Exploring the sex-specific effects of ketamine on perineuronal net coverage around parvalbumin interneurons following early-life adversity

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Abstract

While many patients benefit from typical antidepressants, such as serotonin-reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors, to treat anxiety, a substantial number of people remain with symptoms from treatment resistance or relapse using these first-line medications. Studies have recently shown that ketamine, a non-competitive NMDA receptor antagonist, shows promise in patients with treatment-resistant depression following acute administration for up to two weeks. Due to similar etiology between anxiety and depression, ketamine may also be a novel therapeutic for treatment-refractory anxiety disorders. Early life adversity (ELA) contributes to the development of affective disorders, including anxiety, in both humans and rodents. Given that women are disproportionately impacted by anxiety in humans, this study explores sex differences in the effects of early life adversity and subsequent ketamine treatment. ELA has previously been shown to alter perineuronal net (PNN) density around parvalbumin containing interneurons (PV cells), which in turn, makes those PV cells more susceptible to damage via oxidative stress. Ketamine alternatively restores PNN density in the prefrontal cortex following chronic stress, indicating that in a maternal separations model of anxiety, ketamine may also present similar rescuing effects. Using qPCR, ketamine is hypothesized to increase PNN protein expression in the prefrontal cortex and the ventral hippocampus. Using immunohistochemistry staining, it is expected that ketamine will increase PNN coverage of PV cells. These findings will be analyzed for correlations with changes in anxiety-like behavior presented in the open field with aversive (22 kHz) playback, which measures rat responses to a potential threat cue.

Project Objectives

Ketamine is a non-competitive N-methyl-d-aspartate (NMDA) receptor antagonist with rapid and long-lasting antidepressant effects (Zhang et al., 2021). Its recent efficacy demonstrated in preclinical and clinical trials led to the recent approval of ketamine for treatment-resistant depression by the Food and Drug Administration in the form of a nasal spray (Wajs et al., 2020; Vekhova et al., 2025). While ketamine's therapeutic effects have mainly been studied in the context of major depression, similar underlying etiology between depression and other affective disorders, such as generalized anxiety disorder (GAD), allude to the potential anxiolytic properties of the drug.

Recent studies have highlighted the potential role that ketamine may play in modulating the expression of perineuronal nets (PNNs) around parvalbumin interneurons (PV) (Yu et al., 2022). PV cells regulate inhibition and are associated with emotional regulation in a variety of neuropsychiatric disorders, including GAD. PNNs are extracellular matrices that stabilize synapses and protect fast-spiking interneurons like PV from oxidative stress (Donegan & Lodge, 2017). Thus, the regulation of PNNs is critical for the function of PV cells and the inhibitory circuits they control. Previous studies have linked stressors such as early life adversity (ELA) with maladaptive PNNs around PV interneurons in both human post mortem tissue and animal models (Rahimian et al., 2024). Both male and female rats exhibited a reduced density of PNNs in the infralimbic cortex in adulthood after experiencing maternal separation, a model of ELA (Gildawie et al., 2020). Removing or altering PNNs resulted in increased vulnerability to stress and depressive-like symptoms in a rat model, while ketamine increased the expression of PNNs,

restored GABAergic function, and promoted resilience to stress (Yu et al., 2022). These studies demonstrate one potential mechanism by which ketamine may produce anxiolytic effects.

This study aimed to investigate changes in PNN and PV density following maternal separations and ketamine treatment in early adulthood in a sex-specific manner. Using RNA qPCR analysis, it is hypothesized that early life adversity will reduce expression of PNNs in the infralimbic and prelimbic prefrontal cortex (IL/PL PFC) and the ventral hippocampus (vHPC) and that ketamine treatment will upregulate expression of PNNs around PV cells. With immunohistochemistry, the percentage of PV cells covered with PNNs is expected to reduce significantly following early life adversity while ketamine is hypothesized to rescue the deficits in PNN coverage created by early life adversity. These molecular changes are expected to be paired with decreased anxiety and depressive-like behaviors exhibited in the open field test with aversive (22 kHz) ultrasonic vocalization playback following ketamine treatment.

