Ketamine reverses chronic stress-induced behavioral changes via Ca^{2+}-permeable AMPA receptors in mice

Authors: Joshua C. Flowers[1*], Paige E. Vetter[1*], McKennon J. Wiles[1*], Seung Hyun Roh[2*], Ellison R. Black[2*], Evelina Bouckova[1,3*], Madison H. Wustrau[2*], Rahmi Lee[2], Sang-Hun Lee[2], Seonil Kim[1,2]

[1]Molecular, Cellular and Integrative Neurosciences Program,

[2]Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523, USA

[3]Integrated Biomedical Sciences (IBS) program, The George Washington University, Washington, DC 20037, USA

*These authors contributed equally.

Corresponding author email: seonil.kim@colostate.edu

Conflict of interest statement: The authors declare no competing financial interests.

Abstract

Background and Purpose: Chronic stress affects brain functions leading to the development of mental disorders like anxiety and depression, as well as cognitive decline and social dysfunction. Among many biological changes in chronically stressed brains, disruptions in AMPA Receptor (AMPAR)-mediated synaptic transmission in the hippocampus are associated with stress responses. We have revealed that low-dose ketamine rapidly induces the expression of GluA1-containing, GluA2-lacking Ca^{2+}-Permeable AMPARs (CP-AMPARs), which enhances glutamatergic synaptic strength in hippocampal neurons. Additionally, subanesthetic low-dose ketamine decreases anxiety- and depression-like behaviors in naïve animals. In addition to reducing depression, some research indicates that ketamine may have protective effects against chronic stress in both humans and animals. However, the role of CP-AMPARs in the actions of ketamine's antistress effects is largely unknown.

Experimental Approach: We use whole-cell patch-clamp recordings from CA1 pyramidal neurons in female and male hippocampal slices and multiple behavioral assays including reciprocal social interaction, contextual fear conditioning, and tail suspension test.

Key Results: We demonstrate that low-dose ketamine treatment reverses chronic restraint stress (CRS)-induced social dysfunction, hippocampus-dependent fear memory loss, and depression-like behavior in both female and male mice. Furthermore, we show that the ketamine-induced antistress effects on these behaviors are dependent on CP-AMPARs.

Conclusion and Implications: Our findings suggest that subanesthetic low-dose ketamine rapidly triggers synaptic insertion of CP-AMPARs in the hippocampus, which induces antidepressant and antistress effects.

Keywords

Ketamine

Chronic stress

Ca^{2+}-Permeable AMPA receptors

Behaviors

What is already known

- After demonstrating rapid and robust antidepressant efficacy, the US Food and Drug Administration (FDA) approved esketamine (the S enantiomer form of ketamine) for the treatment of depression in 2019, sparking a surge in clinical and public interest around the world.
- Although the exact mechanisms are largely unknown, one mechanism postulated for ketamine's actions involves the enhancement of excitatory synaptic drive through an increase in AMPA receptor (AMPAR)-mediated synaptic activity.

What this study adds

- This study discovers that a single dose of ketamine rapidly (~one hour) induces synaptic insertion of GluA1-containing, GluA2-lacking Ca^{2+}-Permeable AMPARs (CP-AMPARs), which is required for the antistress effects in animals.

Clinical significance

- Ketamine is typically given in just a single dose or short series of doses rather than repeated treatments because of potential misuse and addictive properties. Furthermore, rapid relapse of depression occurs after cessation of ketamine treatment (18 - 19 days post-infusion).
- Understanding the precise mechanisms behind ketamine's antidepressant effects could lead to the development of new clinical approaches to overcome its primary therapeutic limitation of restricted patient use.
- Unfortunately, the potential sex differences in response to ketamine have been particularly understudied at this time although the hypersensitivity of females to ketamine's effects at lower doses has been shown in several animals' studies. Therefore, our findings may help develop tailored treatment and dosing in clinics.

Introduction

The World Health Organization (WHO) estimates that over 300 million people worldwide have depression (4.4% of the global population), which makes depression the leading cause of disability worldwide. The US Food and Drug Administration (FDA) approved esketamine (the S enantiomer form of ketamine) for the treatment of depression in 2019, sparking a surge in clinical and public interest around the world (Kohtala, 2021). Although the antidepressant effects of ketamine become evident within a few hours or 1 day of a single infusion, the benefits generally disappear within 1 week (Newport et al., 2015). More recent studies have shown that repeated ketamine infusions somewhat sustain the effects (aan het Rot et al., 2010; Murrough et al., 2013; J. L. Phillips et al., 2019). However, relapse of depression still occurs after cessation of treatment (on average, 18–19 days post-treatment) (aan het Rot et al., 2010; Murrough et al., 2013). Moreover, because ketamine is associated with psychomimetic side effects and risk of addiction, it is typically given in just a single dose or short series of doses rather than repeated treatments (Ng, Tse, Ng, & Lau, 2010; Sepulveda Ramos et al., 2022; Silva & Proulx, 2024). Some patients receiving ketamine therapy are also advised to stay on other traditional antidepressants whilst receiving ketamine (Veraart et al., 2021). However, reports have shown that some patients have been inflicted with adverse effects mimicking psychosis following ketamine administration on top of their regular polydrug regimen (Correia-Melo, Silva, Araujo-de-Freitas, & Quarantini, 2017; Paul, Schaaff, Padberg, Moller, & Frodl, 2009; Szymkowicz, Finnegan, & Dale, 2013). It is unclear which medications (or set of medications) negatively interact with ketamine (Veraart et al., 2021). Additionally, ketamine is found to be effective in reducing suicidality in treatment-resistant depression patients (10-20% of the depression patients) (Serafini, Howland, Rovedi, Girardi, & Amore, 2014). For these patients, ketamine + other traditional antidepressants is likely ineffective. Given all these problems, the field requires the development of new strategies that will permit a reduction in the frequency of ketamine administration after response to acute treatment, with maintenance of its beneficial effects for its broader application (Papakostas, 2020; J. L. Phillips et al., 2019). Unfortunately, achieving these goals is considerably more challenging because the exact mechanisms behind ketamine's antidepressant effects are still unclear. Importantly, ketamine-induced neuroplasticity has been proposed as a mechanism underlying its antidepressant effects (Wu, Savalia, & Kwan, 2021). However, still unclear, is when and how neuroplasticity is induced. Specifically, molecular and cellular processes of how ketamine induces neuroplasticity remain to be elucidated.

One mechanism postulated for ketamine's rapid antidepressant actions involves the enhancement of excitatory synaptic drive in the hippocampus (Aleksandrova, Wang, & Phillips, 2020; Kavalali & Monteggia, 2020; Miller, Moran, & Hall, 2016). However, it has been demonstrated that ketamine is a noncompetitive NMDA receptor (NMDAR) antagonist that inhibits excitatory synaptic transmission (Anis, Berry, Burton, & Lodge, 1983). This poses a contradictory question of how ketamine enhances excitatory activity and synaptic Ca^{2+} signaling while blocking NMDARs. This can be explained by an increase in AMPA receptor (AMPAR)-mediated synaptic activity but the mechanisms are unknown (Aleksandrova, Phillips, & Wang, 2017). In addition to reducing depression, some research indicates that ketamine may have protective effects against chronic stress in both humans and animals (Brachman et al., 2016; Feder et al., 2014; Gill et al., 2021; E. H. Lee, Park, Kwon, & Han, 2021; J. H. Ma et al., 2019; L. Ma et al., 2022; Taylor et al., 2018; Y. Yang et al., 2018; Zhou et al., 2021). Chronic stress affects brain functions, resulting in the development of mental disorders like anxiety and depression, as well as cognitive decline and social dysfunction (daSilva et al., 2021; Kessler, 1997; Lupien, McEwen, Gunnar, & Heim, 2009). Among many biological changes in chronically stressed brains, disruptions in AMPAR-mediated synaptic transmission in the hippocampus are associated with stress responses in animal models and individuals with depression (He, Zhou, Wang, & Chen, 2023). Thus, recovery from AMPAR-mediated synaptic dysfunction may be the key target of ketamine's antistress and antidepressant effects.

There are two general types of AMPARs formed through combination of their subunits: GluA2-containing Ca^{2+}-impermeable AMPARs and GluA2-lacking, GluA1-containing Ca^{2+}-Permeable AMPARs (CP-AMPARs) (Diering & Huganir, 2018). Compared to Ca^{2+}-impermeable AMPARs, CP-AMPARs have higher single channel conductance, which contributes to synaptic plasticity (Aoto, Nam, Poon, Ting, & Chen, 2008; Goel & Lee, 2007; Goel et al., 2011; S. Kim, Violette, & Ziff, 2015; S. Kim & Ziff, 2014; H. K. Lee, 2012; Purkey & Dell'Acqua, 2020; Sanderson, Gorski, & Dell'Acqua, 2016; Sanderson, Scott, & Dell'Acqua, 2018; Thiagarajan, Lindskog, & Tsien, 2005). Both preclinical and clinical studies show that chronic stress significantly reduces GluA1 levels in hippocampal synapses, while there are conflicting results describing alterations in hippocampal GluA2 under chronic stress (He et al., 2023). These results suggest that the stress-induced decrease in synaptic GluA1 levels diminishes excitatory synaptic transmission in the hippocampus and exacerbates negative behaviors in chronic stress. Therefore, recovery from GluA1-mediated synaptic

dysfunction may be the key target of ketamine's antidepressant and antistress effects. We have revealed that low-dose ketamine (1 μM, a concentration that blocks approximately 50% of NMDAR-induced currents) (Hare et al., 2019) rapidly induces the expression of CP-AMPARs in cultured hippocampal neurons, which is mediated by increasing GluA1 phosphorylation and its surface expression (Zaytseva et al., 2023). Moreover, we have shown that this ketamine-induced CP-AMPAR expression enhances glutamatergic synaptic strength in cultured neurons (Zaytseva et al., 2023). Additionally, subanesthetic low-dose ketamine (5-10 mg/kg) decreases anxiety- and depression-like behaviors in naïve animals (Zaytseva et al., 2023). In fact, an increase in hippocampal activity is known to improve anxiety- and depression-like behaviors, fear memory, and social behavior in mice (Aleksandrova et al., 2020; Bird & Burgess, 2008; Grieco et al., 2022; Kavalali & Monteggia, 2020; Miller et al., 2016; Okuyama, Kitamura, Roy, Itohara, & Tonegawa, 2016; Sun et al., 2020; Tovote, Fadok, & Luthi, 2015). This suggests that ketamine-induced recovery from disruptions in AMPAR-mediated synaptic transmission and behaviors in stressed animals is likely associated with an increase in synaptic GluA1 expression in the hippocampus (Fischell, Van Dyke, Kvarta, LeGates, & Thompson, 2015; Kallarackal et al., 2013; Li et al., 2011).

Although we have shown that subanesthetic low-dose ketamine selectively increases GluA1 expression in the hippocampus and induces antidepressant effects via CP-AMPAR expression in naïve mice (Zaytseva et al., 2023), much research with hippocampal neural circuits is required to fully comprehend the ketamine's actions on chronic stress-induced behavioral alterations. Here, we use an electrophysiological approach in hippocampal slices to confirm that low-dose ketamine treatment indeed induces synaptic insertion of CP-AMPARs in hippocampal pyramidal neurons. We further show that ketamine at the low dosages reverses chronic restraint stress (CRS)-induced behavioral changes in mice, which is dependent on CP-AMPARs. Our findings thus suggest that subanesthetic low-dose ketamine rapidly triggers synaptic insertion of CP-AMPARs in the hippocampus, which in turn enhances synaptic strength and compensate for reduced NMDAR antagonism-mediated synaptic Ca^{2+} signaling to induce antidepressant and antistress effects.

Methods

Animals

C57Bl6J (Jax 000664) mice were obtained from Jackson laboratory and bred in the animal facility at Colorado State University (CSU). Animals were housed under a 12:12 hour light/dark cycle. To collect brain tissues from animals, mice were deeply anesthetized and euthanized by CO_2 asphyxiation. All efforts were made to minimize animal suffering. CSU's Institutional Animal Care and Use Committee (IACUC) reviewed and approved the animal care and protocol (3408). Results were reported following the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (Kilkenny et al., 2010).

Chronic Restraint stress (CRS)

3-month-old C57Bl6 female and male mice were individually placed into 50 mL polypropylene conical tubes with multiple holes for ventilation, and they were exposed to restraint stress (2 hr/day) for 14 consecutive days. After restraint stress, mice were returned to the home cage. Following 2-week CRS, drug injections, blood collection, and multiple behavior assays were carried out as shown in the experimental timeline in **Fig. 1**.

Drug treatment

Ketamine hydrochloride (VetOne, 510189) was used in both *in vitro* and *in vivo* experiments. 5 mg/kg and 10 mg/kg ketamine were intraperitoneally injected into 3-month-old female and male C57Bl6 mice, respectively (the conditions have been shown to exclusively increase hippocampal GluA1 and induce antidepressant effects on naïve animals' behaviors) (Zaytseva et al., 2023), and saline was administered to controls. We have approval from IACUC to use ketamine and have the United States Drug Enforcement Administration license to use ketamine for research purpose (DEA# RK0573863). 100 μM 1-Naphthyl acetyl spermine trihydrochloride (NASPM, Tocris Bioscience) was used to block CP-AMPARs in slice electrophysiology. 10 mg/kg IEM-1460 (Tocris Bioscience, 1636) was intraperitoneally injected into 3-month-old male and female C57Bl6J mice to inhibit *in vivo* CP-AMPAR activity because it is blood-brain barrier (BBB)-permeable (Adotevi et al., 2020; Szczurowska & Mares, 2015; Wiltgen et al., 2010). Following CRS, we

performed the experiments in the presence or absence of IEM-1460 and compared outcomes to examine whether ketamine treatment reversed the CRS-induced effects via CP-AMPARs.

Brain slice preparation and electrophysiology

The mice were deeply anesthetized with isoflurane, and their brains were removed. The brains were submerged in cold, oxygenated (95% O_2 and 5% CO_2) slicing medium containing (in mM): 85 NaCl, 1.25 NaH_2PO_4, 4 $MgCl_2$, 0.5 $CaCl_2$, 24 $NaHCO_3$, 2.5 KCl, 75 sucrose, and 25 glucose. Horizontal hippocampal slices (350 μm thick) were cut using a vibratome (Leica VT 1000S; Leica Microsystems). The solution was continuously oxygenated with 95% O_2 and 5% CO_2. Slices were initially maintained at 33°C for 30 min, then at room temperature (22 ± 1°C) until they were used for electrophysiological recordings. Whole-cell patch-clamp recording procedures used in the present study were like those described previously (Govindaiah et al., 2018; Kang et al., 2020; Kang et al., 2018). Electrophysiological recordings were performed on CA1 pyramidal cells at 33°C in oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 2.5 KCl, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, and 10 glucose. Slices were superfused with ACSF at a rate of 2.0 ml/min. Individual neurons were visualized using an upright microscope (Olympus BX51WI) with infrared differential interference contrast (DIC) optics. CA1 pyramidal cells in the stratum pyramidale (the pyramidal cell layer), visualized using differential interference contrast microscopy, will be selected based on their morphology as described previously (Kang et al., 2020). Whole-cell patch-clamp recordings were obtained from CA1 pyramidal cells with borosilicate patch pipettes (2–4 MΩ) filled with an internal solution containing (in mM): 126 K-gluconate, 4 KCl, 10 HEPES, 4 ATP-Mg, 0.3 GTP-Na, and 10 phosphocreatine. The pH and osmolarity of the internal solution were adjusted to 7.2 and 290 mOsm, respectively. Recordings were obtained using a MultiClamp 700B amplifier (Molecular Devices). After forming the whole-cell configuration, the recording was allowed to rest for at least 5 min prior to data acquisition. Signals were sampled at 10 kHz, low-pass filtered at 3 kHz using Digidata 1440A analog-to-digital digitizer (Molecular Devices) and stored on computer for subsequent analysis using pClamp 10 software (Molecular Devices). Series resistances were continuously monitored with recordings being discarded if the series resistance increased >20%. We then measured glutamate uncaging-induced excitatory postsynaptic currents (uEPSCs) in hippocampal CA1 neurons one hour following a single dose of ketamine (5 mg/kg for females and 10 mg/kg for males). For

glutamate uncaging, 100 μM MNI-caged L-glutamate was added to ACSF, and epi-illumination photolysis (390 nm, 0.12 mW/mm^2, 50 ms) was applied near the soma of a neuron. 1 μM TTX was added to ACSF for preventing action potential-dependent network activity. The average of uEPSC amplitudes (V_{hold} = -70 and +60 mV) in CA1 pyramidal cells was compared between saline and ketamine treatment. To examine whether CP-AMPARs played a role in uEPSCs, we recorded uEPSCs with a bath application of 100 μM NASPM, a CP-AMPAR blocker. Additionally, we added saline to ACSF as a control for NASPM treatment. Each recording was performed in different slices. We then analyzed the amplitudes of uEPSCs in each condition using Clampfit 10.7 (Molecular Devices).

