Brain response to alcohol after a predator odor stressor

E. Pitrolo[1], R. Tyler[1], J. Besheer[1,2]

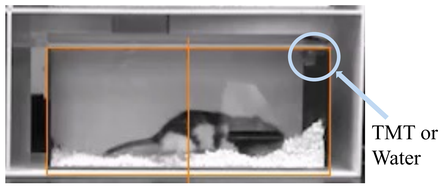
[1]Bowles Center for Alcohol Studies, [2]Department of Psychiatry, University of North Carolina at Chapel Hill

Abstract

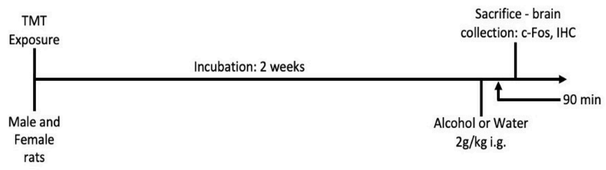
Post-traumatic stress disorder (PTSD) is highly comorbid with alcohol use disorder (AUD). Furthermore, data indicate the rate of comorbidity may be related to sex, as females have a higher rate of comorbid PTSD/AUD than males. Thus, the current study focuses on the neuronal response to alcohol following a stressor in males and females in two brain regions in relation to AUD/PTSD – these are the anterior insular cortex (aIC) and prelimbic cortex (PrL). The current study exposes male and female rats to 2,5-dihydro-2,4,5-trimethylthiazoline (TMT). TMT is a synthetically-made component of fox feces that is a stressor that produces stress-reactive behaviors in rodents and has been used to model some PTSD symptoms. Rats in the current study were exposed to TMT or water for controls and then injected with alcohol or water two weeks later. Rats were sacrificed 90 minutes following alcohol injection. Brain slices were then collected for c-Fos immunohistochemistry. TMT exposure results indicated that TMT produced stress-reactive behaviors in both male and female rats. Results of c-Fos analyses indicate that neuronal activation in the aIC is not altered following TMT exposure and alcohol injection. In the PrL, alcohol was found to significantly increase neuronal activation in males within the control group. Neuronal activation also significantly increased following TMT exposure and alcohol injection in comparison to alcohol injection in control males. TMT significantly increased neuronal activation in males. In females, alcohol significantly decreased neuronal activation in controls and there was no change in neuronal activation in TMT-exposed rats. TMT also significantly decreased neuronal activation in females. The difference in patterns between males and females seen in the current study indicates possible sex differences.

Methods

1. Male (N=32) and female (N=32) Long-Evans rats were utilized. The rats were placed in a chamber, where half of the rats underwent exposure to 10 µL TMT and the other half were exposed to water (Control). The TMT or water was placed on a piece of filter paper in a small metal basket on the right side of the chamber. Exposure lasted 15 minutes and behaviors were analyzed using ANYmaze software. A 2-way RM-ANOVA was run with TMT exposure and time as factors. Post-hoc analysis used Šidák's multiple comparison test.



2. Two weeks following TMT exposure, rats were injected (IG) with 2 g/kg alcohol or water, and were sacrificed 90 minutes later. Brain slices containing the aIC and PrL were then taken using a microtome and collected in cryoprotectant. Slices were then stained for c-Fos and mounted. 2-way ANOVAs were used for analysis with TMT exposure and alcohol administration as factors. If indicated, Šidák's multiple comparison test was used for post-hoc analysis.



Experiment 1

TMT produced stress-reactive behaviors in male and female rats

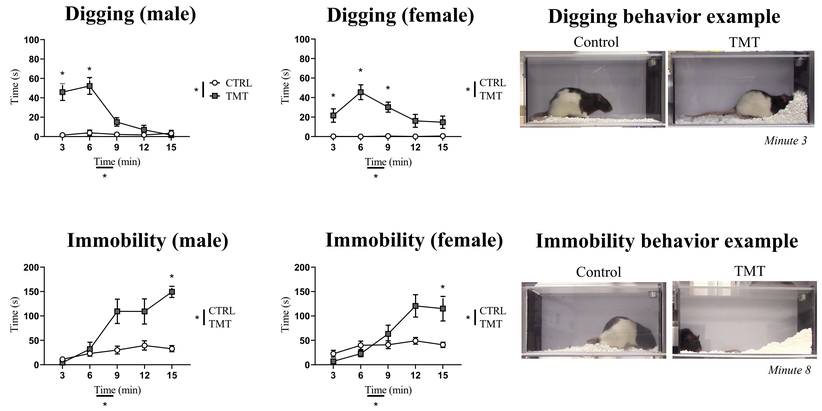


Figure 1: TMT exposure produced stress-reactive behaviors. There was a significant increase in stress-reactive behaviors in the TMT group, as seen in the digging and immobility data. This supports hypothesis 1. * indicates $p < 0.05$.

