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Sex Differences in Effects of Ketamine on Behavior, Spine Density, and Synaptic Proteins in Socially Isolated Rats

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ABSTRACT

BACKGROUND: The mechanistic underpinnings of sex differences in occurrence of depression and efficacy of antidepressant treatments are poorly understood. We examined the effects of isolation stress (IS) and the fast-acting antidepressant ketamine on anhedonia and depression-like behavior, spine density, and synaptic proteins in male and female rats.

METHODS: We used a chronic social IS paradigm to test the effects of ketamine (0, 2.5 mg/kg, and 5 mg/kg) on behavior and levels of synaptic proteins synapsin-1, postsynaptic density protein 95, and glutamate receptor 1 in male rats and female rats in diestrus. Medial prefrontal cortex spine density was also examined in male rats and female rats that received ketamine during either the diestrus or the proestrus phase of their estrous cycle.

RESULTS: Male rats showed anhedonia and depression-like behavior after 8 weeks of IS, concomitant with decreases in spine density and levels of synapsin-1, postsynaptic density protein 95, and glutamate receptor 1 in the medial prefrontal cortex; these changes were reversed by a single injection of ketamine (5 mg/kg). After 11 weeks of IS, female rats showed depression-like behavior but no signs of anhedonia. Although both doses of ketamine rescued depression-like behavior in female rats, the decline observed in synaptic proteins and spine density in IS and in diestrus female rats could not be reversed by ketamine. Spine density was higher in female rats during proestrus than in diestrus.

CONCLUSIONS: Our findings implicate a role for synaptic proteins synapsin-1, postsynaptic density protein 95, and glutamate receptor 1 and medial prefrontal cortex spine density in the antidepressant effects of ketamine in male rats subjected to IS but not in female rats subjected to IS, suggesting dissimilar underlying mechanisms for efficacy of ketamine in the two sexes.

Keywords: Anhedonia, Depression, Ketamine, mPFC, Sex difference, Social isolation

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Interesting sex differences exist in the prevalence rate of depression (1,2), the symptoms of depressive disorders (3,4), and the efficacy of antidepressant medication (5–7). Depression is twice as prevalent among women compared with men. Specific symptoms of depression, such as anxiety, bulimia, and suicidal ideation, are more common in women than in men, who predominantly display symptoms of alcoholism and substance abuse (8,9). Preclinical research has also shown robust sex differences in animal models of depression (10). Despite the existence of compelling evidence for sex differences in depression, the mechanisms underpinning these differences remain largely unexplored. Studies have attributed behavioral differences between male and female animals to sexual dimorphism of the underlying neuronal circuitry and neuroendocrine systems (11), which have genetic (12) and epigenetic (13) underpinnings.

Although some clinical studies have reported higher efficacy of selective serotonin reuptake inhibitors in women than in men (5), collectively, for both sexes, the current available antidepressants, with slow onset of therapeutic effects, have serious limitations (14,15). However, more recently, the *N*-methyl-D-aspartate receptor antagonist ketamine, classically used as an anesthetic, has emerged as a fast-acting antidepressant (16,17). Acute injections of ketamine produce rapid antidepressant effects within a few hours (18–20). Previous studies from our laboratory on rats (21) and other studies on mice (22) have shown female animals to be more sensitive to ketamine than male animals. A single injection of 2.5 mg/kg dose of ketamine induced an antidepressant-like effect in female rats but not in male rats, which responded to doses of ≥5 mg/kg (21). To further investigate sex differences in antidepressant actions of ketamine, we examined its effects on 1) anhedonia and depression-like behavior, 2) spine density in the medial prefrontal cortex (mPFC), and 3) molecular changes in the mPFC synaptoneurosomes in male and female rats that were exposed to chronic social isolation stress (IS). In rats, IS is known to evoke depression and anhedonia-like behavior (23). Previous studies from our laboratory have established the suitability of this stress paradigm for comparative studies between male and female animals (24) because, in contrast

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to chronic unpredictable (25) and chronic mild (26) stress paradigms that were employed to delineate molecular pathways affected by ketamine in male animals, chronic IS does not disrupt the estrous cycle in female animals. Our results highlight interesting differences between male and female rats in their response to IS and ketamine treatment and implicate alternative underlying mechanisms for efficacy of ketamine in male and female rats.

METHODS AND MATERIALS

Animals

Adult male (250–270 g) and female (200–225 g) Sprague Dawley rats (Charles River, Wilmington, MA) were maintained either under pair-housed (PH) condition or in solitary cages under IS on a 12-hour light/dark cycle (lights on at 5:00 AM) with ad libitum access to food and water. All animal protocols were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Florida State University.

