Effects of a Single Ketamine Dose on the Potentiated Startle Response in the Wistar-Kyoto Rat Model of Affective Dysfunction

Alena Lemeshova, 2026
Haney Haidari, 2027
Cherish Zhao, 2028

Background:

The management of treatment-resistant disorders, such as depression and anxiety is one of the most widely researched topics in neuropharmacology. A specific interest has recently been on ketamine, a non-competitive NMDA receptor antagonist, which produces a swift therapeutic response with long term effects (lasting up to 7 days) after a single administration (Gass et al. 2019; Murrough et al. 2013; Yang et al. 2016).

However, the exact mechanism of ketamine's antidepressant effects remains unknown. Furthermore, due to standard poor translational preclinical animal models, the specific Wistar Kyoto rats are used (WKY; Will et al. 2003). This is due to WKY rats being selectively bred to exhibit depressive and anxiety-like symptoms comparable to patients (McAuley et al. 2009). Lastly, only female subjects were used in this project, as they are underrepresented and due to evidence suggesting that estrogen's interactions with ketamine enhances its effectiveness even at lower doses. (Lehmann et al. 1999; Wright et al. 2016)

Hence, this projects utilizes the WKY strain as a model to measure the animals' behavior in an acoustic startle paradigm, assessing the degree of response to a loud unexpected stimulus - as they show maladaptive startle responses, synonymous to human patients with depression (McAuley et al. 2009; Vaidyanathan et al. 2013). We hypothesize that ketamine treatment will normalize this maladaptive behavior both immediately (24 hours after) and longer term (7 days after), and will correlate with the degree of oxidation in the targeted brain areas (Lemeshova 2024; Réus et al. 2015). Lastly, we also hypothesize that ketamine's different efficacies will depend on the phase of the hormonal estrus cycle on the day of administration (Wright et al. 2016).

Methodology, Experimental Procedure, and Timelines

Animals: 44 WKY experimental rats and 12 Wistar control rats were 21 days old upon arrival. After handling and acclimation, at the age of 47 days (the start of their young adult phase), the WKY rats were divided into three experimental groups based on dosage and one control group.

Ketamine Treatment: A single ketamine dose of 5 mg/kg, 10 mg/kg, or 15 mg/kg was intraperitoneally injected (Bærentzen et al. 2024; Manduca et al. 2020; McDonnell et al. 2021). All rats in the experimental group received the treatment at 50 days and were tested 24 hours and 7 days later, with the control group receiving comparable doses of saline.

Behavioral and Molecular Experimentation: Before ketamine treatment, all 47-day-old rats were acclimated in the SR-LAB Startle Response System to remove confounding effects of stress on the expression of the relevant neural markers this study uses, including parvalbumin, after testing (Palmer and Printz, 1999). Both immediate and delayed behavioral trials were conducted using protocols previously used in the Honeycutt laboratory. Upon placement of the rats in the startle box, every experimental session included 30 trials of subsequent 100-millisecond sound cues of 95-, 105-, or 115-dB white noise in a randomized order with 30- to 45-second intervals in between. Average startle response and maximum startle response were assessed as voltage, reported in millivolts.

After the final behavioral testing, brain collection and immunohistochemistry analysis were conducted to examine activation patterns in the basolateral amygdala, hippocampus, and prefrontal cortex, as these areas are associated with anxiety (Campbell and Mcqueen, 2004; Sharp, 2017; Hare and Duman, 2020; Ghasemi et al., 2022). Staining was done for parvalbumin, a protein correlated to neuronal inhibition and stress-related disorders, to measure ketamine's impact on inhibitory behaviors (Gildawie et al., 2020). Oxidative stress was also assessed through analysis of 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a well-established neural marker for oxidative damage, investigating ketamine's potential neuroprotective effects (Gürler et al., 2014; Xiao et al., 2017).

Results

It was concluded that there was a decreasing trend in startle response with increasing ketamine dose. Specifically, the 15 mg/kg ketamine dose significantly suppressed the startle response, potentially indicating an anxiolytic or antidepressant effect at higher doses.

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