



Sex differences in the antidepressant-like effects of ketamine

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ABSTRACT

Current medications for major depression suffer from numerous limitations. Once the right drug for treatment has been determined, it still takes several weeks for it to take effect and improve mood. This time lag is a serious concern for the healthcare community when dealing with patients with suicidal thoughts. However, recent clinical studies have shown that a single low-dose injection of ketamine, an N-methyl D-aspartate receptor (NMDAR) antagonist, has rapid antidepressant effects that are observed within hours and are long lasting. Although major depression affects twice as many women as men, all studies examining the rapid antidepressant effects of ketamine have focused on male subjects. Thus, we have investigated the behavioral and molecular effects of ketamine in both male and female rats and demonstrated greater sensitivity in female rats at a low dose of ketamine, a dose does not have antidepressant-like effects in male rats. The antidepressant-like effects of this low dose of ketamine were completely abolished when female rats were ovariectomized (OVX), and restored when physiological levels of estrogen and progesterone were supplemented, suggesting a critical role for gonadal hormones in enhancing the antidepressant-like effects of ketamine in female rats. In preclinical studies, the mammalian target of rapamycin (mTOR) in the medial prefrontal cortex and the eukaryotic elongation factor (eEF2) in the hippocampus have been proposed as critical mediators of ketamine's rapid antidepressant actions. In our hands, the increased sensitivity of female rats to a low dose of ketamine was not mediated through phosphorylation of mTOR or eEF2.

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1. Introduction

In any given year, more women than men are diagnosed with depression (Holden, 2005; Kessler et al., 2005; Steiner et al., 2005). This has been attributed to the pronounced sex differences that exist in both the anatomy and function of the brain, as well as to the sexually dimorphic hormonal environment (Cosgrove et al., 2008; Kessler, 2003). In particular, women are more likely to suffer from depression and anxiety during periods of when levels of estrogen and progesterone are at their lowest during the premenstrual, postpartum and perimenopausal periods (Douma et al., 2005). In both sexes, the current available antidepressants have serious limitations in that they require weeks to months to ameliorate symptoms, and only one third of patients respond to the first prescribed antidepressant (Trivedi, 2006; Trivedi et al., 2006). Recent clinical studies have shown that acute treatment with ketamine produces rapid antidepressant effects that last for up to 7 days (Berman et al., 2000; Zarate et al., 2006a), and ketamine infusion has been applied by practitioners in off-label use for treatment resistant major depressive disorder (Covvey et al., 2012).

Ketamine is a non-competitive antagonist acting at the NMDA glutamate receptor. Since its discovery more than 50 years ago, ketamine has been used very efficiently in anesthesia and in pain management and is showing great promises for its antidepressant effects. Indeed, unlike classical antidepressants whose therapeutic effects take weeks to be observed, an acute intravenous injection of ketamine is sufficient to induce quick and long-lasting antidepressant effects (Berman et al., 2000; Zarate et al., 2006a, 2006b). The rapid antidepressant effect of ketamine is of utmost interest when dealing with depressed patients who have suicidal thoughts. In this population, a single injection of small dose of ketamine induced a rapid resolution of suicidal ideation (DiazGranados et al., 2010; Price et al., 2009).

In preclinical studies, two independent research groups have identified mTOR in the mPFC and eEF2 in the HPC for mediating the antidepressant effects of ketamine (Autry et al., 2011; Li et al., 2010). Since these studies were carried out only in male subjects, we aimed to determine if ketamine also has antidepressant-like effects in female rats. Our findings clearly show that female rats are much more sensitive to ketamine when compared to male rats, as they respond to a low dose of ketamine (2.5 mg/kg), a dose that is clearly not antidepressant in male rats, and that the gonadal hormones estrogen and progesterone mediate this high sensitivity to

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ketamine in female rats. Understanding the behavioral and molecular basis of sex differences in NMDAR function and responses to estrogen and progesterone are vitally important for designing novel therapeutic agents that have optimal effectiveness in men and women. Due to the multimodal mechanism of action of NMDAR antagonists and estrogen, the rapidity and the breadth of action spectrum, and their combination could lead to more rapid effects – in both men and women – as compared to current antidepressant therapy.

In male rats, administration of low doses of ketamine reportedly leads to rapid phosphorylation of synaptoneurosomal mTOR and other associated proteins (PSD-95, GluR1, and synapsin) in the prefrontal cortex which are responsible for increased formation of dendritic spines (Li et al., 2010). Alternatively, increased BDNF translation mediated by reduced activity of eEF2 kinase within the hippocampus has also been implicated in mediating the antidepressant-like effects of ketamine (Autry et al., 2011). To understand the molecular mechanisms underlying sex differences in the antidepressant-like response to ketamine, we investigated the role of mTOR activation within total protein extracts and synaptoneurosomal fractions of the medial prefrontal cortex as well as hippocampal eEF2 kinase phosphorylation in male and female rats following acute exposure to various doses of ketamine.

2. Methods

2.1. Experimental design

2.1.1. Experiment 1. Sex differences in the antidepressant and anxiolytic-like effects of ketamine

Male and female rats ($n = 6$ per group) were exposed to a single intraperitoneal (i.p.) injection of 0, 2.5, 5.0, or 10.0 mg/kg of ketamine hydrochloride (Butler Schein Animal Health, Inc.), or 20 mg/kg imipramine hydrochloride (Sigma–Aldrich, Co.), and their behaviors tested 30 min later in the forced swim test, novelty suppressed feeding test, or light–dark box. Twenty-four and 48 h later, behaviors were tested in the elevated plus maze and sucrose preference, respectively (Supplementary Fig. S1). The drugs were injected at a volume of 1 ml/kg.