Experimental Designs

Effect of Ketamine on Anhedonia and Depression-like Behavior in Male and Female Rats Subjected to IS. Male (n = 8/group) and female (n = 6/group) rats were housed either under PH condition or under IS. During this period of time, PH and IS groups of animals were tested on the sucrose preference test (SPT) periodically (every other day) to check for the emergence of behavioral abnormalities induced by IS. When a significant decline in preference for sucrose was observed in the IS group and was stable and maintained over the next week, animals were injected with saline/ketamine (2.5 mg/kg, 5 mg/kg; Henry Schein Animal Health, Inc., Dublin, OH) and tested for anhedonia-like behavior on the SPT after 3 hours (day 1) and after 27 hours (day 2) and for depression-like behavior on the forced swim test (FST) on the third day after injection (Figure 1A). Female rats were lavaged for determination of estrous cycle stage, and female rats having consistent cycle lengths of 4–5 days were injected with ketamine and tested behaviorally during diestrus.

Effect of Ketamine on mPFC Spine Density in Male Rats and During Different Phases of Estrous Cycle in Female Rats Subjected to IS. To investigate effects of ketamine on spine density in animals exposed to IS, bilateral stereotactic surgeries were performed to infuse herpes simplex virus–green fluorescent protein (HSV-GFP) into the mPFC of rats of both sexes in the PH and IS groups (n = 4/group). Female rats were lavaged to determine estrous cycle stage. Rats were injected with saline/ketamine (2.5 mg/kg, 5 mg/kg) 3–4 days after surgery. Female rats were injected with ketamine either during diestrus, when gonadal hormone levels are lowest, or during proestrus, when gonadal hormone levels are at peak. Rats were perfused 3 hours after drug injections. Immunohistochemistry with GFP was performed to detect fluorescence. Dendrites belonging to the apical tuft of layer V pyramidal neurons of the mPFC were imaged using a Zeiss confocal microscope under 63× oil objective (Zeiss Inc., Jena, Germany). Spines were counted using Neurolucida Explorer version 9 (MBF Bioscience, Williston, VT).

Effect of Ketamine on Synaptic Proteins in Male and Female Rats Subjected to IS. The male (n = 6/group) and female (n = 5/group) rats in the PH and IS groups that underwent behavioral testing were sacrificed 1 hour after FST, and brain tissues were collected for Western blot assay of the synaptic proteins synapsin-1, postsynaptic density protein 95 (PSD-95), and glutamate receptor 1 (GluR1) on synaptoneurosomal fractions isolated from mPFC.

Behavioral Tests

SPT. The SPT, a two-bottle choice paradigm, was performed as described earlier (21,27). Rats were habituated to drinking from two bottles before testing. For testing of baseline preference for sucrose and for the SPT after the period of stress, rats were given access to two preweighed bottles, one containing water and one containing 0.25% sucrose, for the first 2 hours of the dark cycle. The bottles were weighed at 5:00 PM and 7:00 PM, and the preference for sucrose over water was used as a measure of anhedonia.

FST. The FST, a 2-day procedure, was performed as described previously (28,29). On day 1 and day 2, rats were placed in 30 cm × 45 cm Plexiglas cylinders filled with water, maintained at 25°C for 15 minutes on day 1 and for 5 minutes on day 2. Their behaviors were videotaped and analyzed for immobility time by a scorer blinded to the treatment conditions. Immobility was defined as minimum movement required for remaining afloat (30). Female rats were subjected to the pretest and test when they were in the diestrus stage of their cycle, when the levels of gonadal hormones are at their lowest. Therefore, the FST was conducted 3 days after ketamine injection in male rats and 3–5 days after ketamine injection in female rats.

Virus Delivery and Immunohistochemistry for GFP Staining

To visualize dendrites and spines in the prelimbic region of the mPFC, male and female rats were given bilateral stereotactic injections of the HSV-GFP viral vector (p1005+ HSV plasmid expressing GFP under the control of cytomegalovirus promoter) following standard methods (31). Animals were anesthetized using isoflurane (Henry Schein Animal Health, Dublin, OH) during surgeries. Bregma coordinates used for the surgery were as follows: +3.1 mm anterior, ±0.6 mm lateral, and 3.9 mm ventral to bregma. The virus was delivered at a rate of 0.1 μL/min using 24-gauge syringe needles (Hamilton Laboratory Products, Reno, NV) for a total volume of 1.0 μL per animal on each side. The animals were sacrificed 3–4 days after viral infection when transgene expression is maximal.

Coronal sections (50 μm) of the mPFC encompassing the site of HSV-GFP injection were generated using a Vibratome (Leica Microsystems GmbH, Wetzlar, Germany). To visualize the spines, immunohistochemistry for GFP was performed on the sections. Briefly, after blocking (5% normal goat serum and .3%