Reciprocal social interaction

We performed the reciprocal social interaction test to examine social interaction between two freely moving mice (Bolivar, Walters, & Phoenix, 2007; Matsuo et al., 2009). A test mouse (3-month-old) and a stranger mouse of identical genotypes that were previously housed in different cages were placed into the chamber (40 W x 40 L x 40 H cm) and allowed to explore freely for 20 min. Social behavior was recorded using a camera mounted overhead. Social contacts were determined by the sniffing time that was defined as each instance in which a test mouse's nose came within 2 cm toward a stranger mouse. The total number of reciprocal interactions and total duration of reciprocal interactions were measured manually. Animals that escaped the chamber or showed aggressive behaviors (e.g. attacking a stranger mouse) were excluded from the analysis.

Open field test (OFT)

We measured locomotor activity and anxiety-like behavior using OFT as carried out previously (Kang et al., 2021; Shou, Tran, Snyder, Bleem, & Kim, 2018; Zaytseva et al., 2023). A test mouse was first placed in the center of the open field chamber (40 W x 40 L x 40 H cm) for 5 min. Animals were then allowed to explore the chamber for 20 min. The behavior was recorded by a video camera. A 20 x 20 cm center square was defined as the inside zone. Data were analyzed using the ANY-maze tracking program (Stoelting Co.) to acquire total traveled distance (locomotor activity) and time spent outside and inside (anxiety-like behavior). Animals that escaped the chamber or showed significantly decreased locomotor activity were excluded from the analysis.

Contextual fear conditioning (CFC)

CFC (Habitest Modular System, Coulbourn Instrument) was carried out as described previously (S. Kim et al., 2016; R. Lee, Kim, & Kim, 2024; Shou et al., 2018). On Day 1, a test mouse was placed in a novel rectangular chamber with a grid floor. After a 3-min baseline period, the test animal was given one shock (a 2 sec, 0.5 mA shock) and stayed in the chamber for an additional 1 min after the shock before being returned to the home cage overnight. A contextual memory test was conducted the next day (Day 2) in the same conditioning chamber for 3 min. Fear memory was determined by measuring the percentage of the freezing response (immobility excluding respiration and heartbeat) using an automated tracking program (FreezeFrame). Mice that did not freeze were excluded from the analysis.

Tail suspension test (TST)

It has been suggested that the forced swim test (FST) and TST are based on the same principle: measurement of the duration of immobility when rodents are exposed to an inescapable situation (Castagne, Moser, Roux, & Porsolt, 2011). Importantly, TST is known to be more sensitive to antidepressant agents than FST because the animals remain immobile longer in TST than FST (Cryan, Mombereau, & Vassout, 2005). We thus used TST to examine depression-like behavior as described previously (S. Kim et al., 2016; S. Kim, Shou, Abera, & Ziff, 2018; Zaytseva et al., 2023). A test mouse was suspended by its tails from a rod suspended 20 cm above the tabletop surface with adhesive tape placed 1 cm from the tip of the tail. Animals were immobile when they exhibited no body movement and hung passively for > 3 sec. The time during which mice remained immobile was quantified over a period of 6 min. The behavior was recorded by a video camera and, where data were analyzed using the ANY-maze tracking program to acquire immobility (depression-like behavior). Mice that successfully climbed their tails to escape were excluded from the analysis.

Corticosterone

Corticosterone levels in mice were measured as described previously (Luo et al., 2016; McCosh, Kreisman, Tian, Thomas, & Breen Church, 2024). 50 µl tail-tip whole blood samples were collected and centrifuged at 5,000 rpm for 10 min. 10 µl of serum was harvested and stored at −20°C until assay. 5 µl of

serum samples in each condition was assayed using the DetectX® Corticosterone Multi-Format Enzyme-linked immunosorbent assay (ELISA) kit (Arbor Assays, Inc. K014-H). To prevent possible artifacts during the ELISA experiment, we measured the corticosterone levels twice using the same sample.

Statistical analysis

All behavior tests were blindly scored by more than two investigators. The Franklin A. Graybill Statistical Laboratory at Colorado State University was consulted for statistical analysis in the current study, including sample size determination, randomization, experiment conception and design, data analysis, and interpretation. We used the GraphPad Prism 10 software to determine statistical significance (set at $p < 0.05$). Grouped results of single comparisons were tested for normality with the Shapiro-Wilk normality or Kolmogorov-Smirnov test and analyzed using an unpaired two-tailed Student's t-test when data are normally distributed. Differences between multiple groups were assessed by N-way analysis of variance (ANOVA) with the Tukey test. The graphs were presented as mean ± Standard Deviation (SD). We discard data points that are located further than two SD above or below the average as an outlier.

Results

Subanesthetic low-dose ketamine induces synaptic insertion of CP-AMPARs in hippocampal CA1 pyramidal neurons

The hippocampus is one of the key brain regions controlling cognition, motivation, and social behavior (Felix-Ortiz, Burgos-Robles, Bhagat, Leppla, & Tye, 2016; Hong & Kaang, 2022; W. B. Kim & Cho, 2020; M. L. Phillips, Robinson, & Pozzo-Miller, 2019; Schumacher, Vlassov, & Ito, 2016; Sun et al., 2020; Tao et al., 2022; X. Wang & Zhan, 2022; Watarai, Tao, Wang, & Okuyama, 2021; Yoshida et al., 2021). An increase in hippocampal activity reverses stress-induced memory impairment, social dysfunction, and mood disorder-associated behaviors (Aleksandrova et al., 2020; Bird & Burgess, 2008; Grieco et al., 2022; Kavalali & Monteggia, 2020; Miller et al., 2016; Okuyama et al., 2016; Su, Lu, Fu, Geng, & Chen, 2023; Sun et al., 2020; Tovote et al., 2015). More importantly, the hippocampus is preferably targeted by ketamine at the low doses (P. A. Davoudian, Shao, & Kwan, 2023). This suggests that the hippocampus is the primary brain region to be responsible for ketamine's antistress effects. In fact, we have discovered that subanesthetic low-dose ketamine significantly increases synaptic GluA1 but not GluA2, an indication of CP-AMPAR expression, in the hippocampus of naïve animals (Zaytseva et al., 2023). We thus decided to examine whether low-dose ketamine was able to induce synaptic insertion of CP-AMPARs in hippocampal neurons using whole-cell patch clamping with glutamate uncaging. We intraperitoneally injected 5 mg/kg and 10 mg/kg ketamine to female and male C57Bl6 mice, respectively, and saline was administered to controls. These ketamine concentrations were shown to exclusively increase hippocampal GluA1 and induce antidepressant effects on naïve animals' behaviors (Zaytseva et al., 2023). One hour following injection, hippocampal slices were obtained, and we then measured the amplitudes of glutamate uncaging-induced excitatory postsynaptic currents (uEPSCs) (V_{hold} = -70 and +60 mV) in CA1 pyramidal cells. We found that ketamine injection significantly elevated the amplitudes of uEPSCs (V_{hold} = -70 mV) in these neurons when compared to control cells (CTRL) (FEMALE: CTRL, 86.330 ± 23.107 pA and Ketamine, 113.208 ± 30.563 pA, p = 0.0367. MALE: CTRL, 83.915 ± 27.768 pA and Ketamine, 109.329 ± 28.293 pA, p = 0.0471) (**Fig. 2a and Table 1**). However, when ketamine had no effect on uEPSCs (V_{hold} = +60 mV) (FEMALE: CTRL, 292.223 ± 97.252 pA and Ketamine, 360.238 ± 173.766 pA, p = 0.5017. MALE: CTRL, 286.115 ± 129.521 pA and Ketamine, 372.100 ± 126.540 pA, p = 0.2718) (**Fig. 2b and Table 2**). To examine whether CP-AMPARs were involved in the ketamine's effects on uEPSCs, we added

100 μM NASPM, a CP-AMPAR antagonist, to ACSF and measured the amplitudes of uEPSCs in CA1 pyramidal cells from saline- or ketamine-injected animals. We found that NASPM treatment significantly reduced the amplitudes of uEPSCs (V_{hold} = -70 and + 60 mV) following ketamine treatment (V_{hold} = -70 mV; FEMALE: Ketamine + NASPM, 58.671 ± 22.786 pA, $p < 0.0001$. MALE: Ketamine + NASPM, 55.159 ± 26.805 pA, $p < 0.0001$. V_{hold} = +60 mV; FEMALE: Ketamine + NASPM, 213.793 ± 111.514 pA, $p = 0.0170$. MALE: Ketamine + NASPM, 233.635 ± 130.302 pA, $p = 0.0133$) (**Fig. 2a-2b and Table 1-2**). However, NASPM had no effect on the amplitudes of uEPSCs (V_{hold} = -70 and + 60 mV) measured in slices from saline-injected mice (V_{hold} = -70 mV; FEMALE: NASPM, 61.702 ± 21.324 pA, $p = 0.0575$. MALE: NASPM, 77.500 ± 16.628 pA, $p = 0.9248$. V_{hold} = +60 mV; FEMALE: NASPM, 276.986 ± 97.353 pA, $p = 0.9885$. MALE: NASPM, 284.958 ± 123.642 pA, $p > 0.9999$) (**Fig. 2a-2b and Table 1-2**). These findings demonstrate that subanesthetic low-dose ketamine induces synaptic insertion of CP-AMPARs in hippocampal CA1 pyramidal neurons.

CRS-induced social dysfunction is reversed by ketamine via CP-AMPARs.

Repeated stress can induce the development of social dysfunction (daSilva et al., 2021). Importantly, it has been shown that an increase in hippocampal activity reverses stress-induced social dysfunction (Okuyama et al., 2016; Sun et al., 2020). We thus conducted a reciprocal social interaction test to examine whether chronic stress induced social dysfunction and if ketamine reversed this via CP-AMPARs. For chronic stress in mice, we used chronic restraint stress (CRS) because it has been widely used as a model of chronic psychoemotional stress to induce depression- and anxiety-like behaviors, learning and memory deficits, social dysfunction, and hippocampal neuronal damage in mice (Huang et al., 2015; Park, Seo, Lee, Shin, & Kang, 2018; Yun et al., 2010). After restraint stress, mice were returned to the home cage. Following 2-week CRS, we conducted the reciprocal social interaction test was carried out as shown in the experimental timeline in **Fig. 1**. First, we found that CRS significantly decreased the total number of reciprocal interactions (FEMALE: Naïve, 32.67 ± 8.41 and CRS, 16.83 ± 4.31, $p = 0.0425$. MALE: Naïve, 33.00 ± 10.94 and CRS, 16.77 ± 7.18, $p = 0.0028$) and total time of reciprocal interactions (FEMALE: Naïve, 16.28 ± 3.55 sec and CRS, 7.37 ± 4.83 sec, $p = 0.0315$. MALE: Naïve, 19.03 ± 6.56 sec and CRS, 9.02 ± 2.22 sec, $p = 0.0026$) in both female and male mice when compared to Naïve controls, an indication of social dysfunction (**Fig. 3 and Table 3-4**). This

suggests that CRS significantly disrupted social behavior in animals. We then injected low-dose ketamine to CRS animals and examined whether ketamine reversed CRS-induced social dysfunction one hour after the treatment. We discovered that ketamine markedly increased the total number of reciprocal interactions (FEMALE: CRS + Ket, 44.29 ± 12.41, $p < 0.0001$. MALE: CRS + Ket, 33.25 ± 11.83, $p = 0.0001$) and total time of reciprocal interactions (FEMALE: CRS + Ket, 23.09 ± 6.29 sec, $p < 0.0001$. MALE: CRS + Ket, 17.71 ± 8.13 sec, $p = 0.0009$) in both female and male mice (**Fig. 3 and Table 3-4**). This suggests that subanesthetic low-dose ketamine can restore normal social behavior in chronically stressed animals. Finally, we tested whether these ketamine's effects on social behavior were mediated by CP-AMPARs, we treated IEM-1460 (IEM), a BBB-permeable CP-AMPAR antagonist, with low-dose ketamine in CRS mice and carried out the social behavior test. We revealed that IEM treatment significantly reduced the total number of reciprocal interactions (FEMALE: CRS + Ket + IEM, 19.23 ± 9.08, $p < 0.0001$. MALE: CRS + Ket + IEM, 16.61 ± 7.33, $p < 0.0001$) and total time of reciprocal interactions (FEMALE: CRS + Ket + IEM, 11.60 ± 5.03 sec, $p < 0.0001$. MALE: CRS + Ket + IEM, 12.04 ± 4.38 sec, $p = 0.0286$) in both female and male mice when compared to ketamine-treated CRS mice (**Fig. 3 and Table 3-4**). These findings demonstrate that CRS impairs animals' social behavior, which is reversed by low-dose ketamine treatment, and the ketamine's effects are mediated by CP-AMPARs.

CRS-induced fear memory loss is reversed by ketamine via CP-AMPARs.

Repeated stress can impair learning and memory (E. J. Kim & Kim, 2023). In fact, it has been shown that CRS disrupts hippocampus-dependent fear memory in mice (Yun et al., 2010). We thus examined whether low-dose ketamine reversed CRS-induced hippocampus-dependent fear memory loss via CP-AMPARs. To measure hippocampus-dependent fear memory, CFC was used (Tovote et al., 2015). As shown previously (Yun et al., 2010), we found that freezing was significantly lower in CRS female and male animals compared to naïve controls, an indication of fear memory loss (FEMALE: Naïve, 33.55 ± 11.50% and CRS, 15.24 ± 5.64%, $p = 0.0026$. MALE: Naïve, 26.45 ± 11.14% and CRS, 16.35 ± 7.64%, $p = 0.0133$) (**Fig. 4 and Table 5**). Next, low-dose ketamine was intraperitoneally injected to CRS female and male mice, and we carried out CFC to address the ketamine's effects on fear memory. We discovered that ketamine treatment significantly increased

freezing in CRS female and male mice (FEMALE: CRS + Ket, 28.16 ± 13.92%, p = 0.0454. MALE: CRS + Ket, 27.79 ± 11.50%, p = 0.0021) (**Fig. 4 and Table 5**). This suggests that subanesthetic low-dose ketamine can restore normal hippocampus-dependent fear memory in chronically stressed animals. To address that this ketamine's effect was mediated by CP-AMPARs, we treated ketamine and IEM together in CRS mice. We revealed that blocking CP-AMPARs markedly reduced freezing in ketamine-treated CRS female and male animals compared to ketamine-treated CRS mice (FEMALE: CRS + Ket + IEM, 11.65 ± 4.45%, p = 0.0092. MALE: CRS + Ket + IEM, 19.06 ± 5.34%, p = 0.0322) (**Fig. 4 and Table 5**). This data suggest that CRS disrupts hippocampus-dependent fear memory in animals, which is reversed by low-dose ketamine treatment, and the ketamine's effect is dependent on CP-AMPARs.