2.1.2. Experiment 2. The role of estrogen and progesterone in mediating sex differences in forced swim behavior at a low dose of ketamine

As illustrated in Fig. 3A, 10 days following ovariectomy, female rats ($n = 6$ –10 per group) received subcutaneous injections of 0, 2 or 10 μ g estrogen benzoate (Sigma, St. Louis, MO) in sesame oil 24 h prior to testing, and 0 or 500 μ g progesterone (Sigma, St. Louis, MO) in sesame oil 4 h prior to testing. These doses produce near physiological levels of estrogen (Asarian and Geary, 2002) and progesterone (al-Dahan and Thalmann, 1996) and the cyclic regimen models the four day estrous cycle of intact rats (Yu et al., 2011). OVX and intact female rats were injected (i.p.) with 0 or 2.5 mg/kg ketamine and tested 30 min later in the forced swim test.

2.1.3. Experiment 3. Activation of mTOR in medial prefrontal cortex synaptoneurosomes and sex differences in phosphorylated eEF2 in the hippocampus in response to ketamine

Male and female rats ($n = 6$ per group) were exposed to a single injection (i.p.) of 0, 2.5, or 5.0 mg/kg ketamine and sacrificed 30 min later. The phosphorylation status of mTOR within the entire medial prefrontal cortex and the synaptoneurosomal fraction were determined. The phosphorylation status of eEF2 within the hippocampus was also determined.

2.2. Animals

Adult male (weighing 250–270 g) and female (weighing 200–225 g) Sprague–Dawley rats, were purchased from Charles River (Wilmington, MA, USA). All rats were pair-housed in 43 × 21.5 × 25.5 cm Plexiglas cages and kept on a 12 h:12 h light:dark cycle (lights on at 0700 h) and each cage received identical treatment. Food and water were available *ad libitum* except during testing. All behavioral experiments, except the sucrose preference test, were conducted during the first 4 h of the light phase of the light/dark cycle and all animal protocols were carried out in accordance with the NIH Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Florida State University. For ovariectomy, a 2–3 cm ventral midline incision was made in the lower abdominal region of adult female rats to expose the uterus. Visible blood vessels were ligated, the ovaries removed, and the muscle layers and skin were sutured.

2.3. Behavioral tests

2.3.1. Forced swim test

The FST is a two day procedure performed as described previously (Carrier and Kabbaj, 2012a). On day 1, rats were placed for 15 min in large inescapable Plexiglas cylinders (30 × 45 cm) filled with 25 °C water to a depth of 30 cm in a dimly lit room. On day 2, the rats were again forced to swim for 5 min under the same conditions. Rats were exposed to ketamine before the second swim exposure. The cylinders were emptied and cleaned between rats. Rats' behavior for both swim sessions was videotaped, and the latency to the first immobility, and the total time spent immobile were analyzed by a scorer that was blind to the experimental treatment. Immobility was defined as minimal movements required only to remain afloat (Lucki, 1997).

2.3.2. Novelty suppressed feeding

In the novelty suppressed feeding test, rats were food-deprived for 24 h and then placed in a novel open field (1 m × 1 m) with a small amount of food available in the middle of the field. The amount of time to take the first bite was recorded as the latency to feed. The animal was removed immediately after feeding, or after 15 min had elapsed, whichever came first.

2.3.3. Sucrose preference test

The sucrose preference test consisted of a two-bottle choice paradigm (Carrier and Kabbaj, 2012a; Chen et al., 2012; Dwyer et al., 2011; Kentner et al., 2010). Rats were allowed to drink from two water bottles for five days prior to testing. The rats were given access to two preweighed bottles, one containing water and the other containing 0.25% sucrose for 48 h. The bottles were weighed at 8 am and 5 pm and the preference for sucrose over water was used as a measure of anhedonia. The position of the bottles was counterbalanced daily to prevent a place preference.

2.3.4. Elevated plus maze

Rats were placed into the elevated plus maze (MED Associates Inc., St. Albans, Vermont) facing a closed arm and were allowed to freely explore the maze for 10 min under dim light as described previously (Carrier and Kabbaj, 2012b). Rats' behavior was recorded by a digital camcorder placed directly above the elevated plus maze. Time spent in the open arms and number of entries into the open arms were analyzed in EthoVision XT version 6 (Noldus Information Technology, Leesburg, VA). The elevated plus maze was cleaned with 70% ethanol between trials.

2.3.5. Light–dark box

Rats were placed into the dark compartment (200 × 310 mm) of the dual chamber apparatus (Model LE-812, EB Instruments, Pinellas Park, FL) and allowed to freely explore both compartments for 10 min as described previously (Carrier and Kabbaj, 2012b). Time spent and frequency of entries in the light compartment (310 × 310 mm) were analyzed using PPCWIN software. The apparatus was cleaned with 70% ethanol between trials.

