 48 hr acclimation to ethanol (20% v/v) followed by chronic, intermittent (Mondays, Wednesdays, Fridays), limited 2BC access (2 hr) to ethanol.

Sleep Cycle Analysis: We used OXYmax-CLAMS units to assess rat sleep parameters from photocell-defined motor activity.

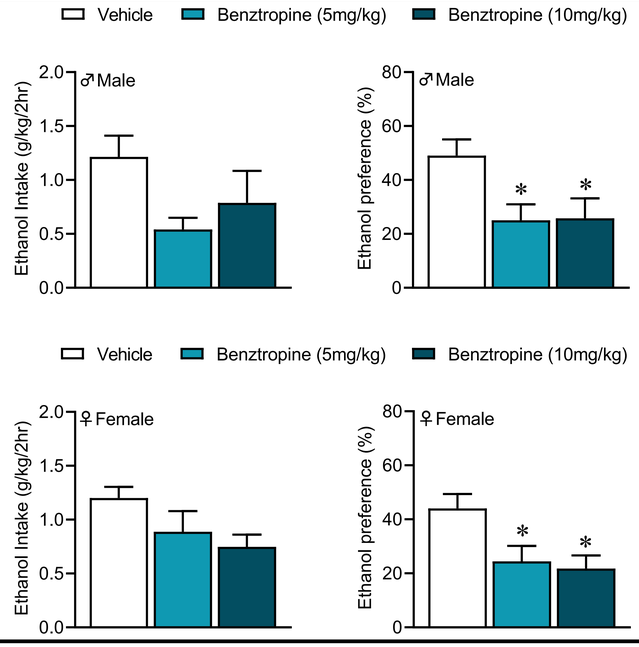
Hyperarousal: Acoustic startle response with several audible intensities (dB) was recorded in a ventilated, sound-proofed isolation chamber containing a Plexiglas cylinder that sat securely on a Plexiglas frame.

Fear Overgeneralization: Rats were tested on a modified chamber box that had numerous features to distinguish it from the original box in which foot shock occurred. It consisted of green plastic and contained holes in the light compartment with an LED light. The dark compartment was covered with black contact paper. The two compartments were separated by a cardboard divider.

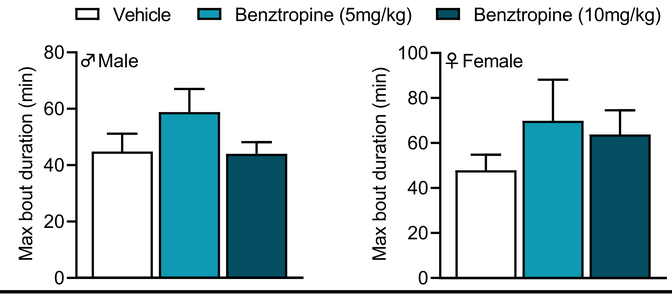
Irritability-like Behavior: A bottle brush was rotated in five phases) forced interaction by touching the whiskers, 3) withdrawing from the rat, 4) rotating upright, and 5) the brush was held upright without rotation. Behaviors assessed included aggressive behaviors, which included biting, boxing, following and mounting, as well as defensive behaviors, including startling, digging, freezing, climbing cage walls, auto-grooming, vocalizing and attempting to escape.

Benztropine Regimen: Benztropine was administered (i.p.) 2 hr prior to each test using an intermediate (5 mg/kg) and high dose (10 mg/kg) of this drug.