CRS-induced depression-like behavior is reversed by ketamine via CP-AMPARs.

Repeated stress is a known trigger for depression in humans and depression-like behavior in animals (Richter-Levin & Xu, 2018; Tafet & Nemeroff, 2016). Indeed, CRS is known to induce depression-like behavior in rodents (Mao, Xu, & Yuan, 2022). Moreover, disruptions in AMPAR-mediated synaptic transmission in the hippocampus are associated with stress responses in animal models and individuals with depression (He et al., 2023). We thus employed TST to examine whether low-dose ketamine reversed CRS-induced depression-like behavior in mice via CP-AMPARs. As shown previously (Mao et al., 2022), CRS significantly elevated immobility in female and male mice when compared to Naïve controls, an indication of depression-like behavior (FEMALE: Naïve, 97.07 ± 42.05 sec and CRS, 168.80 ± 40.18 sec, p = 0.0130. MALE: Naïve, 78.80 ± 29.96 sec and CRS, 139.10 ± 36.04 sec, p = 0.0016) (**Fig. 5 and Table 6**). Importantly, low-dose ketamine treatment markedly decreased immobility in CRS female and male animals, an indication of antidepressant effects (FEMALE: CRS + Ket, 79.43 ± 35.91 sec, p = 0.0006. MALE: CRS + Ket, 90.25 ± 37.03 sec, p = 0.0066) (**Fig. 5 and Table 6**). We further discovered that immobility in IEM and ketamine-treated CRS mice was significantly higher than that ketamine-treated CRS mice (FEMALE: CRS + Ket + IEM, 131.50 ± 61.21 sec, p = 0.0472. MALE: CRS + Ket + IEM, 128.30 ± 31.56 sec, p = 0.0400) (**Fig. 5 and Table 6**). The data show that CRS induces depression-like behavior in animals, which is reversed by low-dose ketamine treatment via CP-AMPARs.

No abnormality in the open field test

As animals' locomotor capacities can influence behavior, we carried out OFT to examine whether there were any abnormalities in animals' locomotion in each condition. We found that there was no difference in total distance traveled, total time spent outside, and total time spent inside between the conditions (**Fig. 6 and Table 7**). Moreover, as time spent outside and inside can be interpreted as anxiety-like behavior (Zaytseva et al., 2023), normal behaviors in OFT indicate that animals used in our study had normal locomotor activity and no anxiety-like behavior. These findings suggest that behavioral alterations we described in the above are not due to changes in animals' movement and anxiety levels.

Ketamine reverses the CRS-induced elevation of corticosterone levels, which is not dependent on CP-AMPARs.

Chronic stress activates the hypothalamic-pituitary-adrenal axis, resulting in the release of glucocorticoid class stress hormones (McEwen, 2007; L. J. Phillips et al., 2006). Cortisol is the primary endogenous adrenal steroid in most mammals, including humans, whereas corticosterone is the primary adrenal corticosteroid in rodents (Raff, Sharma, & Nieman, 2014; Raff, Skelton, & Cowley, 1989; Usa et al., 2007; Yu et al., 2015). Chronic stress can keep these hormone levels high, which in turn alters brain functions (Burke, Sotiropoulos, & Waites, 2024; McEwen, 2007). In fact, CRS is known to increase corticosterone levels in animals, which is associated with stress-induced behavioral changes (Marin, Cruz, & Planeta, 2007). We thus examined whether low-dose ketamine reversed the CRS-induced elevation of corticosterone levels via CP-AMPARs. As shown previously (Tan, Wang, Chen, Long, & Zou, 2017), we found that CRS significantly increased serum corticosterone levels in both female and male animals when compared to naïve mice (FEMALE: Naïve, 831.1 ± 376.8 pg/ml and CRS, 2374 ± 1101 pg/ml, $p = 0.0001$. MALE: Naïve, 756.1 ± 210.6 pg/ml and CRS, 2140 ± 721.1 pg/ml, $p = 0.0005$) (**Fig. 7 and Table 8**). Ketamine is known to significantly decrease the CRS-induced increase in serum corticosterone levels in mice (Tan et al., 2017). Consistent with this finding, we discovered that low-dose ketamine significantly reduced serum corticosterone levels in CRS animals (FEMALE: CRS + Ket, 834.3 ± 372.0 pg/ml, $p = 0.0001$. MALE: CRS + Ket, 1089 ± 851.3 pg/ml, $p = 0.0146$) (**Fig. 7 and Table 8**). Finally, we tested whether the ketamine's effects on corticosterone levels were

mediated by CP-AMPARs. In contrast to behavioral assays, serum corticosterone levels of ketamine-treated CRS animals did not differ in the presence or absence of IEM (FEMALE: CRS + Ket + IEM, 1095 ± 481.9 pg/ml, $p = 0.9247$. MALE: CRS + Ket + IEM, 1082 ± 383.5 pg/ml, $p > 0.9999$) (**Fig. 7 and Table 8**). The data show that low-dose ketamine treatment reverses the CRS-induced increase in serum corticosterone levels, which is independent of CP-AMPARs.

Discussion and Conclusions

Ketamine's unknown mechanisms

It is challenging to understand how ketamine works due to its complex dose-dependent effects, with multiple pharmacologically active metabolites, and various mechanisms associated with the facilitation of synaptic plasticity (Kohtala, 2021). Importantly, the subanesthetic low-dose ketamine-induced increase of glutamatergic synaptic strength is presumed to underlie antidepressant effects in humans and animals (Aleksandrova et al., 2020; Kavalali & Monteggia, 2020; Miller et al., 2016). These actions of ketamine are likely mediated by enhancing AMPAR activity due to its nature of NMDAR antagonism (Aleksandrova et al., 2017). However, the entire role of AMPARs in the actions of ketamine remains undetermined. Additionally, treatment with ketamine is associated with psychomimetic side effects and risk of addiction, which prevent its broader application (Ng et al., 2010). Therefore, it is critical to identify the mechanisms of ketamine's effects, particularly on glutamatergic synapses, to guide the development of safer fast-acting therapy.

The role of CP-AMPARs in ketamine-induced antistress effects

Disruptions of AMPAR-mediated synaptic transmission in diverse cell types in the hippocampus are one of major biological changes in chronically stressed brains of animal models and individuals with depression (He et al., 2023). Importantly, recovery from GluA1-mediated synaptic dysfunction is thought to be the key target of ketamine's antidepressant and antistress effects. Moreover, ketamine-induced recovery from disruptions in AMPAR-mediated synaptic transmission and behaviors in stressed animals is suggested to be associated with an increase in synaptic GluA1 expression in the hippocampus (Fischell et al., 2015; Li et al., 2011). In fact, we have discovered that low-dose ketamine rapidly induces the expression of CP-AMPARs in cultured hippocampal neurons, which is mediated by increasing GluA1 phosphorylation and its surface expression through a reduction of neuronal Ca^{2+} and Ca^{2+}-dependent phosphatase calcineurin (CaN) activity (Zaytseva et al., 2023) (**Fig. 8**). In particular, GluA1-containing CP-AMPARs have higher single channel conductance, which contributes to synaptic plasticity (Aoto et al., 2008; Goel & Lee, 2007; Goel et al., 2011; S. Kim et al., 2015; S. Kim & Ziff, 2014; H. K. Lee, 2012; Purkey & Dell'Acqua, 2020; Sanderson et al., 2016; Sanderson et al., 2018; Thiagarajan et al., 2005). We have indeed shown that ketamine-induced CP-AMPAR expression enhances glutamatergic synaptic strength and chemically-induced long-term potentiation (cLTP) in

cultured neurons (Zaytseva et al., 2023). cLTP is shown to rely on a rise in postsynaptic Ca^{2+} concentration, specifically through the activation of NMDARs (Molnar, 2011). Therefore, when ketamine is treated, CP-AMPAR-mediated Ca^{2+} influx may replace NMDAR-dependent Ca^{2+} signaling. We further show that subanesthetic low-dose ketamine decreases anxiety- and depression-like behaviors in naïve animals (Zaytseva et al., 2023). Importantly, an increase in hippocampal activity is known to improve stress-induced negative behaviors in mice (Aleksandrova et al., 2020; Bird & Burgess, 2008; Grieco et al., 2022; Kavalali & Monteggia, 2020; Miller et al., 2016; Okuyama et al., 2016; Sun et al., 2020; Tovote et al., 2015), which further supports our findings. However, the mechanisms of ketamine's protective effects against stress-induced disorders are not completely understood. Here, our electrophysiological data demonstrate that low-dose ketamine induces synaptic insertion of CP-AMPARs in pyramidal neurons of the hippocampus CA1 area. Additionally, the current study reveals that subanesthetic low-dose ketamine can rapidly reverse social dysfunction, fear memory loss, and depression-like behavior caused by CRS in mice via CP-AMPARs (**Fig.8**). This suggests that low-dose ketamine induces synaptic insertion of CP-AMPARs in the hippocampus, which in turn enhances synaptic strength to reverse hippocampus-dependent behavioral dysfunctions in chronically stressed animals.

The ketamine's rapid onset and long-lasting effects

Given its brief half-life (3 hours in humans and 13 min in mice), the ketamine's rapid onset and long-lasting effects (which persist for days in humans and 24 hours in mice) are not fully understood by synaptic mechanisms (Silva & Proulx, 2024). One potential mechanism underlying the ketamine's rapid onset and long-lasting effects is that ketamine induces neural plasticity (Wu et al., 2021). However, still unclear, is when and how the plasticity is induced. Specifically, molecular and cellular processes of how ketamine induces neural plasticity remain to be elucidated. In addition to strengthening excitatory synaptic drive, low-dose ketamine can enhance neuronal Ca^{2+} signaling in the hippocampus (Zucker, 1999), possibly contributing to the ketamine's rapid onset and long-lasting effects. Although the relationship between ketamine-triggered alteration in Ca^{2+} signaling and functional consequences for downstream processes regulating key aspects of neuronal function is known to be important for the antidepressant and antistress effects (Lisek, Zylinska, & Boczek, 2020), how ketamine enhances synaptic Ca^{2+} signaling is not fully understood. Given that CP-AMPARs are known to

contribute to various forms of synaptic plasticity (Aoto et al., 2008; Cull-Candy & Farrant, 2021; Goel & Lee, 2007; Goel et al., 2011; Guire, Oh, Soderling, & Derkach, 2008; S. Kim et al., 2015; S. Kim & Ziff, 2014; H. K. Lee, 2012; Man, 2011; Purkey & Dell'Acqua, 2020; Sanderson et al., 2016; Sanderson et al., 2018; Thiagarajan et al., 2005), CP-AMPAR-mediated neural plasticity can facilitate the ketamine's effects. Therefore, our findings in which low-dose ketamine rapidly induces hippocampal CP-AMPARs can be significant because ketamine can enhance not only synaptic transmission but also synaptic Ca^{2+} signaling in response to partial antagonism of NMDARs (**Fig. 8**). Therefore, the ketamine-induced synaptic insertion of CP-AMPARs can increase glutamatergic activity and compensate for reduced NMDAR inhibition-mediated synaptic Ca^{2+} signaling enabling the ketamine's rapid onset and long-lasting effects.

Sex differences in ketamine's effects

Our work shows an important finding: female mice have higher ketamine antidepressant responses than male mice, as seen by synaptic insertion of CP-AMPARs following a lower ketamine dosage, which induces the antistress effects. Several studies including both male and female animals consistently demonstrate that females are more sensitive to ketamine (Carrier & Kabbaj, 2013; Dossat, Wright, Strong, & Kabbaj, 2018; Franceschelli, Sens, Herchick, Thelen, & Pitychoutis, 2015; Zanos et al., 2016). These studies collectively suggest that hormonal differences and metabolic factors contribute to the heightened sensitivity of females to ketamine's effects at lower doses. In addition, the distinct pharmacokinetics of ketamine in the animals' brain and plasma could be one reason for the increased ketamine's antistress reactions in female mice (Saland & Kabbaj, 2018). Over the course of three hours after treatment, female rats' medial prefrontal cortex and hippocampus contain higher levels of ketamine and norketamine, a metabolite of ketamine, than do male rats (Saland & Kabbaj, 2018). The study also shows that female rats' slower clearance rates and longer half-lives result in higher post-treatment effects of ketamine and its metabolites (Saland & Kabbaj, 2018). Unfortunately, the potential sex differences in response to ketamine have been particularly understudied at this time, which is an important area for the future investigation in order to develop tailored treatment and dosing in clinics.

Multiple mechanisms underlying ketamine's effects

The mechanisms underlying ketamine's antidepressant effects are not fully understood, although multiple ideas have been proposed. These hypotheses include *1)* direct NMDAR inhibition-mediated mechanism in excitatory neurons (Chen et al., 2024; Kavalali & Monteggia, 2020; S. Ma et al., 2023; Miller et al., 2016; Y. Yang et al., 2018; Zaytseva et al., 2023); *2)* NMDAR inhibition-independent, ketamine metabolite-dependent mechanism (Carrier & Kabbaj, 2013; Franceschelli et al., 2015; Zanos et al., 2016); and *3)* NMDAR inhibition-induced disinhibition- and BDNF-induced protein synthesis-dependent mechanism (Deyama & Duman, 2020; Gerhard et al., 2020). Low-dose MK-801, a NMDAR antagonist, has shown to improve depression-like behavior and working memory in mice (Wesierska, Duda, & Dockery, 2013; B. Yang, Ren, Ma, Chen, & Hashimoto, 2016). Conversely, others suggest that the metabolism of ketamine to (2R,6R)-hydroxynorketamine (HNK), devoid of NMDAR inhibition properties, is important for the antidepressant effect (Carrier & Kabbaj, 2013; Franceschelli et al., 2015; Zanos et al., 2016). Although multiple studies including our own support the NMDAR inhibition-mediated mechanisms, it is important to investigate how our current findings integrate or act independently from other described mechanisms.