2.4. Synaptoneurosomes preparation and western blot

The medial prefrontal cortex (cingulate, infralimbic, and prelimbic regions) and the dorsal hippocampus were tissue punched using a cryostat and frozen at –80 °C until further processing. A crude synaptoneurosomal fraction was prepared from the medial prefrontal cortex as previously described (Li et al., 2010). Briefly, tissue from the medial prefrontal cortex was homogenized in a solution containing 0.32 M sucrose, 20 mM HEPES (pH 7.4), 1 mM EDTA, 1× protease cocktail inhibitor cocktail, 5 mM NaF and 1 mM sodium vanadate and centrifuged at 4 °C for 10 min at 2800 rpm. The pellet contains nuclei and large debris (nuclear fraction). The supernatant was centrifuged at 4 °C for 10 min at 12,000 rpm. Following this centrifugation, the supernatant (cytosolic fraction) was removed and the pellet (crude synaptoneurosomes fraction) was sonicated in 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 2 mM EDTA, 1 mM NaVO₃, 5 mM NaF, and 1× protease inhibitor cocktail. Total proteins were extracted from both hippocampi, or from the medial prefrontal cortex. Protein samples were processed as described previously (Carrier and Kabbaj, 2012a). Equal concentrations of proteins (10–20 μ g) were loaded into 10–12% SDS PAGE gel for electrophoresis. Immunoblots were incubated overnight (4 °C) with phospho-mTOR (1:5000), mTOR (1:1000), phospho-eEF2 (1:1000), eEF2 (1:1000), or GAPDH (1:5000) antibodies (Cell Signaling Technology), washed and incubated 1 h with goat anti-rabbit IR Dye 680LT (Li-COR Biosciences; 1:20,000) or donkey anti-goat IR Dye 800CW (Li-COR; 1:10,000) fluorescent secondary antibodies, and visualized using an Odyssey infrared imaging system (Li-COR Biosciences). Quantification was done using NIH ImageJ (<http://rsbweb.nih.gov/ij/>). Normalized data are expressed as percent of control, with saline injected control animals set to 1.

2.5. Statistical analysis

Results were analyzed using one-way or two-way analysis of variance (ANOVA) followed by post-hoc Fisher tests where appropriate. For behavioral testing, unless specified, interactions were not significant and planned comparison tests were

carried out based on the hypothesis that female rats would be more sensitive to the antidepressant-like effects of ketamine than their male counterparts. p values < 0.05 were considered statistically significant.

3. Results

3.1. Sex differences in the antidepressant and anxiolytic-like effects of ketamine

On day 1 of the forced swim test, during the first 5 min of the 15 min pretest, female rats spent less time immobile compared to male rats indicating greater activity (Fig. 1A; $F_{(1,38)} = 29.765$; $p < 0.0001$). There was no main effect. During the 5 min test, we again observed a sex effect (Fig. 1B; $F_{(1,40)} = 28.148$; $p < 0.0001$) where females spent less total time immobile and a treatment effect ($F_{(3,40)} = 7.448$; $p < 0.001$). Male rats injected with 5.0 or 10.0 mg/kg ketamine spent less time immobile compared to male rats injected with 0 or 2.5 mg/kg ketamine ($p < 0.05$). Female rats injected with 2.5, 5.0, and 10.0 mg/kg ketamine spent less time

immobile compared to female rats injected with 0 mg/kg ketamine ($p < 0.05$). The latency to immobility was affected by treatment (Fig. 1C; $F_{(3,40)} = 6.071$; $p < 0.01$) and there was a trend for a sex effect ($F_{(1,40)} = 2.891$; $p = 0.097$). Male rats injected with 5.0 or 10.0 mg/kg ketamine had a longer latency to immobility compared to male rats injected with 0 or 2.5 mg/kg ketamine ($p < 0.05$). Female rats injected with 10.0 mg/kg ketamine had a longer latency to immobility compared to female rats injected with 0 mg/kg ketamine ($p < 0.05$). There was a trend for longer latency to immobility in female rats injected with 5.0 mg/kg ketamine compared with female rats injected with 0 mg/kg ketamine ($p = 0.11$).

In the novelty suppressed feeding test, we observed a treatment effect (Fig. 2A; $F_{(3,42)} = 5.695$; $p < 0.01$) and an interaction between sex and treatment ($F_{(3,42)} = 3.050$; $p < 0.05$). Male rats injected with 5.0 and 10.0 mg/kg ketamine had a decreased latency to begin eating compared with male rats injected with 0 and 2.5 mg/kg ketamine ($p < 0.05$). Female rats injected with 2.5, 5.0, and 10.0 mg/kg ketamine had a decreased latency to feed compared with female rats injected with 0 mg/kg ketamine ($p < 0.05$).

In the sucrose preference test, there was a treatment effect (Fig. 2B; $F_{(3,36)} = 4.89$; $p < 0.01$) and an interaction between sex and treatment ($F_{(3,36)} = 2.947$; $p < 0.05$). Male rats injected with 5.0 and 10.0 mg/kg ketamine had an increased sucrose preference compared with male rats injected with 0 and 2.5 mg/kg ketamine ($p < 0.01$). There were no significant treatment effects on sucrose preference in female rats.

Acute ketamine injections of 0, 2.5, 5.0, and 10.0 mg/kg had no effect on time spent or number of entries into the open arms of the elevated plus maze in either male or female rats (Table 1; $p > 0.05$). Similarly, there was no treatment effect on time spent or entries into the light box in the light/dark box test in male or female rats (Table 2; $p > 0.05$). A single injection of 20 mg/kg imipramine, a classical antidepressant that requires chronic administration for positive effects (Reus et al., 2010) – used as a negative control – had no effects in the elevated plus maze, light/dark box, or forced swim tests in male and female rats (Table 3; $p > 0.05$).

