Exploring the impact of sex, age, and early life adversity on responses to ultrasonic vocalization playback

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Understanding the effects of early life adversity (ELA) through maternal separation (MS) in rats provides a window to study behavior and mood disorders seen in humans with a history of ELA. We are working to develop and characterize a translational rat model comparable to the human fearful face task. In rats, ultrasonic vocalizations (USVs) are thought to convey similar affective social information as human facial expressions [1,2]. This summer, our study considered how ELA exposure affects responses to aversive (22 kHz) USVs in male and female rats. By exposing ELA and control rats to 22 kHz USVs in a test for hypervigilance, we began to determine how ELA impacts behavioral responses to negative stimuli. Rats were evaluated at two stages: postnatal day (P) 35, and P45 to characterize changes across development. This behavioral model provided us with the opportunity to explore largely understudied factors in behavioral neuroscience–the ways in which encountering ELA affects behavior and emergence of mood disorders across both sex and development.

To measure behavior and impact of USV playback, two behavioral tests were used: the elevated zero maze (EZM) and the open field test (OFT). Both tests aim to use the rat's natural instinct to hide from predictors as a measure of anxiety, with the idea that more time spent in the sheltered, closed arm of the EZM or on the perimeter of the OFT (thigmotaxic zone) the more anxious the rat is [3]. We used the EZM as a measure of anxiety without USV playback to compare baseline anxiety levels of ELA and control rats. Figures A and B show time spent in the open arm of the EZM during a 5-minute trial. Our preliminary results, (n = 2 per group) indicate general trends of greater time spent in open arm for less anxious control rats and more time spent in the closed arm for more anxious ELA rats with an exception for female rats at P35. Initial data for the OFT where 22 kHz playback introduced, show equally interesting results. Figure C shows that for P35 females, USV playback increased time spent in the thigmotaxic zone for control rats less than silence but did not change time spent in the thigmotaxic zone for ELA rats. For the older females (Figure D), control rats were more exploratory in general during silence, but USV playback brought on significantly more time spent in the thigmotaxic zone and thus more anxiety while ELA rats appear unaffected. Overall, these trends show that ELA rats have similar anxiety-like behaviors regardless of playback possibly due to a constant state of anxiety brought on by ELA [4]. In contrast, control rats have unique behavioral shifts due to exposure to 22 kHz playback leading us to infer that ELA may lead to different coping strategies upon exposure to aversive stimuli.

In sum this data shows that both the ELA paradigm and 22 kHz playback have effects on anxiety that vary across development and sex. Continued work on this project will lead to more definitive behavioral results and analysis of physiological changes to the brain from ELA using immunohistochemistry will be integral in understanding the underlying neural mechanisms of ELA and the mood disorders it may cause.

Figures:

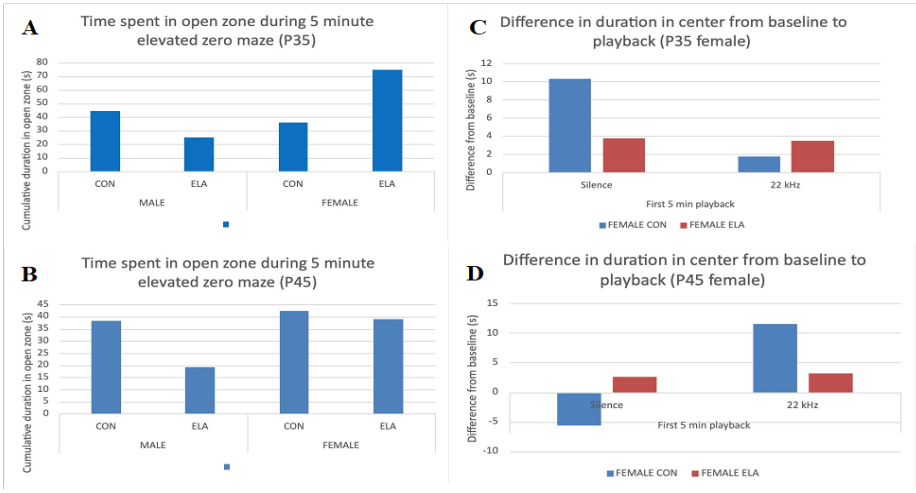

Figure A: Control males and ELA females spent more time in the open zone than their same-sex counterparts. More time in open zone indicates lower levels of anxiety. **Figure B:** Controls of both sexes spent more time in open zones than their same-sex counterparts meaning they are less anxious as expected. **Figure C:** Difference in duration in center between baseline and during first 5 minutes of playback for P35 females. 22 kHz playback led to a shift in behavior for CON rats but had no effect in ELA rats suggesting playback replicates anxiety-like behavior of ELA but does not add to it. **Figure D:** Playback of 22 kHz did not change anxiety levels of ELA in P45 females as indicated by time spent outside of the thigmotaxic zone, but again led to a clear behavioral change in control rats.

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Citations:

1. Granata, L., Valentine, A., Hirsch, J. L., Honeycutt, J., & Brenhouse, H. (2021). Trajectories of Mother-Infant Communication: An Experiential Measure of the Impacts of Early Life Adversity. *Frontiers in Human Neuroscience, 15*(72). doi:10.3389/fnhum.2021.632702
2. Demaestri, C., Brenhouse, H. C., & Honeycutt, J. A. (2019). 22 kHz and 55 kHz ultrasonic vocalizations differentially influence neural and behavioral outcomes: Implications for modeling anxiety via auditory stimuli in the rat. *Behavioural Brain Research, 360*, 134-145. doi:https://doi.org/10.1016/j.bbr.2018.12.5
3. Martinez, R., & Morato, S. (2004). Thigmotaxis and exploration in adult and pup rats. *Revista de Etologia*, *6*(1), 49-54.
4. Grassi-Oliveira, R., Honeycutt, J. A., Holland, F. H., Ganguly, P., & Brenhouse, H. C. (2016). Cognitive impairment effects of early life stress in adolescents can be predicted with early biomarkers: Impacts of sex, experience, and cytokines. *Psychoneuroendocrinology, 71*, 19-30. doi:https://doi.org/10.1016/j.psyneuen.2016.04.016