Brain region-specific actions of ketamine

Research identifying the mechanisms of the antidepressant effects of ketamine has largely focused on the hippocampus, since it is known to be dysfunctional in depressive disorders (Carreno et al., 2016; Duman, Aghajanian, Sanacora, & Krystal, 2016; Moda-Sava et al., 2019; Zanos et al., 2016). More recently, other areas of the brain have been investigated, including the lateral habenula (Chen et al., 2024; S. Ma et al., 2023; Y. Yang et al., 2018). A recent study shows that in CRS animals, ketamine selectively inhibited NMDARs in lateral habenula neurons, but not in hippocampal pyramidal neurons. This suggests that the lateral habenula-NMDAR inhibition–dependent mechanism likely occurs more upstream in the cascade of ketamine signaling *in vivo* (Chen et al., 2024). In contrast, another recent study using a systematic and unbiased mapping approach that provides a comprehensive coverage of all brain regions discovers that ketamine preferably targets the hippocampus (Pasha A. Davoudian, Shao, & Kwan, 2022). The hippocampus is a crucial brain area that governs learning, memory, social interaction, and depression (Bird & Burgess, 2008; Campbell & Macqueen, 2004; Fortin, Agster, & Eichenbaum, 2002; Ghasemi, Navidhamidi, Rezaei, Azizikia, & Mehranfard, 2022; Jarrard, 1993; Xu et al., 2021). Research also demonstrates that an increase in hippocampal activity can be

protective against stress (Aleksandrova et al., 2020; Bird & Burgess, 2008; Grieco et al., 2022; Kavalali & Monteggia, 2020; Miller et al., 2016; Okuyama et al., 2016; Sun et al., 2020; Tovote et al., 2015). Importantly, one of the most vulnerable targets of stress is hippocampus, because it abundantly expresses both glucocorticoid and mineralocorticoid receptors (Smith, 1996; Uno, Tarara, Else, Suleman, & Sapolsky, 1989). Moreover, stress changes hippocampal neural activity and synaptic plasticity, activating hippocampal glucocorticoid receptors, and decreasing neuronal cell survival and neurogenesis (Egeland, Zunszain, & Pariante, 2015; J. J. Kim & Diamond, 2002; Ryu et al., 2016). This suggests that the hippocampus is the important brain region responsible for ketamine's effects. Since ketamine is delivered systemically in most studies, many regions in the brain can potentially be responsive to ketamine. Therefore, it is crucial to identify the crosstalk from the hippocampus to other brain regions including the lateral habenula for better understanding of the ketamine's therapeutic effects.

Potential role of ketamine's anti-inflammatory properties

Chronic stress is known to increase the release of corticosterone in animals (McEwen, 2007; L. J. Phillips et al., 2006). In fact, we discover that ketamine significantly reduces serum corticosterone levels in CRS mice, which is consistent with the previous finding (Tan et al., 2017). In contrast to ketamine-induced protective effects against CRS-induced behavioral changes, this effect is not dependent on CP-AMPAR expression. Interestingly, chronic stress can induce disorders through increased pre-inflammatory cytokines, reactive microglia numbers, and up-regulated regulatory molecules (Liu, Wang, & Jiang, 2017). Moreover, chronic inflammatory responses directly affect brain structure and function, resulting in pathological behaviors in animals (McEwen, Nasca, & Gray, 2016). Importantly, ketamine is known to be associated with anti-inflammatory effects in humans (Dale, Somogyi, Li, Sullivan, & Shavit, 2012; Nikkheslat, 2021) and animals (Ommati et al., 2023; Spencer et al., 2022; N. Wang et al., 2015), suggesting that its ability to reduce the inflammation may play a role in its effects on ketamine's protective effects against stress. Therefore, it is possible that the mechanism underlying the ketamine-induced decrease in stress hormones following chronic stress can be mediated by the hypothalamic-pituitary-adrenal axis rather than the hippocampus. It is thus possible that hypothalamic neurons may not express CP-AMPARs in response to ketamine treatment. In fact, there are multiple mechanisms proposed to explain the ketamine's anti-inflammatory effects, including the

involvement of the gut microbiome (Getachew et al., 2018; Zhang et al., 2017) and priming of Type 1 T helper (Th1)-type immune response (Ohta, Ohashi, & Fujino, 2009). However, clinical relevance of ketamine-induced inflammatory modulation is still unclear. Therefore, further studies are needed to identify the precise mechanisms of its anti-inflammatory properties.

Conclusions

The current study addresses how chronic stress and ketamine affect hippocampal synaptic activity and hippocampus-dependent behaviors, providing a new evidence-based idea that NMDAR inhibition by ketamine leads to the antistress effects through synaptic insertion of CP-AMPARs. Therefore, our work advances current understandings on the mechanisms of ketamine's antistress effects to guide the developments of new therapies.

Acknowledgments

We thank members of the Kim laboratory for their generous support. This work is supported by Student Experiential Learning Grants and College Research Council Shared Research Program from College of Veterinary Medicine and Biomedical Sciences, Colorado State University and a research grant from Korea Ginseng Corporation.

References

aan het Rot, M., Collins, K. A., Murrough, J. W., Perez, A. M., Reich, D. L., Charney, D. S., & Mathew, S. J. (2010). Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biol Psychiatry, 67*(2), 139-145. doi:10.1016/j.biopsych.2009.08.038

Adotevi, N., Lewczuk, E., Sun, H., Joshi, S., Dabrowska, N., Shan, S., … Kapur, J. (2020). alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid Receptor Plasticity Sustains Severe, Fatal Status Epilepticus. *Ann Neurol, 87*(1), 84-96. doi:10.1002/ana.25635

Aleksandrova, L. R., Phillips, A. G., & Wang, Y. T. (2017). Antidepressant effects of ketamine and the roles of AMPA glutamate receptors and other mechanisms beyond NMDA receptor antagonism. *J Psychiatry Neurosci, 42*(4), 222-229. doi:10.1503/jpn.160175

Aleksandrova, L. R., Wang, Y. T., & Phillips, A. G. (2020). Ketamine and its metabolite, (2R,6R)-HNK, restore hippocampal LTP and long-term spatial memory in the Wistar-Kyoto rat model of depression. *Mol Brain, 13*(1), 92. doi:10.1186/s13041-020-00627-z

Anis, N. A., Berry, S. C., Burton, N. R., & Lodge, D. (1983). The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. *Br J Pharmacol, 79*(2), 565-575. doi:10.1111/j.1476-5381.1983.tb11031.x

Aoto, J., Nam, C. I., Poon, M. M., Ting, P., & Chen, L. (2008). Synaptic signaling by all-trans retinoic acid in homeostatic synaptic plasticity. *Neuron, 60*(2), 308-320. doi:10.1016/j.neuron.2008.08.012

Bird, C. M., & Burgess, N. (2008). The hippocampus and memory: insights from spatial processing. *Nature reviews. Neuroscience, 9*(3), 182-194. doi:10.1038/nrn2335

Bolivar, V. J., Walters, S. R., & Phoenix, J. L. (2007). Assessing autism-like behavior in mice: variations in social interactions among inbred strains. *Behav Brain Res, 176*(1), 21-26. doi:10.1016/j.bbr.2006.09.007

Brachman, R. A., McGowan, J. C., Perusini, J. N., Lim, S. C., Pham, T. H., Faye, C., … Denny, C. A. (2016). Ketamine as a Prophylactic Against Stress-Induced Depressive-like Behavior. *Biol Psychiatry, 79*(9), 776-786. doi:10.1016/j.biopsych.2015.04.022

Burke, M. R., Sotiropoulos, I., & Waites, C. L. (2024). The multiple roles of chronic stress and glucocorticoids in Alzheimer's disease pathogenesis. *Trends Neurosci*doi:10.1016/j.tins.2024.08.015

Campbell, S., & Macqueen, G. (2004). The role of the hippocampus in the pathophysiology of major depression. *J Psychiatry Neurosci, 29*(6), 417-426. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15644983

Carreno, F. R., Donegan, J. J., Boley, A. M., Shah, A., DeGuzman, M., Frazer, A., & Lodge, D. J. (2016). Activation of a ventral hippocampus-medial prefrontal cortex pathway is both necessary and sufficient for an antidepressant response to ketamine. *Mol Psychiatry, 21*(9), 1298-1308. doi:10.1038/mp.2015.176

Carrier, N., & Kabbaj, M. (2013). Sex differences in the antidepressant-like effects of ketamine. *Neuropharmacology, 70*, 27-34. doi:10.1016/j.neuropharm.2012.12.009

Castagne, V., Moser, P., Roux, S., & Porsolt, R. D. (2011). Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Curr Protoc Neurosci, Chapter 8*, Unit 8 10A. doi:10.1002/0471142301.ns0810as55

Chen, M., Ma, S., Liu, H., Dong, Y., Tang, J., Ni, Z., … Hu, H. (2024). Brain region-specific action of ketamine as a rapid antidepressant. *Science, 385*(6709), eado7010. doi:10.1126/science.ado7010

Correia-Melo, F. S., Silva, S. S., Araujo-de-Freitas, L., & Quarantini, L. C. (2017). S-(+)-ketamine-induced dissociative symptoms as a traumatic experience in patients with treatment-resistant depression. *Braz J Psychiatry, 39*(2), 188-189. doi:10.1590/1516-4446-2016-2070

Cryan, J. F., Mombereau, C., & Vassout, A. (2005). The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev, 29*(4-5), 571-625. doi:10.1016/j.neubiorev.2005.03.009

Cull-Candy, S. G., & Farrant, M. (2021). Ca(2+) -permeable AMPA receptors and their auxiliary subunits in synaptic plasticity and disease. *J Physiol, 599*(10), 2655-2671. doi:10.1113/JP279029

Dale, O., Somogyi, A. A., Li, Y., Sullivan, T., & Shavit, Y. (2012). Does intraoperative ketamine attenuate inflammatory reactivity following surgery? A systematic review and meta-analysis. *Anesth Analg, 115*(4), 934-943. doi:10.1213/ANE.0b013e3182662e30

daSilva, A. W., Huckins, J. F., Wang, W., Wang, R., Campbell, A. T., & Meyer, M. L. (2021). Daily perceived stress predicts less next day social interaction: Evidence from a naturalistic mobile sensing study. *Emotion, 21*(8), 1760-1770. doi:10.1037/emo0000994

Davoudian, P. A., Shao, L.-X., & Kwan, A. C. (2022). Shared and distinct brain regions targeted for immediate early gene expression by ketamine and psilocybin. *bioRxiv*, 2022.2003.2018.484437. doi:10.1101/2022.03.18.484437

Davoudian, P. A., Shao, L. X., & Kwan, A. C. (2023). Shared and Distinct Brain Regions Targeted for Immediate Early Gene Expression by Ketamine and Psilocybin. *ACS Chem Neurosci, 14*(3), 468-480. doi:10.1021/acschemneuro.2c00637

Deyama, S., & Duman, R. S. (2020). Neurotrophic mechanisms underlying the rapid and sustained antidepressant actions of ketamine. *Pharmacol Biochem Behav, 188*, 172837. doi:10.1016/j.pbb.2019.172837

Diering, G. H., & Huganir, R. L. (2018). The AMPA Receptor Code of Synaptic Plasticity. *Neuron, 100*(2), 314-329. doi:10.1016/j.neuron.2018.10.018

Dossat, A. M., Wright, K. N., Strong, C. E., & Kabbaj, M. (2018). Behavioral and biochemical sensitivity to low doses of ketamine: Influence of estrous cycle in C57BL/6 mice. *Neuropharmacology, 130*, 30-41. doi:10.1016/j.neuropharm.2017.11.022

Duman, R. S., Aghajanian, G. K., Sanacora, G., & Krystal, J. H. (2016). Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat Med, 22*(3), 238-249. doi:10.1038/nm.4050

Egeland, M., Zunszain, P. A., & Pariante, C. M. (2015). Molecular mechanisms in the regulation of adult neurogenesis during stress. *Nature reviews. Neuroscience, 16*(4), 189-200. doi:10.1038/nrn3855

Feder, A., Parides, M. K., Murrough, J. W., Perez, A. M., Morgan, J. E., Saxena, S., … Charney, D. S. (2014). Efficacy of intravenous ketamine for treatment of chronic posttraumatic stress disorder: a randomized clinical trial. *JAMA Psychiatry, 71*(6), 681-688. doi:10.1001/jamapsychiatry.2014.62

Felix-Ortiz, A. C., Burgos-Robles, A., Bhagat, N. D., Leppla, C. A., & Tye, K. M. (2016). Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex. *Neuroscience, 321*, 197-209. doi:10.1016/j.neuroscience.2015.07.041

Fischell, J., Van Dyke, A. M., Kvarta, M. D., LeGates, T. A., & Thompson, S. M. (2015). Rapid Antidepressant Action and Restoration of Excitatory Synaptic Strength After Chronic Stress by Negative Modulators of

Alpha5-Containing GABAA Receptors. *Neuropsychopharmacology, 40*(11), 2499-2509. doi:10.1038/npp.2015.112

Fortin, N. J., Agster, K. L., & Eichenbaum, H. B. (2002). Critical role of the hippocampus in memory for sequences of events. *Nature neuroscience, 5*(5), 458-462. doi:10.1038/nn834

Franceschelli, A., Sens, J., Herchick, S., Thelen, C., & Pitychoutis, P. M. (2015). Sex differences in the rapid and the sustained antidepressant-like effects of ketamine in stress-naive and "depressed" mice exposed to chronic mild stress. *Neuroscience, 290*, 49-60. doi:10.1016/j.neuroscience.2015.01.008

Gerhard, D. M., Pothula, S., Liu, R. J., Wu, M., Li, X. Y., Girgenti, M. J., … Duman, R. S. (2020). GABA interneurons are the cellular trigger for ketamine's rapid antidepressant actions. *J Clin Invest, 130*(3), 1336-1349. doi:10.1172/JCI130808

Getachew, B., Aubee, J. I., Schottenfeld, R. S., Csoka, A. B., Thompson, K. M., & Tizabi, Y. (2018). Ketamine interactions with gut-microbiota in rats: relevance to its antidepressant and anti-inflammatory properties. *BMC Microbiol, 18*(1), 222. doi:10.1186/s12866-018-1373-7

Ghasemi, M., Navidhamidi, M., Rezaei, F., Azizikia, A., & Mehranfard, N. (2022). Anxiety and hippocampal neuronal activity: Relationship and potential mechanisms. *Cogn Affect Behav Neurosci, 22*(3), 431-449. doi:10.3758/s13415-021-00973-y

Gill, H., Gill, B., Rodrigues, N. B., Lipsitz, O., Rosenblat, J. D., El-Halabi, S., … McIntyre, R. S. (2021). The Effects of Ketamine on Cognition in Treatment-Resistant Depression: A Systematic Review and Priority Avenues for Future Research. *Neurosci Biobehav Rev, 120*, 78-85. doi:10.1016/j.neubiorev.2020.11.020

Goel, A., & Lee, H. K. (2007). Persistence of experience-induced homeostatic synaptic plasticity through adulthood in superficial layers of mouse visual cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 27*(25), 6692-6700. doi:10.1523/JNEUROSCI.5038-06.2007

Goel, A., Xu, L. W., Snyder, K. P., Song, L., Goenaga-Vazquez, Y., Megill, A., … Lee, H. K. (2011). Phosphorylation of AMPA receptors is required for sensory deprivation-induced homeostatic synaptic plasticity. *PLoS One, 6*(3), e18264. doi:10.1371/journal.pone.0018264

Govindaiah, G., Kang, Y. J., Lewis, H. E. S., Chung, L., Clement, E. M., Greenfield, L. J., Jr., … Lee, S. H. (2018). Group I metabotropic glutamate receptors generate two types of intrinsic membrane oscillations

in hippocampal oriens/alveus interneurons. *Neuropharmacology, 139*, 150-162. doi:10.1016/j.neuropharm.2018.06.035

Grieco, S. F., Castren, E., Knudsen, G. M., Kwan, A. C., Olson, D. E., Zuo, Y., … Xu, X. (2022). Psychedelics and Neural Plasticity: Therapeutic Implications. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 42*(45), 8439-8449. doi:10.1523/JNEUROSCI.1121-22.2022

Guire, E. S., Oh, M. C., Soderling, T. R., & Derkach, V. A. (2008). Recruitment of calcium-permeable AMPA receptors during synaptic potentiation is regulated by CaM-kinase I. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 28*(23), 6000-6009. doi:10.1523/JNEUROSCI.0384-08.2008

Hare, B. D., Shinohara, R., Liu, R. J., Pothula, S., DiLeone, R. J., & Duman, R. S. (2019). Optogenetic stimulation of medial prefrontal cortex Drd1 neurons produces rapid and long-lasting antidepressant effects. *Nat Commun, 10*(1), 223. doi:10.1038/s41467-018-08168-9