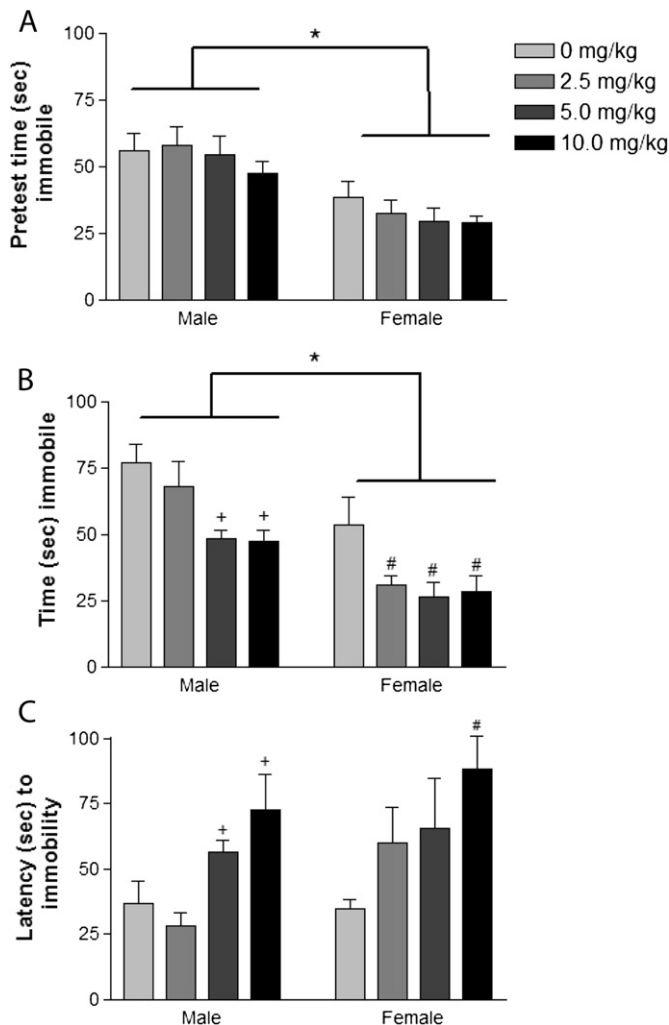


Fig. 1. Sex differences in the forced swim test following acute ketamine exposure. A) Female rats spent less total time immobile compared to male rats during the first 5 min of the 15 min pretest indicative of greater activity. B) Male rats injected with 5.0 and 10.0 mg/kg ketamine, and female rats injected with 2.5, 5.0, and 10.0 mg/kg ketamine, spent less time immobile during the 5 min forced swim test. C) Male rats injected with 5.0 and 10.0 mg/kg ketamine, and female rats injected with 10.0 mg/kg ketamine had a longer latency to immobility. * $p < 0.0001$; # $p < 0.05$ compared to 0 mg/kg ketamine-treated female rats; + $p < 0.05$ compared to 0 and 2.5 mg/kg ketamine-treated male rats.

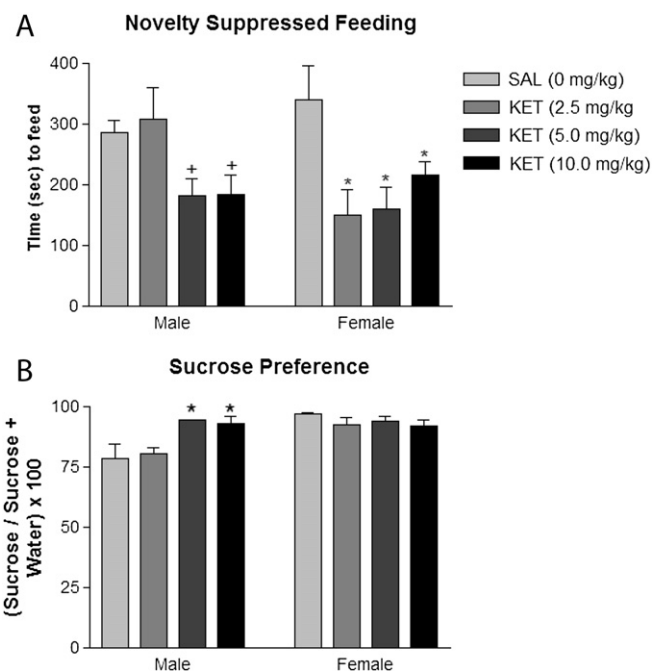


Fig. 2. Sex differences in the novelty suppressed feeding and sucrose preference tests. A) Male rats injected with 5.0 and 10.0 mg/kg ketamine and females injected with 2.5, 5.0, and 10.0 mg/kg ketamine had decreased latency to feed. B) Male rats injected with 5.0 and 10.0 mg/kg ketamine had an increased sucrose preference.

Table 1

Elevated plus maze. Time spent and number of entries into the open arms were not affected by ketamine treatment male or female rats.

Ketamine dose	Time (s) in open arms		Entries into open arms	
	Male	Female	Male	Female
0 mg/kg	23.072 ± 6.613	53.174 ± 10.861	5.87 ± 3.264	19.045 ± 9.705
2.5 mg/kg	54.521 ± 15.854	37.070 ± 11.256	11.475 ± 5.088	28.258 ± 14.094
5.0 mg/kg	52.219 ± 40.868	45.646 ± 13.721	10.508 ± 6.432	22.685 ± 10.293
10.0 mg/kg	14.715 ± 8.491	62.095 ± 17.816	5.886 ± 2.654	24.617 ± 8.366

3.2. The role of estrogen and progesterone in mediating sex differences in forced swim behavior at a low dose of ketamine

