The role of pubertal hormones in female rats' development to anxiety-like behavior in an early life adversity model

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Abstract

Early life adversity (ELA) can be characterized by a series of stressful or traumatic experiences that occur in an individuals early development that is associated with an increased risk in emergence of affective/psychiatric disorders such as depression and anxiety. The fact that women are twice as likely to develop these affective disorders highlights a necessity to better characterize sex-specific differences in neural development. Brain regions such as the prefrontal cortex (PFC) have been shown to play an important role in the affective processing in both rodents and humans, which makes it a region of interest. Following ELA, parvalbumin (PV)-containing inhibitory interneuron density is altered in the PFC in a sex-dependent manner, which makes this cell type a cell type of interest. One drug that has recently demonstrated promising potential for treatment of affective disorders is ketamine, a noncompetitive glutamate NMDA receptor antagonist that may regulate excitatory neuronal output by blocking the inhibitory function of PV neurons. Research in our lab suggests that post-pubertal/adult female rats show a hypervigilant behavioral phenotype while prepubertal/juvenile female rats show more typical anxiety-like behavior as those shown across ages in male rats. Pubertal hormones might be mediating this unique behavioral phenotype in adult females as such that older female rats look different than their male rats or prepubertal female rats. We hoped to further investigate this hypothesis by replicating preliminary data.

Project Objectives

This project aims to further characterize the potential role of female pubertal hormones in female rats' sensitivity to acute ketamine treatment and subsequent anxiety-like behavior following early life adversity. While the preliminary data showed interesting results, replicability of data drives this continued research design. This research works to identify the neural mechanisms through which female pubertal hormones may alter behavior and PV neuron density in the PFC. We hope to utilize ovariectomies, in order to isolate the potential effects of pubertal hormones on behavior and neural circuitry in female rats from each experimental group (ELA or control). We aim to study the long term effects of acute ketamine treatment, which has previously been shown to alleviate depressive-like symptoms in rodents (Garcia et al. 2009). Our goal is to measure and characterize behavior through the use of an open field test (OFT) with aversive 22kHz ultrasonic vocalization (USV) playback to test for behavioral hypervigilance and anxiety-like behavioral phenotypes (Shieh & Carter, 2015).

Methodology Used

Early Life Adversity (ELA) via Maternal Separation (MS)

The MS paradigm is a well-recognized model of ELA that is used to simulate a similar environment in rodents to one resulting from psychosocial caregiver deprivation in humans. Pups are randomly assigned to the experimental (ELA) or control group (CON). From postnatal day 2 to 20 (P2-P20), all ELA pups are separated from their dam (rat mother) and isolated in small cups or small cages containing bedding for 3.5-4 hours per day. CON pups were undisturbed unless they were being handled to acclimate them to experimenters.

Ovariectomy (OVX) Surgical Procedure

On P24-25, all animals (CON and ELA) either underwent prepuberal bilateral dorsal ovariectomy (OVX) or a Sham surgery as outlined in previous research (eg., Steele & Bennett, 2011; Sinclair et al., 2019;

Klump et al., 2021). Rats were injected with a dose of 2.5mg/kg body weight of Meloxicam subcutaneously as preoperative analgesic. Rats then underwent initial anesthesia before surgery and consistent oxygen and isoflurane throughout surgery. For OVX animals, both ovaries were removed. For Sham animals, a similar surgical procedure was performed until ovaries were visualized, from which point the ovaries were tucked back in. Internal and external suturing was performed. Antibiotic ointment was applied to external sutures. Following surgery, all rats were housed individually for recovery for one week in order to prevent rough play or over-grooming.

Ketamine Treatment
At the time of early adulthood (P54-55), all rats either received intraperitoneal (IP) injection of either a 15 mg/kg dosage of ketamine hydrochloride (KET; Wang et al., 2011) or an equivalent volume of 0.9% physiological saline (SAL).

Open Field Test (OFT) with 22kHz Ultrasonic Vocalization (USV) Playback
On P59-60, all animals underwent modified OFT testing. Rats were placed individually in a square field with walls on each side and their behavior was recorded over the course of 10 minutes. An audio containing 5 minutes of silence and 5 minutes of aversive playback (22kHz) was played through an ultrasonic speaker. Behavior was analyzed using Ethovision XT. The duration of time spent and frequency in the center zone, total distance traveled, acceleration, and time spent immobile were compared
between silence and 22 kHz playback conditions.

Results Obtained
We hope to compare the averaged data across conditions using a three-way ANOVA of Surgery (OVX/Sham) x Treatment (SAL/KET) x Rearing (CON/ELA) with follow-up post-hoc Šídák's multiple comparisons test. However, a small sample size prevented data collection for ELAxSHAMxSAL and ELAxShamxKet conditions. Results will be obtained once data is collected from these two group conditions.

Significance and Interpretation of Results
Due to small sample size, significance results were not obtained. We expect that with a larger sample size for each condition, significant effects will start to emerge. If data is replicated, we expect that ELA x Sham x Saline female rats will enter the center more frequently, demonstrating a pubertal hormone driven hypervigilant behavior. We expect ELA x OVX x Saline and ELA x OVX x Ket conditions to result in a reduced frequency of center entries. In the future we also hope to visualize the density and intensity of PV-containing neurons and quantify estradiol receptor-α (ERα) presence using immunohistochemistry staining.

Figures/Charts

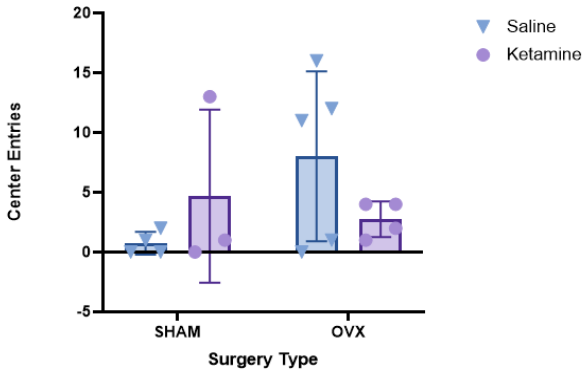

Fig 1. Effect of surgery and treatment on OFT center entries in control animals. Number of times entering into the center zone of the open field during last five minutes of playback (5:00-10:00 of aversive 22kHz USV playback). No statistical Significance was found.

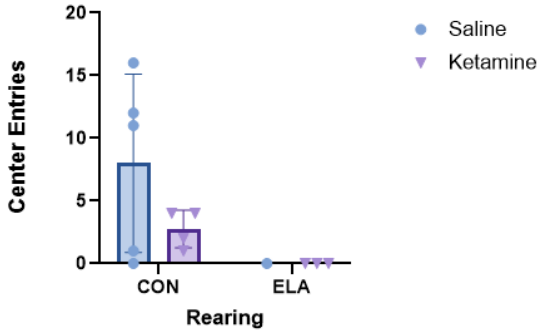

Fig 2. Effect of rearing and treatment on OFT center entries in OVX animals. Number of times entering into the center zone of the open field during last five minutes of playback (5:00-10:00 of aversive 22kHz USV playback). No statistical significance was found. Trend may suggest main effect of rearing.

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