He, J. G., Zhou, H. Y., Wang, F., & Chen, J. G. (2023). Dysfunction of Glutamatergic Synaptic Transmission in Depression: Focus on AMPA Receptor Trafficking. *Biol Psychiatry Glob Open Sci, 3*(2), 187-196. doi:10.1016/j.bpsgos.2022.02.007

Hong, I., & Kaang, B. K. (2022). The complexity of ventral CA1 and its multiple functionalities. *Genes Brain Behav, 21*(7), e12826. doi:10.1111/gbb.12826

Huang, P., Li, C., Fu, T., Zhao, D., Yi, Z., Lu, Q., … Xu, X. (2015). Flupirtine attenuates chronic restraint stress-induced cognitive deficits and hippocampal apoptosis in male mice. *Behav Brain Res, 288*, 1-10. doi:10.1016/j.bbr.2015.04.004

Jarrard, L. E. (1993). On the role of the hippocampus in learning and memory in the rat. *Behav Neural Biol, 60*(1), 9-26. doi:10.1016/0163-1047(93)90664-4

Kallarackal, A. J., Kvarta, M. D., Cammarata, E., Jaberi, L., Cai, X., Bailey, A. M., & Thompson, S. M. (2013). Chronic stress induces a selective decrease in AMPA receptor-mediated synaptic excitation at hippocampal temporoammonic-CA1 synapses. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 33*(40), 15669-15674. doi:10.1523/JNEUROSCI.2588-13.2013

Kang, Y. J., Clement, E. M., Park, I. H., Greenfield, L. J., Jr., Smith, B. N., & Lee, S. H. (2021). Vulnerability of cholecystokinin-expressing GABAergic interneurons in the unilateral intrahippocampal kainate mouse model of temporal lobe epilepsy. *Exp Neurol, 342*, 113724. doi:10.1016/j.expneurol.2021.113724

Kang, Y. J., Clement, E. M., Sumsky, S. L., Xiang, Y., Park, I. H., Santaniello, S., … Lee, S. H. (2020). The critical role of persistent sodium current in hippocampal gamma oscillations. *Neuropharmacology, 162*, 107787. doi:10.1016/j.neuropharm.2019.107787

Kang, Y. J., Lewis, H. E. S., Young, M. W., Govindaiah, G., Greenfield, L. J., Jr., Garcia-Rill, E., & Lee, S. H. (2018). Cell Type-specific Intrinsic Perithreshold Oscillations in Hippocampal GABAergic Interneurons. *Neuroscience, 376*, 80-93. doi:10.1016/j.neuroscience.2018.02.014

Kavalali, E. T., & Monteggia, L. M. (2020). Targeting Homeostatic Synaptic Plasticity for Treatment of Mood Disorders. *Neuron, 106*(5), 715-726. doi:10.1016/j.neuron.2020.05.015

Kessler, R. C. (1997). The effects of stressful life events on depression. *Annu Rev Psychol, 48*, 191-214. doi:10.1146/annurev.psych.48.1.191

Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M., Altman, D. G., & Group, N. C. R. R. G. W. (2010). Animal research: reporting in vivo experiments: the ARRIVE guidelines. *Br J Pharmacol, 160*(7), 1577-1579. doi:10.1111/j.1476-5381.2010.00872.x

Kim, E. J., & Kim, J. J. (2023). Neurocognitive effects of stress: a metaparadigm perspective. *Mol Psychiatry, 28*(7), 2750-2763. doi:10.1038/s41380-023-01986-4

Kim, J. J., & Diamond, D. M. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nature reviews. Neuroscience, 3*(6), 453-462. doi:10.1038/nrn849

Kim, S., Pick, J. E., Abera, S., Khatri, L., Ferreira, D. D., Sathler, M. F., … Ziff, E. B. (2016). Brain region-specific effects of cGMP-dependent kinase II knockout on AMPA receptor trafficking and animal behavior. *Learn Mem, 23*(8), 435-441. doi:10.1101/lm.042960.116

Kim, S., Shou, J., Abera, S., & Ziff, E. B. (2018). Sucrose withdrawal induces depression and anxiety-like behavior by Kir2.1 upregulation in the nucleus accumbens. *Neuropharmacology, 130*, 10-17. doi:10.1016/j.neuropharm.2017.11.041

Kim, S., Violette, C. J., & Ziff, E. B. (2015). Reduction of increased calcineurin activity rescues impaired homeostatic synaptic plasticity in presenilin 1 M146V mutant. *Neurobiol Aging, 36*(12), 3239-3246. doi:10.1016/j.neurobiolaging.2015.09.007

Kim, S., & Ziff, E. B. (2014). Calcineurin mediates synaptic scaling via synaptic trafficking of Ca2+-permeable AMPA receptors. *PLoS Biol, 12*(7), e1001900. doi:10.1371/journal.pbio.1001900

Kim, W. B., & Cho, J. H. (2020). Encoding of contextual fear memory in hippocampal-amygdala circuit. *Nat Commun, 11*(1), 1382. doi:10.1038/s41467-020-15121-2

Kohtala, S. (2021). Ketamine-50 years in use: from anesthesia to rapid antidepressant effects and neurobiological mechanisms. *Pharmacol Rep, 73*(2), 323-345. doi:10.1007/s43440-021-00232-4

Lee, E. H., Park, J. Y., Kwon, H. J., & Han, P. L. (2021). Repeated exposure with short-term behavioral stress resolves pre-existing stress-induced depressive-like behavior in mice. *Nat Commun, 12*(1), 6682. doi:10.1038/s41467-021-26968-4

Lee, H. K. (2012). Ca-permeable AMPA receptors in homeostatic synaptic plasticity. *Front Mol Neurosci, 5*, 17. doi:10.3389/fnmol.2012.00017

Lee, R., Kim, G., & Kim, S. (2024). Co-activation of selective nicotinic acetylcholine receptor subtypes is required to reverse hippocampal network dysfunction and prevent fear memory loss in Alzheimer's disease. *bioRxiv*doi:10.1101/2024.07.08.602576

Li, N., Liu, R. J., Dwyer, J. M., Banasr, M., Lee, B., Son, H., … Duman, R. S. (2011). Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biol Psychiatry, 69*(8), 754-761. doi:10.1016/j.biopsych.2010.12.015

Lisek, M., Zylinska, L., & Boczek, T. (2020). Ketamine and Calcium Signaling-A Crosstalk for Neuronal Physiology and Pathology. *Int J Mol Sci, 21*(21)doi:10.3390/ijms21218410

Liu, Y. Z., Wang, Y. X., & Jiang, C. L. (2017). Inflammation: The Common Pathway of Stress-Related Diseases. *Front Hum Neurosci, 11*, 316. doi:10.3389/fnhum.2017.00316

Luo, E., Stephens, S. B., Chaing, S., Munaganuru, N., Kauffman, A. S., & Breen, K. M. (2016). Corticosterone Blocks Ovarian Cyclicity and the LH Surge via Decreased Kisspeptin Neuron Activation in Female Mice. *Endocrinology, 157*(3), 1187-1199. doi:10.1210/en.2015-1711

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature reviews. Neuroscience, 10*(6), 434-445. doi:10.1038/nrn2639

Ma, J. H., Wang, S. Y., Yu, H. Y., Li, D. Y., Luo, S. C., Zheng, S. S., … Duan, K. M. (2019). Prophylactic use of ketamine reduces postpartum depression in Chinese women undergoing cesarean section(). *Psychiatry Res, 279*, 252-258. doi:10.1016/j.psychres.2019.03.026

Ma, L., Wang, L., Chang, L., Shan, J., Qu, Y., Wang, X., … Hashimoto, K. (2022). A key role of miR-132-5p in the prefrontal cortex for persistent prophylactic actions of (R)-ketamine in mice. *Transl Psychiatry, 12*(1), 417. doi:10.1038/s41398-022-02192-6

Ma, S., Chen, M., Jiang, Y., Xiang, X., Wang, S., Wu, Z., … Hu, H. (2023). Sustained antidepressant effect of ketamine through NMDAR trapping in the LHb. *Nature*doi:10.1038/s41586-023-06624-1

Man, H. Y. (2011). GluA2-lacking, calcium-permeable AMPA receptors--inducers of plasticity? *Curr Opin Neurobiol, 21*(2), 291-298. doi:10.1016/j.conb.2011.01.001

Mao, Y., Xu, Y., & Yuan, X. (2022). Validity of chronic restraint stress for modeling anhedonic-like behavior in rodents: a systematic review and meta-analysis. *J Int Med Res, 50*(2), 3000605221075816. doi:10.1177/03000605221075816

Marin, M. T., Cruz, F. C., & Planeta, C. S. (2007). Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. *Physiol Behav, 90*(1), 29-35. doi:10.1016/j.physbeh.2006.08.021

Matsuo, N., Tanda, K., Nakanishi, K., Yamasaki, N., Toyama, K., Takao, K., … Miyakawa, T. (2009). Comprehensive behavioral phenotyping of ryanodine receptor type 3 (RyR3) knockout mice: decreased social contact duration in two social interaction tests. *Front Behav Neurosci, 3*, 3. doi:10.3389/neuro.08.003.2009

McCosh, R. B., Kreisman, M. J., Tian, K., Thomas, S. A., & Breen Church, K. M. (2024). Norepinephrine Neurons in the Nucleus of the Solitary Tract Suppress Luteinizing Hormone Secretion in Female Mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 44*(34)doi:10.1523/JNEUROSCI.0084-24.2024

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev, 87*(3), 873-904. doi:10.1152/physrev.00041.2006

McEwen, B. S., Nasca, C., & Gray, J. D. (2016). Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. *Neuropsychopharmacology, 41*(1), 3-23. doi:10.1038/npp.2015.171

Miller, O. H., Moran, J. T., & Hall, B. J. (2016). Two cellular hypotheses explaining the initiation of ketamine's antidepressant actions: Direct inhibition and disinhibition. *Neuropharmacology, 100*, 17-26. doi:10.1016/j.neuropharm.2015.07.028

Moda-Sava, R. N., Murdock, M. H., Parekh, P. K., Fetcho, R. N., Huang, B. S., Huynh, T. N., … Liston, C. (2019). Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation. *Science, 364*(6436)doi:10.1126/science.aat8078

Molnar, E. (2011). Long-term potentiation in cultured hippocampal neurons. *Semin Cell Dev Biol, 22*(5), 506-513. doi:10.1016/j.semcdb.2011.07.017

Murrough, J. W., Perez, A. M., Pillemer, S., Stern, J., Parides, M. K., aan het Rot, M., … Iosifescu, D. V. (2013). Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. *Biol Psychiatry, 74*(4), 250-256. doi:10.1016/j.biopsych.2012.06.022

Newport, D. J., Carpenter, L. L., McDonald, W. M., Potash, J. B., Tohen, M., Nemeroff, C. B., … Treatments. (2015). Ketamine and Other NMDA Antagonists: Early Clinical Trials and Possible Mechanisms in Depression. *Am J Psychiatry, 172*(10), 950-966. doi:10.1176/appi.ajp.2015.15040465

Ng, S. H., Tse, M. L., Ng, H. W., & Lau, F. L. (2010). Emergency department presentation of ketamine abusers in Hong Kong: a review of 233 cases. *Hong Kong Med J, 16*(1), 6-11. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20124567

Nikkheslat, N. (2021). Targeting inflammation in depression: Ketamine as an anti-inflammatory antidepressant in psychiatric emergency. *Brain Behav Immun Health, 18*, 100383. doi:10.1016/j.bbih.2021.100383

Ohta, N., Ohashi, Y., & Fujino, Y. (2009). Ketamine inhibits maturation of bone marrow-derived dendritic cells and priming of the Th1-type immune response. *Anesth Analg, 109*(3), 793-800. doi:10.1213/ane.0b013e3181adc384

Okuyama, T., Kitamura, T., Roy, D. S., Itohara, S., & Tonegawa, S. (2016). Ventral CA1 neurons store social memory. *Science, 353*(6307), 1536-1541. doi:10.1126/science.aaf7003

Ommati, M. M., Mobasheri, A., Niknahad, H., Rezaei, M., Alidaee, S., Arjmand, A., … Heidari, R. (2023). Low-dose ketamine improves animals' locomotor activity and decreases brain oxidative stress and

inflammation in ammonia-induced neurotoxicity. *J Biochem Mol Toxicol, 37*(11), e23468. doi:10.1002/jbt.23468

Papakostas, G. I. (2020). Maintaining Rapid Antidepressant Effects Following Ketamine Infusion: A Major Unmet Need. *J Clin Psychiatry, 81*(2)doi:10.4088/JCP.19r12859

Park, M. J., Seo, B. A., Lee, B., Shin, H. S., & Kang, M. G. (2018). Stress-induced changes in social dominance are scaled by AMPA-type glutamate receptor phosphorylation in the medial prefrontal cortex. *Sci Rep, 8*(1), 15008. doi:10.1038/s41598-018-33410-1

Paul, R., Schaaff, N., Padberg, F., Moller, H. J., & Frodl, T. (2009). Comparison of racemic ketamine and S-ketamine in treatment-resistant major depression: report of two cases. *World J Biol Psychiatry, 10*(3), 241-244. doi:10.1080/15622970701714370

Phillips, J. L., Norris, S., Talbot, J., Birmingham, M., Hatchard, T., Ortiz, A., … Blier, P. (2019). Single, Repeated, and Maintenance Ketamine Infusions for Treatment-Resistant Depression: A Randomized Controlled Trial. *Am J Psychiatry, 176*(5), 401-409. doi:10.1176/appi.ajp.2018.18070834

Phillips, L. J., McGorry, P. D., Garner, B., Thompson, K. N., Pantelis, C., Wood, S. J., & Berger, G. (2006). Stress, the hippocampus and the hypothalamic-pituitary-adrenal axis: implications for the development of psychotic disorders. *Aust N Z J Psychiatry, 40*(9), 725-741. doi:10.1080/j.1440-1614.2006.01877.x

Phillips, M. L., Robinson, H. A., & Pozzo-Miller, L. (2019). Ventral hippocampal projections to the medial prefrontal cortex regulate social memory. *Elife, 8*doi:10.7554/eLife.44182

Purkey, A. M., & Dell'Acqua, M. L. (2020). Phosphorylation-Dependent Regulation of Ca(2+)-Permeable AMPA Receptors During Hippocampal Synaptic Plasticity. *Front Synaptic Neurosci, 12*, 8. doi:10.3389/fnsyn.2020.00008

Raff, H., Sharma, S. T., & Nieman, L. K. (2014). Physiological basis for the etiology, diagnosis, and treatment of adrenal disorders: Cushing's syndrome, adrenal insufficiency, and congenital adrenal hyperplasia. *Compr Physiol, 4*(2), 739-769. doi:10.1002/cphy.c130035

Raff, H., Skelton, M. M., & Cowley, A. W., Jr. (1989). Feedback control of vasopressin and corticotrophin secretion in conscious dogs: effect of hypertonic saline. *J Endocrinol, 122*(1), 41-48. doi:10.1677/joe.0.1220041

Richter-Levin, G., & Xu, L. (2018). How could stress lead to major depressive disorder? *IBRO Rep, 4*, 38-43. doi:10.1016/j.ibror.2018.04.001

Ryu, J. R., Hong, C. J., Kim, J. Y., Kim, E. K., Sun, W., & Yu, S. W. (2016). Control of adult neurogenesis by programmed cell death in the mammalian brain. *Mol Brain, 9*, 43. doi:10.1186/s13041-016-0224-4

Saland, S. K., & Kabbaj, M. (2018). Sex Differences in the Pharmacokinetics of Low-dose Ketamine in Plasma and Brain of Male and Female Rats. *J Pharmacol Exp Ther, 367*(3), 393-404. doi:10.1124/jpet.118.251652