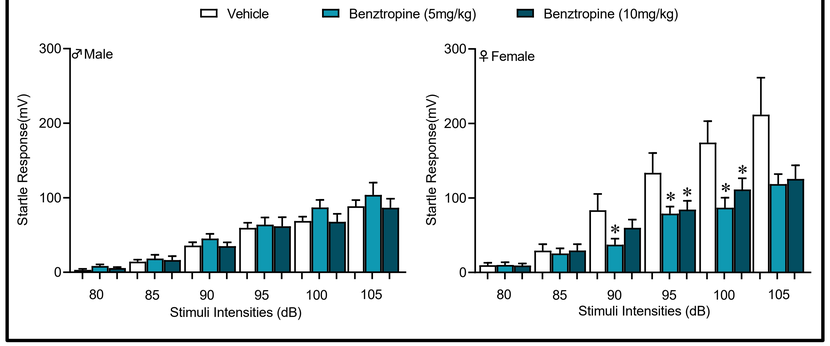
BENZTROPINE REDUCES 2BC ETHANOL PREFERENCE IN PTSD/AUD COMORBID-LIKE RATS



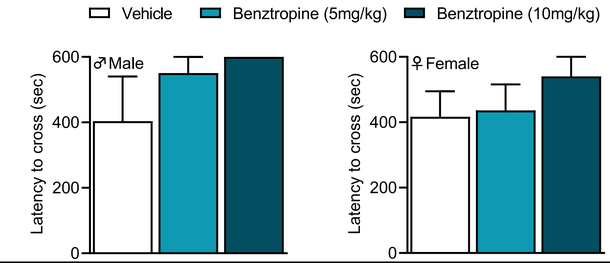
BENZTROPINE DOES NOT RESTORE SLEEP DISTRUBANCES IN PTSD/AUD COMORBID-LIKE RATS



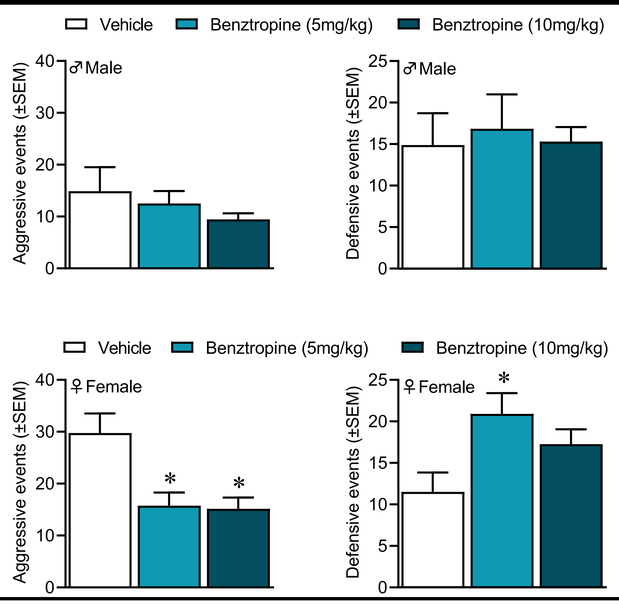
BENZTROPINE REDUCES HYPERAROUSAL IN FEMALE PTSD/AUD COMORBID-LIKE RATS



BENZTROPINE DOES NOT RESTORE FEAR MEMORY AVOIDANCE IN PTSD/AUD COMORBID-LIKE RATS



BENZTROPINE REDUCES IRRITABILITY-LIKE BEHAVIOR IN FEMALE PTSD/AUD COMORBID-LIKE RATS



CONCLUSIONS

Male and female PTSD/ AUD comorbid-like rats display elevated levels ethanol drinking, disrupted sleep patterns, elevated hyperarousal, disrupted fear memory avoidance and irritability-like behavior as previously observed in Steinman et al (2020). Benztropine, an FKBP5 inhibitor, reduced ethanol preference in both male and female PTSD/AUD comorbid-like rats, suggesting that FKBP5 may serve to decrease enhanced ethanol consumption induced by stress disorders. Benztropine contains sex-dependent restorative effects, with this drug uniquely reducing hyperarousal and irritability-like behavior only in female PTSD/AUD comorbid-like rats. Future work will investigate the underlying neurobiological mechanisms related to FKBP5 that influence stress and alcohol use vulnerability in a sex-dependent manner.

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