OVX female rats spent more time immobile in the forced swim test compared to intact female rats (Fig. 3B; $F_{(1,23)} = 108.704$; $p < 0.0001$). There was a treatment effect ($F_{(1,23)} = 5.567$; $p < 0.05$) such that injection of 2.5 mg/kg in intact female rats reduced immobility time compared with female rats injected with 0 mg/kg ($p < 0.05$), however OVX female rats were not affected by the same treatment ($p > 0.05$). Similarly, injection of 2.5 mg/kg ketamine had no effect in OVX female rats receiving 2 µg estrogen (Fig. 3C; $t_{12} = 0.990$; $p > 0.05$), 10 µg estrogen (Fig. 3D; $t_{12} = 0.003$; $p > 0.05$), or progesterone (Fig. 3E; $t_{12} = 0.077$; $p > 0.05$) compared to female rats injected with 0 mg/kg ketamine. OVX female rats receiving both 2 µg estrogen and 500 µg progesterone exhibited decreased immobility when injected with 2.5 mg/kg ketamine compared with OVX female rats injected with 0 mg/kg ketamine (Fig. 3F; $t_{12} = 5.661$; $p < 0.05$).

3.3. Activation of mTOR in medial prefrontal cortex synaptoneuroosomes in response to ketamine

Male rats injected with 0, 2.5, or 5.0 mg/kg ketamine did not differ in phosphorylated mTOR (Fig. 4A; $F_{(2,10)} = 2.008$; $p > 0.05$) or total mTOR (Fig. 4B; $F_{(2,10)} = 0.828$; $p > 0.05$) in total protein preparations from the medial prefrontal cortex. Similarly, injection of 0 or 2.5 mg/kg ketamine in female rats had no effect on phosphorylated mTOR (Fig. 4A; $t_7 = 0.045$; $p > 0.05$) or total mTOR (Fig. 4B; $t_7 = 0.099$; $p > 0.05$) in total protein preparations from the medial prefrontal cortex. Injection of 5.0 mg/kg ketamine increased phosphorylated mTOR in synaptoneurosome preparations from the medial prefrontal cortex in both male (Fig. 4C; $F_{(2,11)} = 8.791$; $p < 0.01$) and female ($F_{(2,11)} = 10.773$; $p < 0.01$) rats compared to rats injected with 0 or 2.5 mg/kg ketamine. The synaptoneurosome protein fraction containing increased levels of phosphorylated mTOR was highly concentrated in post synaptic density proteins such as PSD-95 (Fig. 4D).

3.4. Sex differences in phosphorylation of eEF2 in the hippocampus in response to ketamine

Injection of 5.0 mg/kg ketamine in male rats decreased phosphorylated eEF2 in total protein preparations from the hippocampus compared to male rats injected with 0 or 2.5 mg/kg ketamine

(Fig. 5; $F_{(2,11)} = 6.621$; $p < 0.05$). Female rats injected with 0, 2.5, or 5.0 mg/kg ketamine did not differ in phosphorylated eEF2 in total hippocampal protein preparations ($F_{(2,11)} = 2.270$; $p > 0.05$).

4. Discussion

Our findings clearly show that female rats are more sensitive to ketamine when compared to male rats, as they respond to a low dose of ketamine (2.5 mg/kg), a dose that clearly does not have antidepressant-like effects in male rats, and that the gonadal hormones estrogen and progesterone mediate this high sensitivity to ketamine in female rats. Ketamine did not affect the phosphorylation status of mTOR in total protein preparations from the medial prefrontal cortex of either female or male rats. We observed increased phosphorylation of mTOR specifically within synaptoneuroosomes isolated from the medial prefrontal cortex following 5.0 mg/kg, but not 2.5 mg/kg ketamine in both male and female rats. Following injection of 5.0 mg/kg ketamine, we detected reduced phosphorylation of eEF2 kinase within the hippocampus of male rats only. Thus, the antidepressant-like response and greater sensitivity of female rats at the 2.5 mg/kg ketamine dose is mediated by an alternative mechanism from mTOR and eEF2 activation.

Activation of mTOR in the medial prefrontal cortex has been proposed as a critical mediator of the rapid antidepressant actions following ketamine exposure (Li et al., 2010). In a series of elegant studies, Li et al. (2010) demonstrated acute treatment with ketamine led to the rapid phosphorylation of synaptoneuroosomal mTOR and other associated proteins that led to a rapid elevation of synaptic proteins' synthesis (e.g. PSD-95, GluR1 and synapsin) in synaptoneuroosomes and increased the number and function of spines within the medial prefrontal cortex. The implication of spines in depression is supported by studies showing that chronic stress leads to reduced spine density and retraction of dendritic branches in the medial prefrontal cortex and hippocampus (Radley et al., 2006; Shansky et al., 2009; Shansky and Morrison, 2009) effects that are reversed by classical antidepressant treatments (Bessa et al., 2009; Hajszan et al., 2009; Norrholm and Quimet, 2001; Wood et al., 2004) as well as by acute ketamine treatment (Li et al., 2010). Within total protein preparations from the medial prefrontal cortex, we were unable to detect changes in phosphorylation of mTOR following ketamine exposure. In agreement with Li et al. (2010), we observed an increase in phosphorylation of mTOR specifically within synaptoneuroosomes of the medial prefrontal cortex following 5.0 mg/kg ketamine in both male and female rats.

Table 2

Light/dark box. Time spent and entries (%) were not affected by ketamine treatment in male or female rats.

Ketamine dose	Time (s) in light box		Entries (%) into white box	
	Male	Female	Male	Female
0 mg/kg	176.833 ± 24.085	190.500 ± 31.655	49.073 ± 0.927	48.773 ± 1.274
2.5 mg/kg	139.833 ± 34.158	247.000 ± 15.898	50.908 ± 1.964	49.843 ± 0.645
5.0 mg/kg	96.667 ± 28.093	165.500 ± 37.494	35.670 ± 7.531	40.535 ± 8.136
10.0 mg/kg	71.000 ± 34.163	100.667 ± 31.458*	32.575 ± 10.326	42.222 ± 8.462

* $p < 0.05$ compared to 0 mg/kg.