Sanderson, J. L., Gorski, J. A., & Dell'Acqua, M. L. (2016). NMDA Receptor-Dependent LTD Requires Transient Synaptic Incorporation of Ca(2)(+)-Permeable AMPARs Mediated by AKAP150-Anchored PKA and Calcineurin. *Neuron, 89*(5), 1000-1015. doi:10.1016/j.neuron.2016.01.043

Sanderson, J. L., Scott, J. D., & Dell'Acqua, M. L. (2018). Control of Homeostatic Synaptic Plasticity by AKAP-Anchored Kinase and Phosphatase Regulation of Ca(2+)-Permeable AMPA Receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*doi:10.1523/JNEUROSCI.2362-17.2018

Schumacher, A., Vlassov, E., & Ito, R. (2016). The ventral hippocampus, but not the dorsal hippocampus is critical for learned approach-avoidance decision making. *Hippocampus, 26*(4), 530-542. doi:10.1002/hipo.22542

Sepulveda Ramos, C., Thornburg, M., Long, K., Sharma, K., Roth, J., Lacatusu, D., … Jacobs, R. J. (2022). The Therapeutic Effects of Ketamine in Mental Health Disorders: A Narrative Review. *Cureus, 14*(3), e23647. doi:10.7759/cureus.23647

Serafini, G., Howland, R. H., Rovedi, F., Girardi, P., & Amore, M. (2014). The role of ketamine in treatment-resistant depression: a systematic review. *Curr Neuropharmacol, 12*(5), 444-461. doi:10.2174/1570159X12666140619204251

Shou, J., Tran, A., Snyder, N., Bleem, E., & Kim, S. (2018). Distinct Roles of GluA2-lacking AMPA Receptor Expression in Dopamine D1 or D2 Receptor Neurons in Animal Behavior. *Neuroscience, 398*, 102-112. doi:10.1016/j.neuroscience.2018.12.002

Silva, J. C. H., & Proulx, C. D. (2024). Locking away depression. *Science, 385*(6709), 608-609. doi:10.1126/science.adq9566

Smith, M. A. (1996). Hippocampal vulnerability to stress and aging: possible role of neurotrophic factors. *Behav Brain Res, 78*(1), 25-36. doi:10.1016/0166-4328(95)00220-0

Spencer, H. F., Berman, R. Y., Boese, M., Zhang, M., Kim, S. Y., Radford, K. D., & Choi, K. H. (2022). Effects of an intravenous ketamine infusion on inflammatory cytokine levels in male and female Sprague-Dawley rats. *J Neuroinflammation, 19*(1), 75. doi:10.1186/s12974-022-02434-w

Su, T., Lu, Y., Fu, C., Geng, Y., & Chen, Y. (2023). GluN2A mediates ketamine-induced rapid antidepressant-like responses. *Nature neuroscience, 26*(10), 1751-1761. doi:10.1038/s41593-023-01436-y

Sun, Q., Li, X., Li, A., Zhang, J., Ding, Z., Gong, H., & Luo, Q. (2020). Ventral Hippocampal-Prefrontal Interaction Affects Social Behavior via Parvalbumin Positive Neurons in the Medial Prefrontal Cortex. *iScience, 23*(3), 100894. doi:10.1016/j.isci.2020.100894

Szczurowska, E., & Mares, P. (2015). An antagonist of calcium permeable AMPA receptors, IEM1460: Anticonvulsant action in immature rats? *Epilepsy Res, 109*, 106-113. doi:10.1016/j.eplepsyres.2014.10.020

Szymkowicz, S. M., Finnegan, N., & Dale, R. M. (2013). A 12-month naturalistic observation of three patients receiving repeat intravenous ketamine infusions for their treatment-resistant depression. *J Affect Disord, 147*(1-3), 416-420. doi:10.1016/j.jad.2012.10.015

Tafet, G. E., & Nemeroff, C. B. (2016). The Links Between Stress and Depression: Psychoneuroendocrinological, Genetic, and Environmental Interactions. *J Neuropsychiatry Clin Neurosci, 28*(2), 77-88. doi:10.1176/appi.neuropsych.15030053

Tan, S., Wang, Y., Chen, K., Long, Z., & Zou, J. (2017). Ketamine Alleviates Depressive-Like Behaviors via Down-Regulating Inflammatory Cytokines Induced by Chronic Restraint Stress in Mice. *Biol Pharm Bull, 40*(8), 1260-1267. doi:10.1248/bpb.b17-00131

Tao, K., Chung, M., Watarai, A., Huang, Z., Wang, M. Y., & Okuyama, T. (2022). Disrupted social memory ensembles in the ventral hippocampus underlie social amnesia in autism-associated Shank3 mutant mice. *Mol Psychiatry, 27*(4), 2095-2105. doi:10.1038/s41380-021-01430-5

Taylor, J. H., Landeros-Weisenberger, A., Coughlin, C., Mulqueen, J., Johnson, J. A., Gabriel, D., … Bloch, M. H. (2018). Ketamine for Social Anxiety Disorder: A Randomized, Placebo-Controlled Crossover Trial. *Neuropsychopharmacology, 43*(2), 325-333. doi:10.1038/npp.2017.194

Thiagarajan, T. C., Lindskog, M., & Tsien, R. W. (2005). Adaptation to synaptic inactivity in hippocampal neurons. *Neuron, 47*(5), 725-737. doi:10.1016/j.neuron.2005.06.037

Tovote, P., Fadok, J. P., & Luthi, A. (2015). Neuronal circuits for fear and anxiety. *Nature reviews. Neuroscience, 16*(6), 317-331. doi:10.1038/nrn3945

Uno, H., Tarara, R., Else, J. G., Suleman, M. A., & Sapolsky, R. M. (1989). Hippocampal damage associated with prolonged and fatal stress in primates. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 9*(5), 1705-1711. doi:10.1523/JNEUROSCI.09-05-01705.1989

Usa, K., Singh, R. J., Netzel, B. C., Liu, Y., Raff, H., & Liang, M. (2007). Renal interstitial corticosterone and 11-dehydrocorticosterone in conscious rats. *Am J Physiol Renal Physiol, 293*(1), F186-192. doi:10.1152/ajprenal.00484.2006

Veraart, J. K. E., Smith-Apeldoorn, S. Y., Bakker, I. M., Visser, B. A. E., Kamphuis, J., Schoevers, R. A., & Touw, D. J. (2021). Pharmacodynamic Interactions Between Ketamine and Psychiatric Medications Used in the Treatment of Depression: A Systematic Review. *Int J Neuropsychopharmacol, 24*(10), 808-831. doi:10.1093/ijnp/pyab039

Wang, N., Yu, H. Y., Shen, X. F., Gao, Z. Q., Yang, C., Yang, J. J., & Zhang, G. F. (2015). The rapid antidepressant effect of ketamine in rats is associated with down-regulation of pro-inflammatory cytokines in the hippocampus. *Ups J Med Sci, 120*(4), 241-248. doi:10.3109/03009734.2015.1060281

Wang, X., & Zhan, Y. (2022). Regulation of Social Recognition Memory in the Hippocampal Circuits. *Front Neural Circuits, 16*, 839931. doi:10.3389/fncir.2022.839931

Watarai, A., Tao, K., Wang, M. Y., & Okuyama, T. (2021). Distinct functions of ventral CA1 and dorsal CA2 in social memory. *Curr Opin Neurobiol, 68*, 29-35. doi:10.1016/j.conb.2020.12.008

Wesierska, M. J., Duda, W., & Dockery, C. A. (2013). Low-dose memantine-induced working memory improvement in the allothetic place avoidance alternation task (APAAT) in young adult male rats. *Front Behav Neurosci, 7*, 203. doi:10.3389/fnbeh.2013.00203

Wiltgen, B. J., Royle, G. A., Gray, E. E., Abdipranoto, A., Thangthaeng, N., Jacobs, N., … Vissel, B. (2010). A role for calcium-permeable AMPA receptors in synaptic plasticity and learning. *PLoS One, 5*(9)doi:10.1371/journal.pone.0012818

Wu, H., Savalia, N. K., & Kwan, A. C. (2021). Ketamine for a Boost of Neural Plasticity: How, but Also When? *Biol Psychiatry, 89*(11), 1030-1032. doi:10.1016/j.biopsych.2021.03.014

Xu, S., Jiang, M., Liu, X., Sun, Y., Yang, L., Yang, Q., & Bai, Z. (2021). Neural Circuits for Social Interactions: From Microcircuits to Input-Output Circuits. *Front Neural Circuits, 15*, 768294. doi:10.3389/fncir.2021.768294

Yang, B., Ren, Q., Ma, M., Chen, Q. X., & Hashimoto, K. (2016). Antidepressant Effects of (+)-MK-801 and (-)-MK-801 in the Social Defeat Stress Model. *Int J Neuropsychopharmacol, 19*(12)doi:10.1093/ijnp/pyw080

Yang, Y., Cui, Y., Sang, K., Dong, Y., Ni, Z., Ma, S., & Hu, H. (2018). Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nature, 554*(7692), 317-322. doi:10.1038/nature25509

Yoshida, K., Drew, M. R., Kono, A., Mimura, M., Takata, N., & Tanaka, K. F. (2021). Chronic social defeat stress impairs goal-directed behavior through dysregulation of ventral hippocampal activity in male mice. *Neuropsychopharmacology, 46*(9), 1606-1616. doi:10.1038/s41386-021-00990-y

Yu, T., Xu, H., Wang, W., Li, S., Chen, Z., & Deng, H. (2015). Determination of endogenous corticosterone in rodent's blood, brain and hair with LC-APCI-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci, 1002*, 267-276. doi:10.1016/j.jchromb.2015.08.035

Yun, J., Koike, H., Ibi, D., Toth, E., Mizoguchi, H., Nitta, A., … Yamada, K. (2010). Chronic restraint stress impairs neurogenesis and hippocampus-dependent fear memory in mice: possible involvement of a brain-specific transcription factor Npas4. *J Neurochem, 114*(6), 1840-1851. doi:10.1111/j.1471-4159.2010.06893.x

Zanos, P., Moaddel, R., Morris, P. J., Georgiou, P., Fischell, J., Elmer, G. I., … Gould, T. D. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature, 533*(7604), 481-486. doi:10.1038/nature17998

Zaytseva, A., Bouckova, E., Wiles, M. J., Wustrau, M. H., Schmidt, I. G., Mendez-Vazquez, H., … Kim, S. (2023). Ketamine's rapid antidepressant effects are mediated by Ca(2+)-permeable AMPA receptors. *Elife, 12*doi:10.7554/eLife.86022

Zhang, J. C., Yao, W., Dong, C., Yang, C., Ren, Q., Ma, M., & Hashimoto, K. (2017). Blockade of interleukin-6 receptor in the periphery promotes rapid and sustained antidepressant actions: a possible role of gut-microbiota-brain axis. *Transl Psychiatry, 7*(5), e1138. doi:10.1038/tp.2017.112

Zhou, Y., Wang, C., Lan, X., Zheng, W., Li, H., Chao, Z., … Ning, Y. (2021). The potential pro-cognitive effects with intravenous subanesthetic ketamine in adults with treatment-resistant major depressive or bipolar disorders and suicidality. *J Psychiatr Res, 144*, 312-319. doi:10.1016/j.jpsychires.2021.10.037

Zucker, R. S. (1999). Calcium- and activity-dependent synaptic plasticity. *Curr Opin Neurobiol, 9*(3), 305-313. doi:10.1016/s0959-4388(99)80045-2

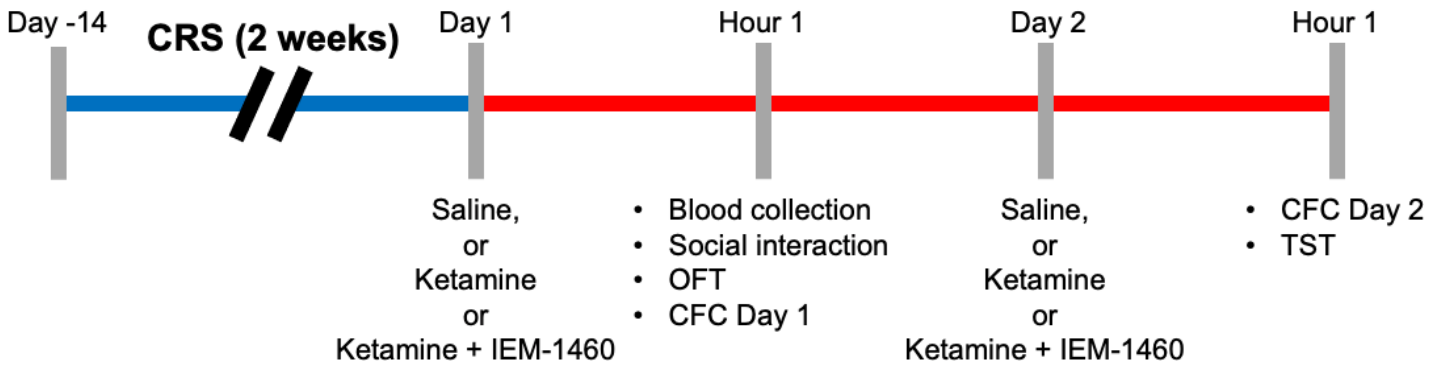

Figure 1. Experimental timeline of chronic restraint stress (CRS) that is applied to mice for 2 weeks, drug treatment, blood collection, and behavioral tests - reciprocal social interaction test, open-field test (OFT), contextual fear conditioning (CFC) day 1 and 2, and tail suspension test (TST). Single intraperitoneal injection of ketamine (5 mg/kg for females and 10 mg/kg for males) or saline in CRS mice in the presence or absence of IEM-1460 (10 mg/kg for both females and males). We optimized the test order from least to most stressful in order to prevent the possibility that multiple behavioral tests with the same mouse can induce stress, especially if the tests are performed too closely together.