Methodology Used

This study used 48 male and 49 female Sprague-Dawley rats born across ten litters. Maternal separations were conducted for 3.5-4 hours a day from postnatal day 2 to 21 to induce an anxiety-like phenotype by disrupting parent-offspring bond formation (Figure 1). At the beginning of young adulthood (P46-48), the rats were given either a 15 mg/kg intraperitoneal ketamine injection or an equivalent saline injection. A week later, the rats were tested in the open field for three minutes of silence followed by three minutes with 22 kHz ultrasonic vocalization playback to observe differences in anxiety-like behavior following ketamine treatment. The rats were then sacrificed using rapid decapitations and one hemisphere was saved for immunohistochemistry while the other was microdissected to collect the IL/PL PFC and the vHPC for qPCR. Brains were sliced using a microtome and slices will be processed with immunohistochemistry staining for PNN and PV proteins in the fall. The microdissected tissue will soon be analyzed for changes in protein expression of PNN, PV, and BDNF proteins using qPCR.

Results Obtained

In the open field behavioral test, female rats that experienced early life adversity showed significant increases in the time spent in the center of the field one week during 22 kHz playback after a single 15 mg/kg intraperitoneal injection of ketamine (Figure 2B). In the first three minutes of the open field test (silence), control females that were given ketamine exhibited a significant reduction in the time before they entered the center (latency to center) (Figure 2E). Molecular results will be processed and correlated with the behavioral data in the fall.

Significance and Interpretation of Results

The results of the open field test indicate that the female rats, both control and early life adversity animals, showed significant changes in behavior following a single dose of 15 mg/kg ketamine, while the male rats did not exhibit any significant behavioral changes under the same conditions (Figure 2). Ketamine increased willingness to enter the center of the open field in the female rats, demonstrating a reduction in avoidance and anxiety-like behavior. This data demonstrates a sex-specific effect of this ketamine treatment paradigm and the potential therapeutic effects of a single ketamine dose in females. In the fall, changes in PNN density around PV cells and PNN expression may allude to why some of these sex-specific behavioral effects were observed.

