Effects of a clinically relevant therapeutic dose of ketamine on the startle response in the Wistar Kyoto rat model of affective dysfunction

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In many anxiety and depressive-like disorders, including Post Traumatic Stress Disorder (PTSD) and Major Depressive Disorder (MDD) [1], the focus for treatment is on drugs that target monoamine neurotransmitters, including selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs) [2]. However, these common pharmacological treatments have a high percentage of individuals who are "treatment resistant." Due to differences in brain chemistry or neuroanatomy, treatments like SSRIs are only effective in about 15% of patients [2]. Different treatments come to the forefront of research; one of these is ketamine. Ketamine is a non-competitive N-methyl-d-Aspartate receptor (NMDAR) antagonist, meaning it inhibits NMDAR activity [4]. Our focus is pyramidal cells, which receive excitatory and inhibitory neuronal input. This means modulations to pyramidal cells impact the excitatory:inhibitory (E:I) balance. One main modulator of pyramidal cell activity is parvalbumin (PV) cells, which are fast-spiking, inhibitory, GABAergic, calcium-buffered cells. These fast-spiking cells are often depolarized, leaving the PCP site open for a non-competitive antagonist (such as ketamine) to inhibit the activity of these cells. When ketamine blocks the PV NMDARs, the cell cannot depolarize [3, 4]. This inhibits the signal of the PV cells, permitting the pyramidal cell to send its signal and continue its original intended pathway. This shifts the E:I balance within the brain, allowing events to be recalled and recorded as more nuanced and with a less inhibited tone. In this study, we utilized the Wistar Kyoto (WKY) rat model of affective dysfunction and hypervigilance. This model translationally represents antidepressant resistance to SSRI-like treatments, as well as aligning neuropathologically with symptoms of hypervigilance [6]. Thus, we applied the WKY model as a genetic predisposition model of hypervigilance to measure response to ketamine in those who are genetically likely to have hypervigilant and anxiety-like behavior. Our focus on female WKYs stems from the lack of research in females, as well as the high likelihood of females developing psychiatric disorders like PTSD, MDD, and other anxiety-like disorders. They have also been shown to have a lower responsiveness level to ketamine as a therapeutic treatment compared to males; thus, females are of particular interest in this study [5].

We received cohorts of 9-12 female WKY rats at post-natal day (P)26. Beginning at P31, animals were briefly handled daily. At P39, they began habituating to transportation to our behavior room. At P41, animals began habituation to the acoustic startle response (ASR) box, which involved 27 minutes of white noise at 45 decibels (dB). On P46, animals received an injection of saline or ketamine, given in doses of 5, 10, or 15 mg/kg. On both P47 and P54, animals underwent behavioral testing. They were given 5 minutes of acclimating white noise in the ASR box, before a string of 30 startle pulses of either 95, 105, or 115 dB, spaced 30 to 45 seconds apart. Following the second testing, animals were euthanized with CO_2 and were perfused with phosphate-buffered saline (PBS), then 4% paraformaldehyde in PBS. The brains were collected and maintained in 4% paraformaldehyde for 1 week, then cryoprotected in 30% sucrose.

Results show that following a 24-hour delay, higher doses of ketamine (15 mg/kg) appear to be significantly beneficial in reducing startle response. However, lower doses (10 mg/kg) appear to have long-lasting effects, reducing startle response after a 1-week delay. This suggests that lower doses may selectively target PV cells, while a higher therapeutic dose may be having an off target effect, potentially interacting with μ-opioid receptors [7]. In the future, we hope to use immunohistochemistry to tag PV, cFos (a marker for neuronal activation), and 8-oxo-dG (a marker for oxidative stress) to research regarding the occurring neurochemical activity.

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