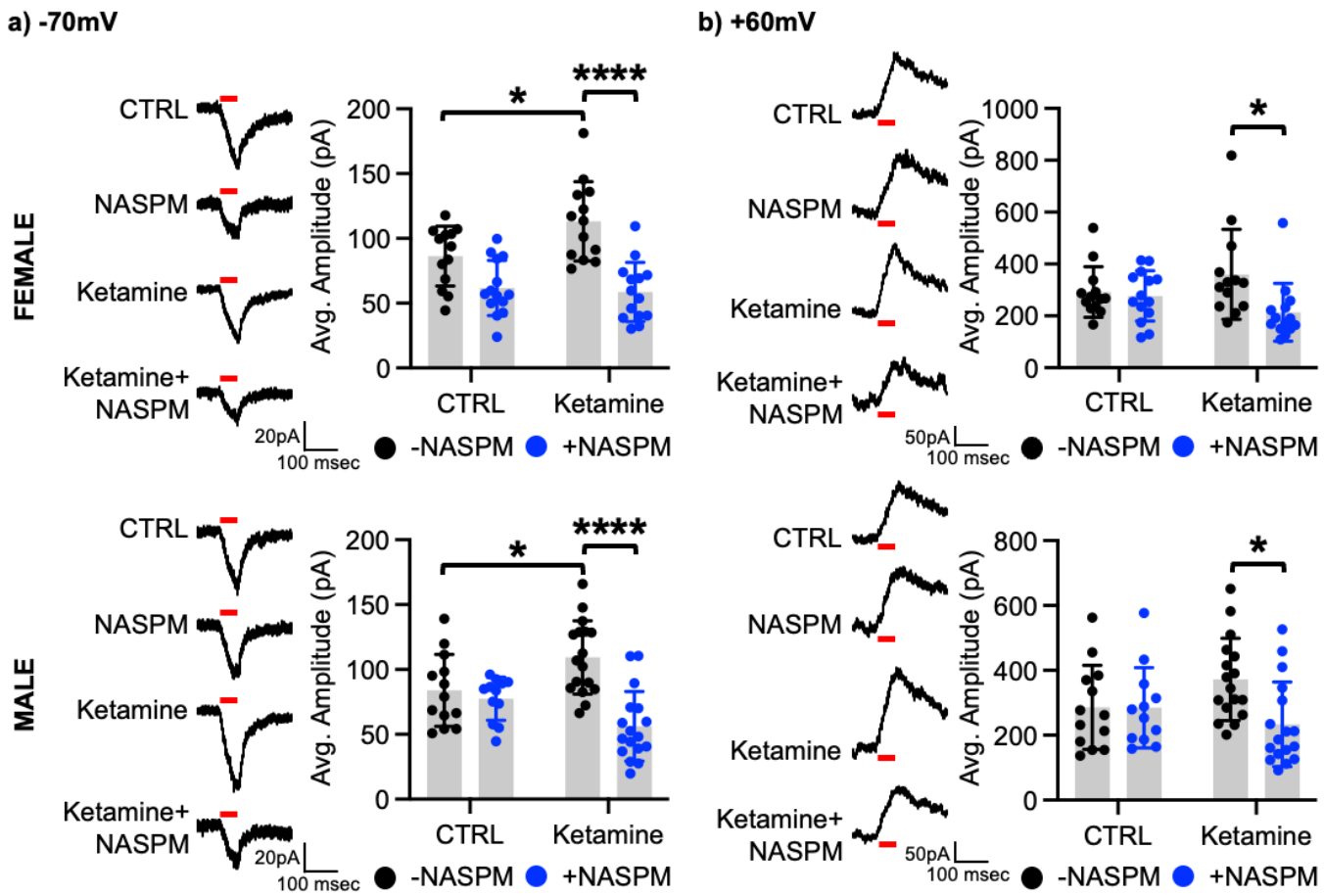

Figure 2. Subanesthetic low-dose ketamine induces synaptic insertion of CP-AMPARs in hippocampal CA1 pyramidal neurons. Representative traces and summary of the amplitudes of uEPSCs (**a)** V_{hold} = -70 mV and **b)** V_{hold} = +60 mV) in each condition (n = number of cells [n = number of animals]. FEMALE: CTRL = 13 [3], NASPM = 14 [3], Ketamine = 13 [3], and Ketamine + NASPM = 14 [3]. MALE: CTRL = 13 [4], NASPM = 14 [4], Ketamine = 13 [3], and Ketamine + NASPM = 14 [3]). *$p < 0.05$ and ****$p < 0.0001$, Two-Way ANOVA, Tukey test. A bar indicates photostimulation. Ketamine: 5 mg/kg for females and 10 mg/kg for males. NASPM: 100 μM.

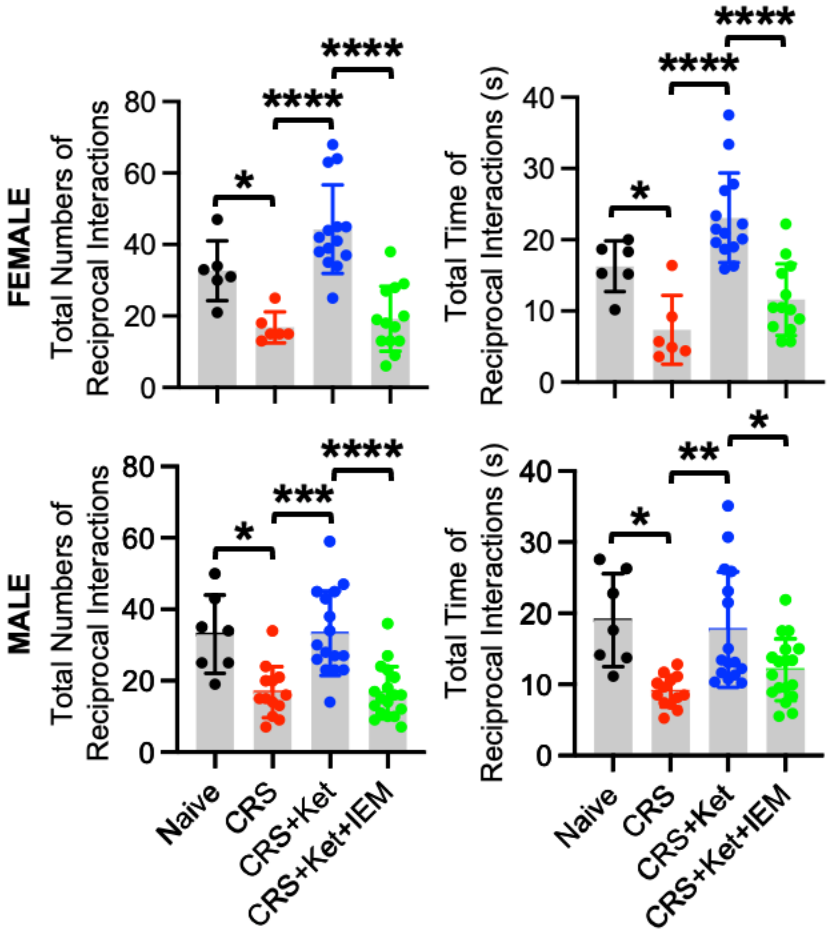

Figure 3. CRS-induced social dysfunction is reversed by ketamine via CP-AMPARs. Summary of total numbers and time of reciprocal interactions in each condition (n = number of animals. FEMALE: Naïve = 5, CRS = 6, CRS + Ket = 14, and CRS + Ket + IEM = 13. MALE: Naïve = 7, CRS = 12, CRS + Ket = 14, and CRS + Ket + IEM = 17). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$, One-Way ANOVA, Tukey test. Ketamine: 5 mg/kg for females and 10 mg/kg for males. IEM-1460: 10 mg/kg for both females and males.

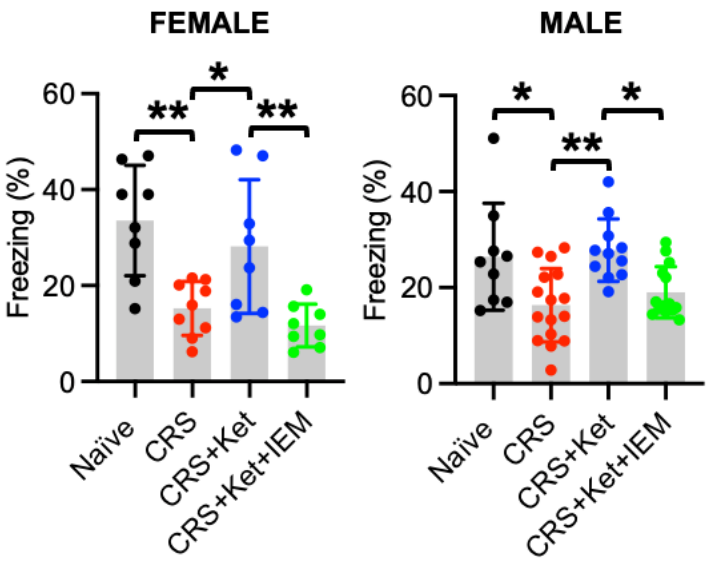

Figure 4. CRS-induced fear memory loss is reversed by ketamine via CP-AMPARs. Summary of fear memory in each condition (n = number of animals. FEMALE: Naïve = 8, CRS = 9, CRS + Ket = 8, and CRS + Ket + IEM = 8. MALE: Naïve = 9, CRS = 15, CRS + Ket = 11, and CRS + Ket + IEM = 14). *$p < 0.05$ and **$p < 0.01$, One-Way ANOVA, Tukey test. Ketamine: 5 mg/kg for females and 10 mg/kg for males. IEM-1460: 10 mg/kg for both females and males.

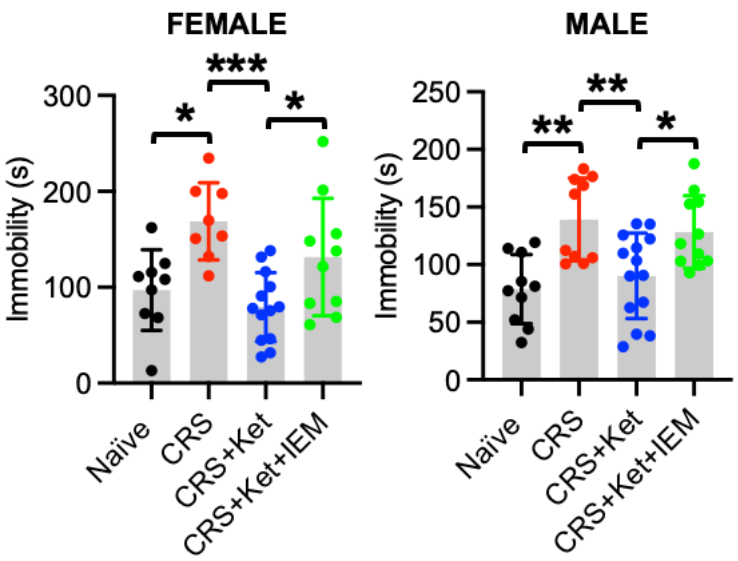

Figure 5. CRS-induced depression-like behavior is reversed by ketamine via CP-AMPARs. Summary of immobility in each condition (n = number of animals. FEMALE: Naïve = 9, CRS = 8, CRS + Ket = 13, and CRS + Ket + IEM = 10. MALE: Naïve = 10, CRS = 10, CRS + Ket = 14, and CRS + Ket + IEM = 11). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, One-Way ANOVA, Tukey test. Ketamine: 5 mg/kg for females and 10 mg/kg for males. IEM-1460: 10 mg/kg for both females and males.

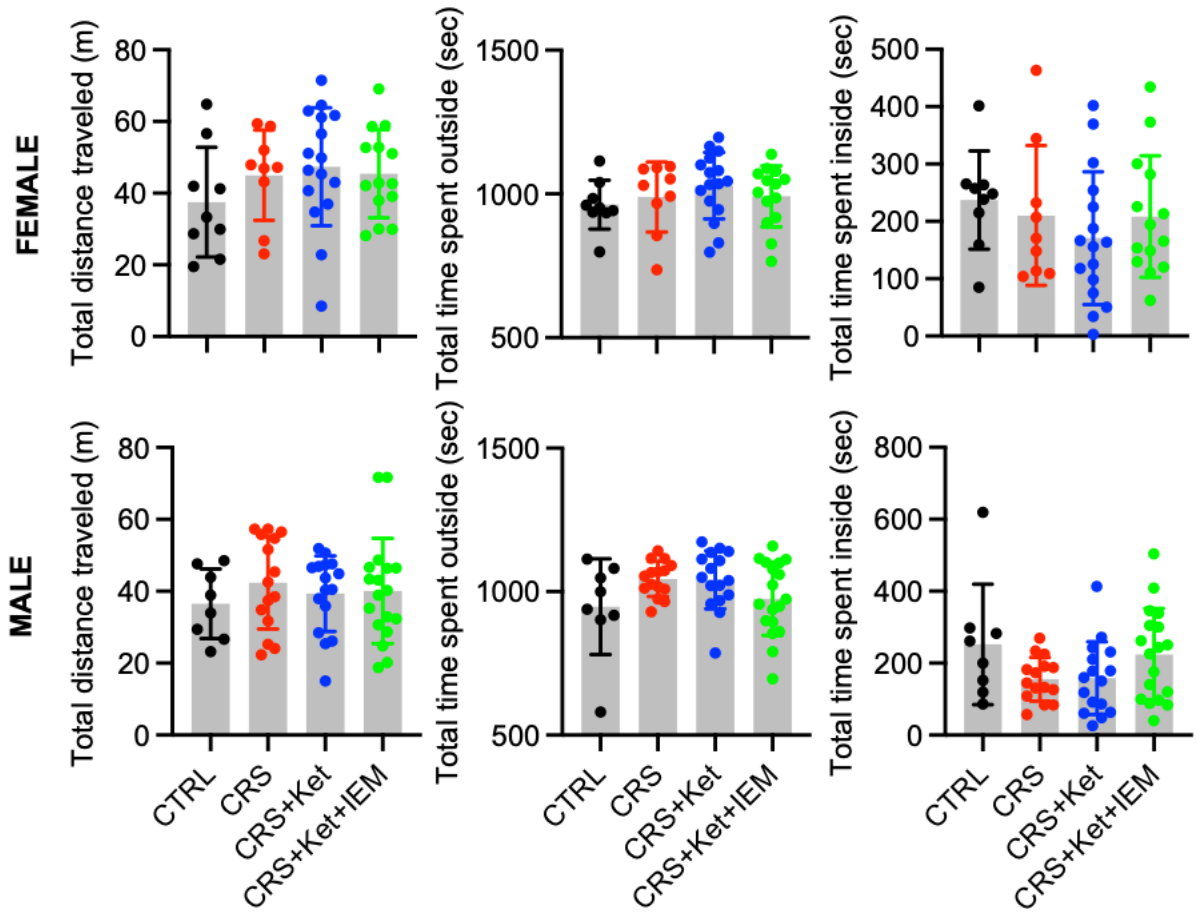

Figure 6. No abnormality in the open field test. Summary of total distance traveled, total time spent outside, and total time spent inside in each condition (n = number of animals. FEMALE: Naïve = 9, CRS = 9, CRS + Ket = 16, and CRS + Ket + IEM = 14. MALE: Naïve = 8, CRS = 15, CRS + Ket = 16, and CRS + Ket + IEM = 18). Ketamine: 5 mg/kg for females and 10 mg/kg for males. IEM-1460: 10 mg/kg for both females and males.

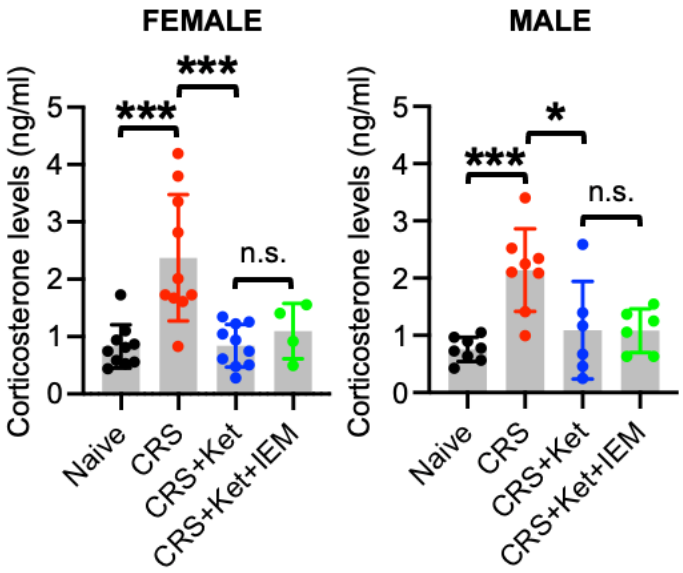

Figure 7. Ketamine reverses the CRS-induced elevation of corticosterone levels, which is not dependent on CP-AMPARs. Summary of serum corticosterone levels in each condition (n = number of animals with duplication. FEMALE: Naïve = 5, CRS = 5, CRS + Ket = 5, and CRS + Ket + IEM = 2. MALE: Naïve = 4, CRS = 4, CRS + Ket = 3, and CRS + Ket + IEM = 3). *$p < 0.05$ and ***$p < 0.001$, One-Way ANOVA, Tukey test. n.s. – not significant. Ketamine: 5 mg/kg for females and 10 mg/kg for males. IEM-1460: 10 mg/kg for both females and males.