Table 3

Anxiolytic and antidepressant-like effects of acute imipramine treatment. A single injection of 20 mg/kg imipramine had no anxiolytic effects in the elevated plus maze or light/dark box. Imipramine increased latency to feed in the novelty suppressed feeding test in both male and female rats and increased latency to immobility in the forced swim test only in male rats.

Behavior test	Male		Female	
	0 mg/kg	20 mg/kg	0 mg/kg	20 mg/kg
Elevated plus maze				
Time (s) in open arms	23.072 ± 6.613	48.615 ± 33.477	53.174 ± 10.861	51.405 ± 18.417
Entries into open arms	5.87 ± 3.264	6.504 ± 4.076	19.045 ± 9.705	10.803 ± 2.871
Light–dark box				
Time (s) in light box	176.833 ± 24.085	107.833 ± 36.542	190.500 ± 31.655	142.333 ± 30.555
Entries (%) into light box	49.073 ± 0.927	45.732 ± 3.265	48.773 ± 1.274	47.143 ± 1.844
Forced swim test				
Time (s) immobile	76.593 ± 7.151	65.466 ± 7.297	53.231 ± 10.732	53.053 ± 9.479

We did not observe increased phosphorylation of mTOR in female rats following 2.5 mg/kg ketamine, suggesting that mTOR activation within synaptoneurosome of the medial prefrontal cortex does not mediate the increased sensitivity and antidepressant-like effect at 2.5 mg/kg ketamine in female rats.

Another recent study indicated that the rapid antidepressant actions of ketamine were mediated through inhibition of spontaneous hippocampal NMDAR-mEPSCs, leading to reduced activity of the kinase eEF2 and rapid increases in BDNF translation (Autry et al., 2011). Enhanced BDNF secretion in the hippocampus by classical antidepressants and ketamine is likely a critical factor in the improvement of depression symptoms (Autry et al., 2011; Banasr et al., 2011, but see Lindholm et al., 2012). Following injection of 5.0 mg/kg ketamine, we also observed reduced activity of eEF2 kinase in male rats. Decreased eEF2 phosphorylation was not seen at 2.5 mg/kg ketamine in male rats, or at any ketamine dose in female rats. The sex difference in eEF2 kinase phosphorylation

strongly supports an alternative molecular mechanism responsible for the increased sensitivity and antidepressant effects of ketamine in female rats. Alterations in AMPA receptor function have recently been implicated in the antidepressant-like effects of ketamine in male mice (Maeng et al., 2008), thus future studies examining the potential effects of enhanced AMPA throughput in critical neuronal circuits in female rodents may further elucidate sex differences in the rapid antidepressant-like effects of ketamine.

Our study has shown that acute administration of ketamine had antidepressant-like, but not anxiolytic effects in several behavioral tests. A previous study has shown that low dose ketamine injection increased anxiety-like behaviors in several behavioral tests (Silvestre et al., 1997), however individually housed Wistar rats were used prior to drug administration. Our group has recently shown (Carrier and Kabbaj, 2012b) that two weeks of chronic social isolation is sufficient to induce anxiety and depressive-like behaviors compared to pair-housed controls. The difference in the

