Assessing sex differences in threat responding to posttraumatic stress disorder (PTSD)–relevant challenges in mice

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Introduction

- Posttraumatic stress disorder (PTSD) is a fear-associated disorder that affects approximately 6 million individuals annually with higher prevalence in war veterans and females. PTSD is characterized by maladaptive threat responding and persisting trauma-associated fear memories.
- There is an interest in identifying intrinsic factors that may promote PTSD phenotype.
- Previous studies: PTSD patients have increased sensitivity to CO₂ inhalation (Muhtz et al., 2011).
- Loss of pre-habitual fear extinction deficits in male mice (McMurray et al., 2020).
- High sensitivity to CO₂ correlated with passive coping to stress and fear.
- FosB activation in the amygdala is correlated with passive coping to stress and fear.

Results

(a) Passive Coping

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fear Acquisition</th>
<th>Fear Extinction</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ Low</td>
<td>Air: 0% (n=5)</td>
<td>Fire: 0% (n=5)</td>
</tr>
<tr>
<td>CO₂ High</td>
<td>Air: 0% (n=5)</td>
<td>Fire: 0% (n=5)</td>
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(b) Active Coping

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Methods

Subjects: Male and female C57Bl/6 mice (Jackson Laboratories). Mice were 8 weeks old at arrival, pair housed and acclimated for 2 weeks before behavioral studies began.

CO₂ Inhalation: On Day 1, mice habituated to a 30 s CO₂ exposure (10%). Day 2, mice were exposed to 10% CO₂ or air for 10 min. On Day 3, mice were returned to the context for 5 min. Mice received two fear conditioning sessions.

Rearing: mice were placed in a stress environment and rearing was monitored.

FosB: mice were treated with FosB and stained for FosB.

Data Analysis: Group data was analyzed using ANOVA and multiple comparison tests.

Immunostaining

(a) FosB immunostaining (females) in the BLA (n=9).

Conclusions/Future Directions

- We used a CO₂ inhalation-PTSD paradigm to show how an interoceptive threat, namely CO₂ modulates PTSD risk in males and females separately.
- Our data revealed heterogeneous freezing and rearing behaviors to CO₂ in male and female mice similar to CO₂ variance observed in humans.
- Females demonstrated significantly more active coping behavior compared to males during CO₂ exposure and fear extinction.
- Females also had alterations in FosB cell counts in BLA/CA when exposed to CO₂, which was not observed in male mice (consistent with Kessler et al., 2017 study).
- FosB is merely an indication of neuron recruitment and thus does not indicate if the neurons were activated or inhibited. Further research can delve into this phenomenon.
- Future direction could include investigating the different circuits that male mice may recruit that females may or may not also recruit, including the prefrontal cortex, hippocampus and locus coeruleus of the brainstem.
- Future direction could also include evaluating male and female differences with the female estrogen cycle as an independent variable.

Supported by VA Merit Award (Sab; 2018EX00175-04), NIH Grant F32MH117913 (KMU)