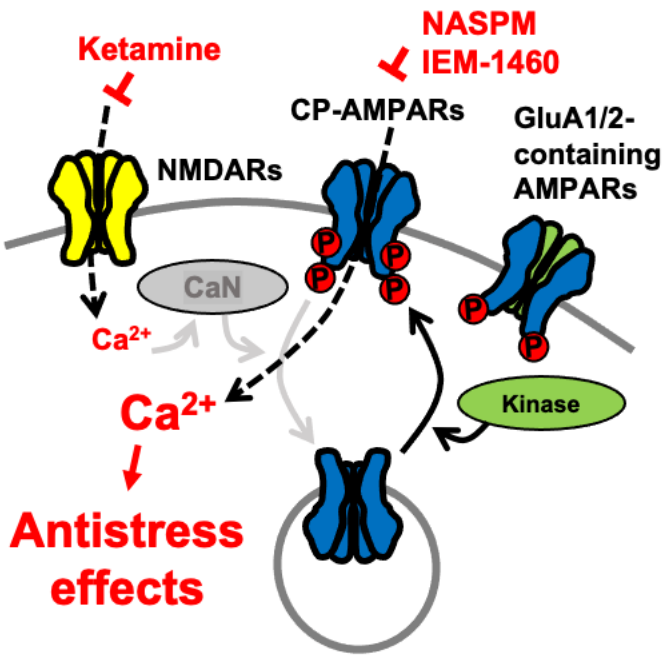

Figure 8. A schematic model of ketamine's antistress effects. Because ketamine is a noncompetitive NMDAR antagonist, a therapeutic dose is enough to block NMDAR-mediated Ca^{2+} influx in excitatory synapses. This can lower the activity of calcineurin (CaN), a Ca^{2+}-dependent phosphatase that dephosphorylates the AMPA receptor (AMPAR) subunit GluA1, and promote GluA1 phosphorylation, resulting in increased synaptic insertion of GluA2-lacking, GluA1-containing Ca^{2+}-permeable AMPARs (CP-AMPARs). When ketamine is administered, CP-AMPAR-mediated Ca^{2+} influx may replace NMDAR-dependent Ca^{2+} signaling. This increases neural plasticity, which leads to antistress effects in animals. Therefore, inhibition of CP-AMPARs using antagonists such as NASPM and IEM-1460 prevents the ketamine's effects.

Table 1. Statistical analysis of the amplitudes of uEPSCs (V_{hold} = -70 mV) in hippocampal CA1 pyramidal neurons

Female (-70 mV)					
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	*P* value
Interaction	3015	1	3015	F (1, 50) = 4.979	*P*=0.0302
Row Factor (Saline vs. Ket)	1917	1	1917	F (1, 50) = 3.165	*P*=0.0813
Column Factor (Saline vs. NASPM)	21122	1	21122	F (1, 50) = 34.88	*P*<0.0001
Residual	30277	50	605.5		
Tukey's multiple comparisons test	Predicted (LS) mean diff.		95.00% CI of diff.		Adjusted *P* Value
CTRL vs. NASPM	24.63		-0.5609 to 49.82		0.0575
CTRL vs. Ketamine	-26.88		-52.53 to -1.227		0.0367
CTRL vs. Ketamine + NASPM	27.66		2.470 to 52.85		0.0262
NASPM vs. Ketamine	-51.51		-76.69 to -26.32		<0.0001
NASPM vs. Ketamine + NASPM	3.031		-21.69 to 27.75		0.9879
Ketamine vs. Ketamine + NASPM	54.54		29.35 to 79.72		<0.0001
Male (-70 mV)					
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	*P* value
Interaction	7866	1	7866	F (1, 55) = 11.82	*P*=0.0011
Row Factor (Saline vs. Ket)	59.69	1	59.69	F (1, 55) = 0.08970	*P*=0.7657
Column Factor (Saline vs. NASPM)	12776	1	12776	F (1, 55) = 19.20	*P*<0.0001
Residual	36598	55	665.4		
Tukey's multiple comparisons test	Predicted (LS) mean diff.		95.00% CI of diff.		Adjusted *P* Value
CTRL vs. NASPM	6.415		-20.94 to 33.77		0.9248
CTRL vs. Ketamine	-25.41		-50.59 to -0.2344		0.0471
CTRL vs. Ketamine + NASPM	27.76		2.577 to 52.94		0.0253
NASPM vs. Ketamine	-31.83		-57.60 to -6.062		0.0097
NASPM vs. Ketamine + NASPM	21.34		-4.426 to 47.11		0.1376
Ketamine vs. Ketamine + NASPM	53.17		29.73 to 76.61		<0.0001

Table 2. Statistical analysis of the amplitudes of uEPSCs (V_{hold} = +60 mV) in hippocampal CA1 pyramidal neurons

Female (+60 mV)					
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	*P* value
Interaction	58023	1	58023	F (1, 50) = 3.814	*P*=0.0564
Row Factor (Saline vs. Ket)	78.38	1	78.38	F (1, 50) = 0.005152	*P*=0.9431
Column Factor (Saline vs. NASPM)	88106	1	88106	F (1, 50) = 5.791	*P*=0.0198
Residual	760699	50	15214		
Tukey's multiple comparisons test	Predicted (LS) mean diff.		95.00% CI of diff.		Adjusted *P* Value
CTRL vs. NASPM	15.24		-111.0 to 141.5		0.9885
CTRL vs. Ketamine	-68.02		-196.6 to 60.56		0.5017
CTRL vs. Ketamine + NASPM	78.43		-47.83 to 204.7		0.3602
NASPM vs. Ketamine	-83.25		-209.5 to 43.00		0.3082
NASPM vs. Ketamine + NASPM	63.19		-60.70 to 187.1		0.5327
Ketamine vs. Ketamine + NASPM	146.4		20.19 to 272.7		0.0170
Male (+60 mV)					
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	*P* value
Interaction	67842	1	67842	F (1, 55) = 4.158	*P*=0.0462
Row Factor (Saline vs. Ket)	4323	1	4323	F (1, 55) = 0.2650	*P*=0.6088
Column Factor (Saline vs. NASPM)	70148	1	70148	F (1, 55) = 4.300	*P*=0.0428
Residual	897324	55	16315		
Tukey's multiple comparisons test	Predicted (LS) mean diff.		95.00% CI of diff.		Adjusted *P* Value
CTRL vs. NASPM	1.157		-134.3 to 136.6		>0.9999
CTRL vs. Ketamine	-85.98		-210.7 to 38.70		0.2718
CTRL vs. Ketamine + NASPM	52.48		-72.20 to 177.2		0.6818
NASPM vs. Ketamine	-87.14		-214.7 to 40.45		0.2799
NASPM vs. Ketamine + NASPM	51.32		-76.27 to 178.9		0.7116
Ketamine vs. Ketamine + NASPM	138.5		22.39 to 254.5		0.0133

Table 3. Statistical analysis of the total numbers of reciprocal interactions in the reciprocal social interaction test.

Female					
ANOVA table	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	5447	3	1816	F (3, 35) = 18.49	*P*<0.0001
Residual (within columns)	3437	35	98.21		
Total	8885	38			

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted *P* Value
Naive vs. CRS	15.83	0.4028 to 31.26	0.0425
Naive vs. CRS + Ket	-11.62	-24.66 to 1.422	0.0952
Naive vs. CRS + Ket + IEM	13.44	0.2451 to 26.63	0.0446
CRS vs. CRS + Ket	-27.45	-40.49 to -14.41	<0.0001
CRS vs. CRS + Ket + IEM	-2.397	-15.59 to 10.79	0.9608
CRS + Ket vs. CRS + Ket + IEM	25.05	14.76 to 35.35	<0.0001

Male					
ANOVA table	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	3594	3	1198	F (3, 50) = 13.76	*P*<0.0001
Residual (within columns)	4352	50	87.03		
Total	7945	53			

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted *P* Value
Naive vs. CRS	16.23	4.608 to 27.85	0.0028
Naive vs. CRS + Ket	-0.2500	-11.49 to 10.99	>0.9999
Naive vs. CRS + Ket + IEM	16.39	5.345 to 27.43	0.0014
CRS vs. CRS + Ket	-16.48	-25.74 to -7.223	0.0001
CRS vs. CRS + Ket + IEM	0.1581	-8.866 to 9.182	>0.9999
CRS + Ket vs. CRS + Ket + IEM	16.64	8.120 to 25.16	<0.0001

Table 4. Statistical analysis of the total time of reciprocal interactions in the reciprocal social interaction test.

Female					
ANOVA table	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	1401	3	466.9	F (3, 35) = 16.40	*P*<0.0001
Residual (within columns)	996.4	35	28.47		
Total	2397	38			

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted *P* Value
Naive vs. CRS	8.917	0.6088 to 17.22	0.0315
Naive vs. CRS + Ket	-6.802	-13.82 to 0.2191	0.0605
Naive vs. CRS + Ket + IEM	4.683	-2.419 to 11.79	0.3005
CRS vs. CRS + Ket	-15.72	-22.74 to -8.698	<0.0001
CRS vs. CRS + Ket + IEM	-4.233	-11.34 to 2.869	0.3876
CRS + Ket vs. CRS + Ket + IEM	11.49	5.943 to 17.03	<0.0001

Male					
ANOVA table	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	788.4	3	262.8	F (3, 50) = 8.035	*P*=0.0002
Residual (within columns)	1635	50	32.71		
Total	2424	53			

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted *P* Value
Naive vs. CRS	10.01	2.888 to 17.14	0.0026
Naive vs. CRS + Ket	1.322	-5.565 to 8.210	0.9563
Naive vs. CRS + Ket + IEM	6.990	0.2196 to 13.76	0.0406
CRS vs. CRS + Ket	-8.691	-14.37 to -3.016	0.0009
CRS vs. CRS + Ket + IEM	-3.024	-8.555 to 2.508	0.4734
CRS + Ket vs. CRS + Ket + IEM	5.667	0.4452 to 10.89	0.0286

Table 5. Statistical analysis of contextual fear conditioning.

Female					
ANOVA table	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	2638	3	879.4	F (3, 29) = 9.531	*P*=0.0002
Residual (within columns)	2676	29	92.27		
Total	5314	32			
Tukey's multiple comparisons test	Mean Diff.		95.00% CI of diff.		Adjusted *P* Value
Naive vs. CRS	18.31		5.595 to 31.03		0.0026
Naive vs. CRS + Ket	5.393		-7.692 to 18.48		0.6786
Naive vs. CRS + Ket + IEM	21.90		8.811 to 34.98		0.0005
CRS vs. CRS + Ket	-12.92		-25.63 to -0.2016		0.0454
CRS vs. CRS + Ket + IEM	3.584		-9.132 to 16.30		0.8682
CRS + Ket vs. CRS + Ket + IEM	16.50		3.417 to 29.59		0.0092

Male					
ANOVA table	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	1164	3	387.9	F (3, 46) = 6.701	*P*=0.0008
Residual (within columns)	2663	46	57.89		
Total	3826	49			
Tukey's multiple comparisons test	Mean Diff.		95.00% CI of diff.		Adjusted *P* Value
Naive vs. CRS	10.11		1.656 to 18.56		0.0133
Naive vs. CRS + Ket	-1.334		-10.45 to 7.781		0.9796
Naive vs. CRS + Ket + IEM	7.394		-1.270 to 16.06		0.1190
CRS vs. CRS + Ket	-11.44		-19.38 to -3.496		0.0021
CRS vs. CRS + Ket + IEM	-2.712		-10.13 to 4.710		0.7649
CRS + Ket vs. CRS + Ket + IEM	8.728		0.5568 to 16.90		0.0322

Table 6. Statistical analysis of the tail suspension test.

Female					
ANOVA table	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	45229	3	15076	F (3, 36) = 7.272	*P*=0.0006
Residual (within columns)	74637	36	2073		
Total	119866	39			

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted *P* Value
Naive vs. CRS	-71.77	-131.4 to -12.18	0.0130
Naive vs. CRS + Ket	17.64	-35.54 to 70.81	0.8084
Naive vs. CRS + Ket + IEM	-34.42	-90.77 to 21.92	0.3669
CRS vs. CRS + Ket	89.41	34.30 to 144.5	0.0006
CRS vs. CRS + Ket + IEM	37.35	-20.82 to 95.52	0.3239
CRS + Ket vs. CRS + Ket + IEM	-52.06	-103.6 to -0.4780	0.0472

Male					
ANOVA table	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	27140	3	9047	F (3, 41) = 7.801	*P*=0.0003
Residual (within columns)	47549	41	1160		
Total	74689	44			

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted *P* Value
Naive vs. CRS	-60.31	-101.1 to -19.53	0.0016
Naive vs. CRS + Ket	-11.45	-49.20 to 26.30	0.8484
Naive vs. CRS + Ket + IEM	-49.48	-89.32 to -9.640	0.0097
CRS vs. CRS + Ket	48.86	11.11 to 86.61	0.0066
CRS vs. CRS + Ket + IEM	10.83	-29.01 to 50.67	0.8854
CRS + Ket vs. CRS + Ket + IEM	-38.03	-74.77 to -1.292	0.0400

Table 7. Summary of the open field test.

	FEMALE				MALE			
	CTRL	CRS	CRS + Ket	CRS + Ket + IEM	CTRL	CRS	CRS + Ket	CRS + Ket + IEM
Total distance traveled (m)	37.52 ± 15.31	45.02 ± 12.62	47.37 ± 16.47	45.43 ± 12.24	36.55 ± 9.70	42.37 ± 12.86	39.34 ± 10.56	40.07 ± 14.65
Total time spent outside (sec)	962.8 ± 85.57	989.6 ± 122.0	1029 ± 115.9	991.8 ± 106.0	947.6 ± 167.2	1044 ± 60.74	1042 ± 101.2	975.9 ± 128.1
Total time spent inside (sec)	237.2 ± 85.57	210.4 ± 122.0	170.8 ± 115.9	208.2 ± 106.0	252.4 ± 167.2	155.6 ± 60.74	158.4 ± 101.2	224.1 ± 128.1

Table 8. Statistical analysis of corticosterone levels.

Female					
ANOVA table	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	15981461	3	5327154	F (3, 30) = 11.31	*P*<0.0001
Residual (within columns)	14132346	30	471078		
Total	30113807	33			

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted *P* Value
Naive vs. CRS	-1543	-2378 to -708.6	0.0001
Naive vs. CRS + Ket	-12.17	-846.8 to 822.4	>0.9999
Naive vs. CRS + Ket + IEM	-264.2	-1368 to 839.9	0.9145
CRS vs. CRS + Ket	1531	696.5 to 2366	0.0001
CRS vs. CRS + Ket + IEM	1279	175.0 to 2383	0.0183
CRS + Ket vs. CRS + Ket + IEM	-252.0	-1356 to 852.1	0.9247

Male					
ANOVA table	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	8562805	3	2854268	F (3, 24) = 8.244	*P*=0.0006
Residual (within columns)	8309607	24	346234		
Total	16872412	27			

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted *P* Value
Naive vs. CRS	-1384	-2196 to -572.3	0.0005
Naive vs. CRS + Ket	-332.8	-1209 to 543.9	0.7239
Naive vs. CRS + Ket + IEM	-325.8	-1202 to 550.9	0.7366
CRS vs. CRS + Ket	1051	174.5 to 1928	0.0146
CRS vs. CRS + Ket + IEM	1058	181.5 to 1935	0.0139
CRS + Ket vs. CRS + Ket + IEM	7.000	-930.2 to 944.2	>0.9999