✓ Hypothesis 1: TMT will produce behavioral adaptations in both male and female rats in comparison to controls

- Increases in stress-reactive behaviors such as digging and immobility in the TMT group indicate that TMT produced behavioral responses indicative of stress and fear in both males and females
- Indicates that TMT can be used in this study as a stressor

Experiment 2

Anterior insular cortex (aIC) c-Fos immunohistochemistry

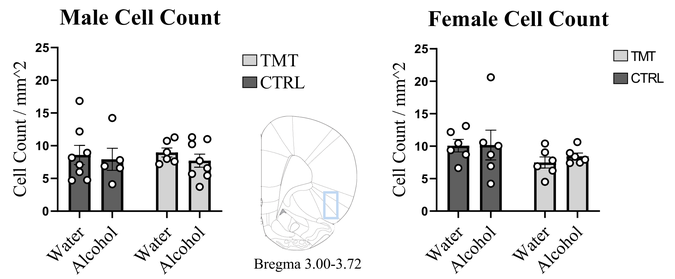


Figure 2: aIC c-Fos IHC in male and female rats. This figure shows the results of male and female c-Fos IHC in the aIC. No significant differences were found.

X Hypothesis 2 (aIC): TMT will increase c-Fos expression in comparison to controls
X Hypothesis 3 (aIC): Expression of c-Fos following alcohol injection will be higher in TMT than in the control group

- The current finding differs from acute TMT exposure. The current study measured neuronal activation 2 weeks following TMT stressor to assess lasting adaptations, while previous data assessed immediate changes 100 min after TMT.
- Possible that the acute aIC changes return to baseline levels 2 weeks following a stressor

Experiment 3

Prelimbic cortex (PrL) c-Fos immunohistochemistry

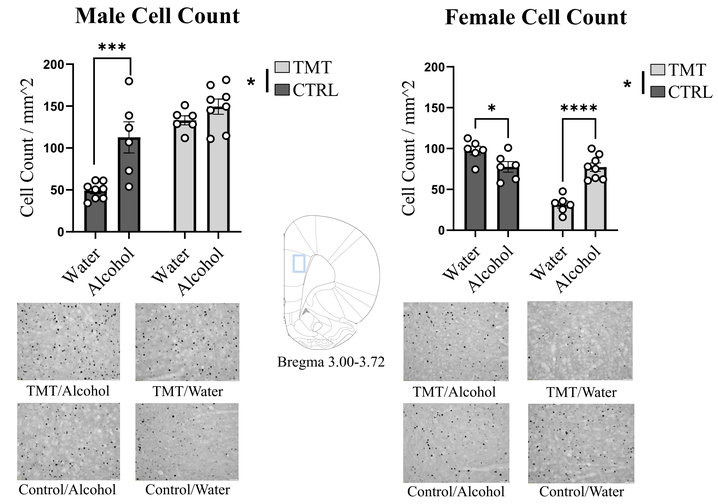


Figure 3: PrL c-Fos IHC in male and female rats. This figure shows the results of male and female c-Fos IHC in the PrL. Males and females showed significant differences between and within the TMT and control groups. * indicates $p < 0.05$

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✓	X
X	X

Hypothesis 2 (PrL): TMT will increase c-Fos expression in comparison to controls
Hypothesis 3 (PrL): Expression of c-Fos following alcohol injection will be higher in TMT than in the control group

Males:
- Both TMT and alcohol increased neuronal activation in the PrL.
- Additive effect of TMT and alcohol

Females:
- Alcohol decreases neuronal activation in the control group but increases activation in the TMT group.
- TMT significantly decreased neuronal activation.

Conclusions

- TMT produced stress-reactive behaviors in male and female rats, indicated by increases in digging and immobility in the TMT group.
- The aIC c-Fos IHC did not indicate differences in neuronal activity, different from existing data on acute effects of TMT exposure.
- Effects of TMT and alcohol are different in male and female PrL data, indicating potential sex differences.
- Further research is needed on sex differences in PTSD/AUD comorbidity based on PrL c-Fos IHC results
- The effects seen in PrL data likely reflect changes in glutamatergic, projection neurons, as the majority of cells in the PrL are excitatory. Further research is needed to determine the cell-type specificity of the c-Fos changes.

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