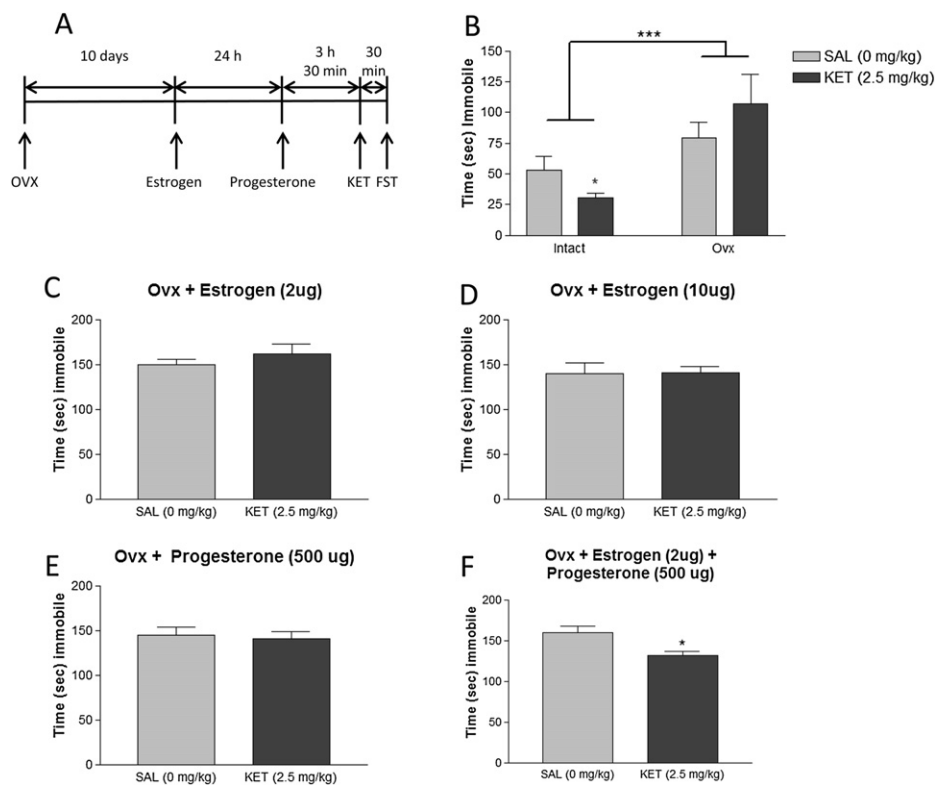


Fig. 3. Estrogen and progesterone are required for the antidepressant response to low dose ketamine in female rats. A) Timeline of estrogen and progesterone replacements. B) Intact female rats spent less time immobile compared to OVX female rats. Injection of 2.5 mg/kg ketamine reduced immobility time in intact, but not OVX female rats. Injection of 2.5 mg/kg ketamine was without effect in OVX female rats receiving (B) estrogen (2 µg), (C) estrogen (10 µg), or (D) progesterone. Injection of 2.5 mg/kg ketamine reduced immobility time in OVX female rats receiving (E) estrogen (2 µg) and progesterone compared with OVX female rats injected with 0 mg/kg ketamine. *** $p < 0.0001$; * $p < 0.05$.

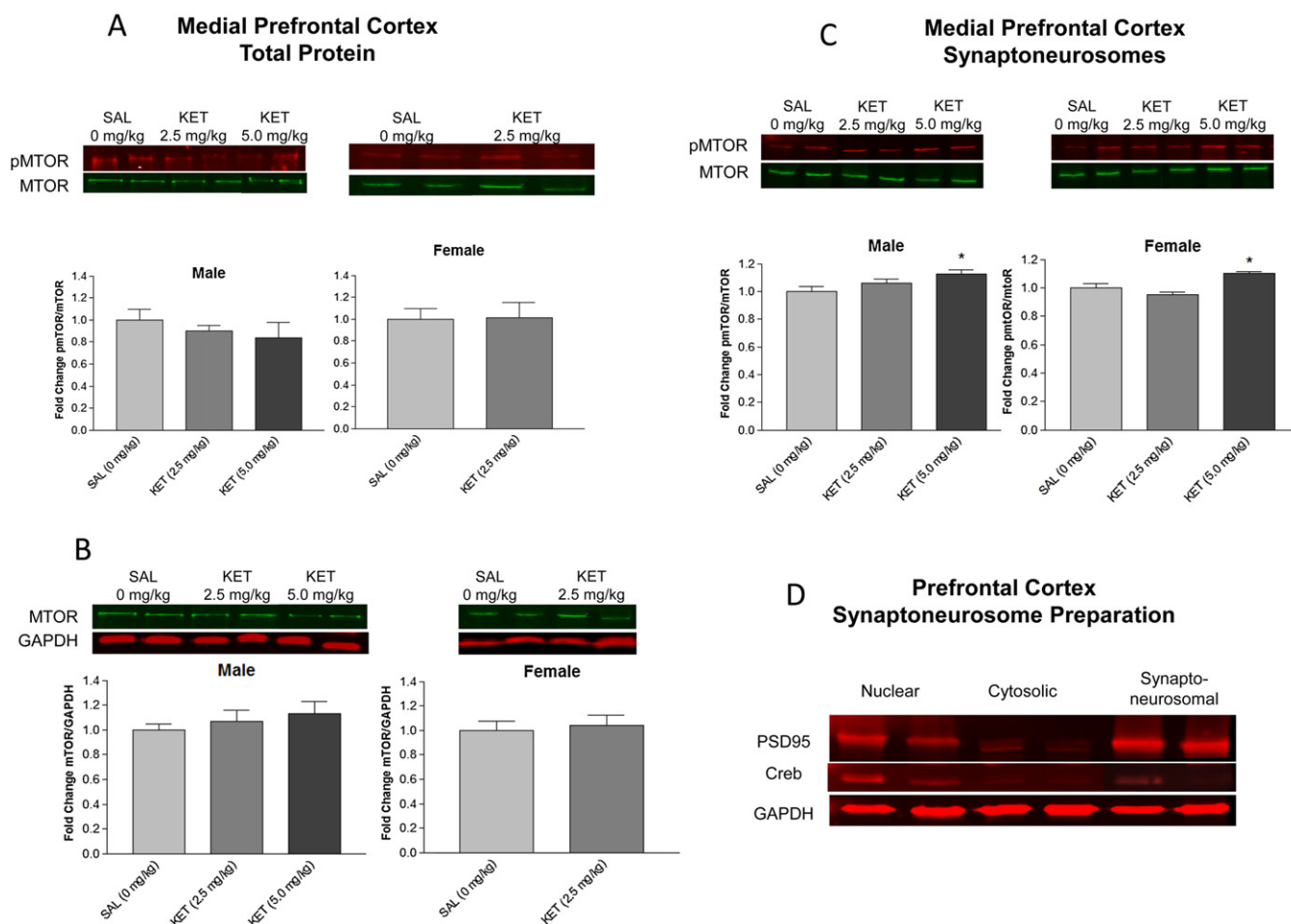


Fig. 4. Activation of mTOR in the medial prefrontal cortex. Injection of 0, 2.5, or 5.0 mg/kg ketamine in male rats, or 0 and 2.5 mg/kg in female rats had no effect on (A) phosphorylated mTOR or (B) total mTOR in total protein preparations from the medial prefrontal cortex. (C) Injection of 5.0 mg/kg ketamine increased phosphorylated mTOR in synaptoneurosomes protein preparations from the medial prefrontal cortex in both male and female rats. (D) The synaptoneurosomal fraction was concentrated in post synaptic density proteins such as PSD-95. * $p < 0.01$.