Figures

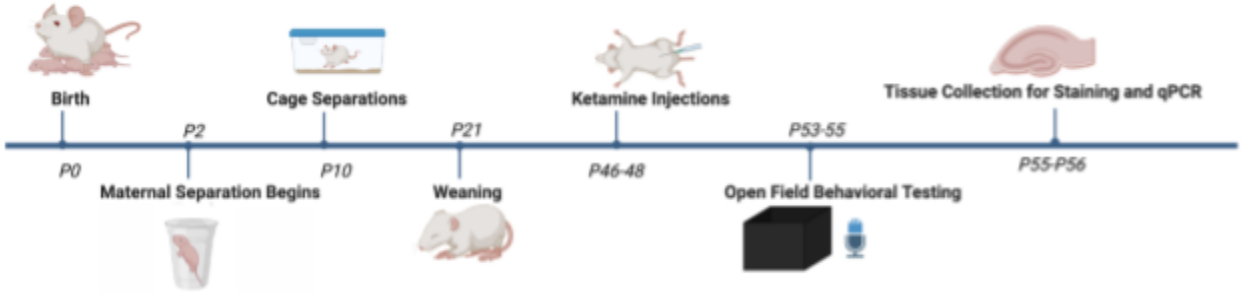

Figure 1. Project timeline

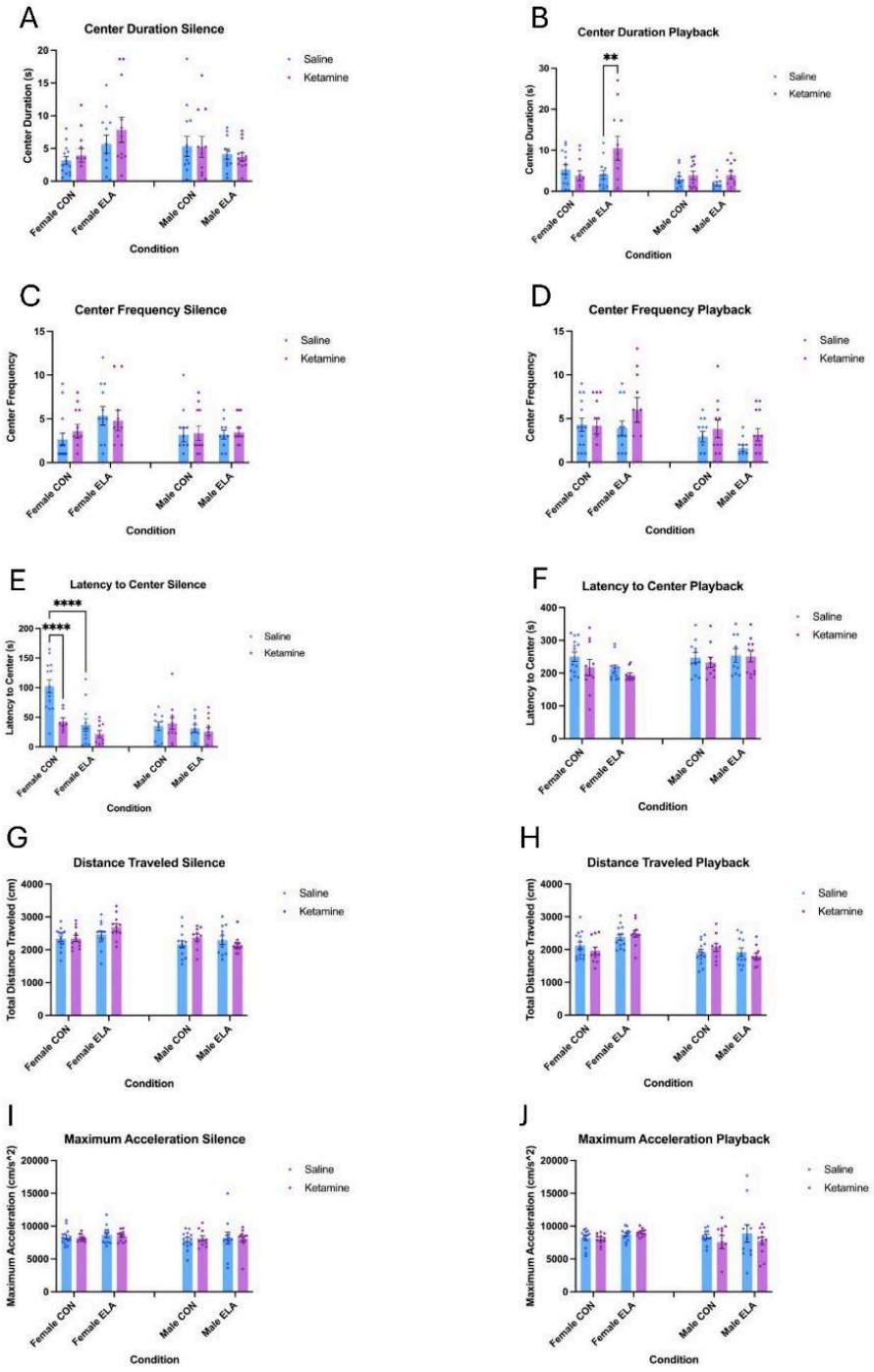

Figure 2. Open field behavior in silence and 22 kHz aversive playback. One week following ketamine or saline injections (15 mg/kg), the P53-P55 rats were tested in the open field with three minutes of silence followed by three minutes of 22 kHz aversive playback. **A,B.** The female rats that underwent early life adversity showed significant increases in time spent in the center of the open field during playback ($p<0.01$). **C,D.** No significant changes were observed following ketamine treatment in the number of center entries (center frequency). **E,F.** Control females showed significantly lower times to enter the center (latency to center) following ketamine treatment during the first three minutes in the open field ($p<0.0001$). **G,H.** No significant changes in total distance traveled were observed following ketamine treatment. **I,J.** No significant changes in maximum acceleration were observed following ketamine treatment.

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