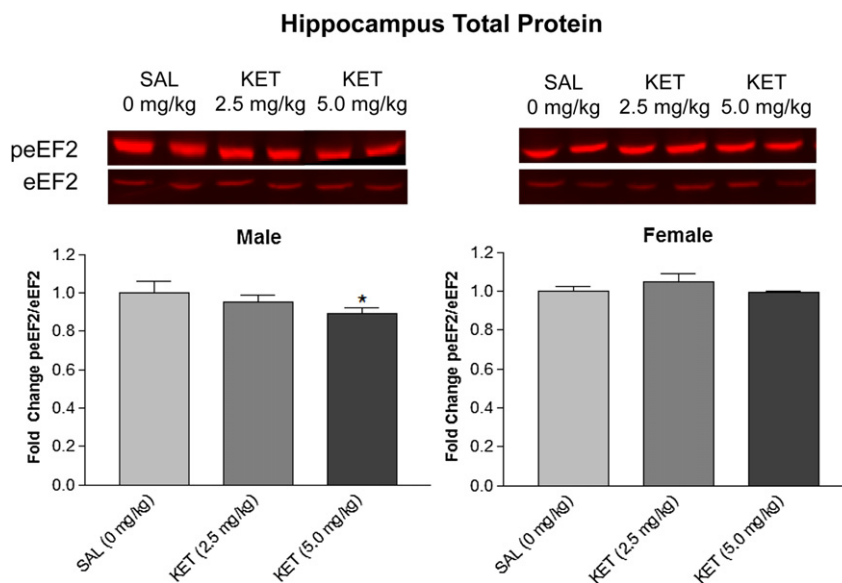


Fig. 5. Sex differences in phosphorylation of eEF2 following acute ketamine exposure. Injection of 5.0 mg/kg ketamine reduced phosphorylated eEF2 in male but not female rats. * $p < 0.05$.

social component of housing conditions between these two studies likely explains this discrepancy. Interestingly, in the present study, the antidepressant-like effects of ketamine were without effect in female rats. Intact control female rats consumed greater than 95% sucrose creating a ceiling effect such that increased sucrose preference with ketamine treatment was undetectable.

The antidepressant-like response at 2.5 mg/kg ketamine is abolished in OVX female rats. Only when OVX female rats receive physiological replacements of both estrogen and progesterone that mimic the 4-day estrous cycle in intact female rats, is the 2.5 mg/kg injection effective at eliciting an antidepressant-like response. Interestingly, estrogen and progesterone have many similarities with classical antidepressants and could be good candidates for enhancing ketamine's effects in female rats – and maybe in male rats as well. Estrogen and progesterone are released from the ovaries in a sequential manner and interact to induce neurophysiological changes (Fink, 1988; Mani et al., 1994). In fact, synergistic actions between estrogen and progesterone are essential in behavioral and molecular paradigms. Indeed, progestins and estrogens act through steroid–receptor complexes within the ventromedial hypothalamus (Molenda et al., 2002), and through non-genomic, membrane mediated mechanisms within the ventral tegmental area (Frye et al., 2006) to facilitate lordosis in female rodents. Interestingly, progesterone has also been shown to work synergistically with estrogen to modulate dopamine (D₅) receptor expression in hypothalamic neurons – possibly protecting against the onset and incidence of psychosis in schizophrenia (Lee et al., 2001). While the antidepressant-like effects of ketamine are not affected by progesterone alone, our data suggest that progesterone may enhance the effect of estrogen to increase sensitivity in female rodents.

The mechanism whereby estrogen and progesterone may potentiate the antidepressant-like effects of ketamine is unknown. However, it is reasonable to suggest that they could in fact be acting synergistically on the same molecular pathways targeted by ketamine (like BDNF) or could be targeting additional molecular pathways not influenced by ketamine; as such the outcome would be an enhanced physiological response. Enhanced BDNF secretion in the medial prefrontal cortex could be a major mechanism by which classical antidepressants and ketamine exert their effects (Duman and Monteggia, 2006; Krishnan and Nestler, 2008; Li et al., 2010, but see Lindholm et al., 2012). So far, and to the best of our knowledge, there are no studies that examined if BDNF translation in the medial prefrontal cortex is affected by estrogen and progesterone. But, in female rats, dendritic spines density in the medial prefrontal cortex is greater during proestrus (when estrogen is high) when compared to diestrus (when estrogen is low) (Chen et al., 2009). Also, the reduced density of dendritic spines and decreased PSD-95 following OVX was restored to normal by estrogen treatment (Chen et al., 2009; Wallace et al., 2006).

5. Conclusions

We have demonstrated that female rats exhibit an antidepressant-like response following injection of 2.5 mg/kg ketamine and that this response at the same dose is absent in male rats. The greater sensitivity to 2.5 mg/kg ketamine is completely abolished in OVX female rats and restored with physiological levels of both estrogen and progesterone administered in a cyclic manner to mimic the four-day estrous cycle in intact female rats. Injection of 5.0 mg/kg ketamine resulted in increased phosphorylation of mTOR within synaptoneurosome, but not total protein preparations from the prefrontal cortex in both male and female rats. Additionally, within the hippocampus, 5.0 mg/kg ketamine induced reductions of eEF2 kinase activation likely mediating increased

BDNF translation; however this effect was completely absent in female rats. We conclude that the antidepressant-like behavioral effects seen in female rats following low dose ketamine administration are mediated by an alternative mechanism to that observed in male rats. Future studies are underway to elucidate the molecular pathway(s) and brain sites mediating increased sensitivity of females to ketamine.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.neuropharm.2012.12.009>.

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