Ketamine reverses chronic stress-induced behavioral changes via the expression of 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*], 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

*These authors contributed equally.

Corresponding author email: seonil.kim@colostate.edu

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

Abstract

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. Here, we use whole-cell patch-clamp recordings from CA1 pyramidal neurons in female and male hippocampal slices to affirm that subanesthetic low-dose ketamine treatment induces CP-AMPAR expression in these cells. Using multiple behavioral assays including reciprocal social interaction, contextual fear conditioning, and tail suspension test, 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-AMPAR expression. From this, our findings suggest that subanesthetic low-dose ketamine rapidly triggers the expression of CP-AMPARs in the hippocampus, which induces antidepressant and antistress effects.

Introduction

An extensively postulated mechanism for ketamine's rapid antidepressant actions involves the enhancement of excitatory synaptic drive in the hippocampus (Miller et al., 2016; Aleksandrova et al., 2020; Kavalali and Monteggia, 2020). However, it has been demonstrated that ketamine is a noncompetitive NMDA receptor (NMDAR) antagonist that inhibits excitatory synaptic transmission (Anis et al., 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 et al., 2017). In addition to reducing depression, some research indicates that ketamine may have protective effects against chronic stress in both humans and animals (Feder et al., 2014; Brachman et al., 2016; Taylor et al., 2018; Yang et al., 2018; Ma et al., 2019; Gill et al., 2021; Lee et al., 2021; Zhou et al., 2021; Ma et al., 2022). Chronic stress affects brain functions, resulting in the development of mental disorders like anxiety and depression, as well as cognitive decline and social dysfunction (Kessler, 1997; Lupien et al., 2009; daSilva et al., 2021). 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 et al., 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 and Huganir, 2018). Compared to Ca^{2+}-impermeable AMPARs, CP-AMPARs have higher single channel conductance, which contributes to synaptic plasticity (Thiagarajan et al., 2005; Goel and Lee, 2007; Aoto et al., 2008; Goel et al., 2011; Lee, 2012; Kim and Ziff, 2014; Kim et al., 2015a; Sanderson et al., 2016; Sanderson et al., 2018; Purkey and Dell'Acqua, 2020). 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 (Bird and Burgess, 2008; Tovote et al., 2015; Miller et al., 2016; Okuyama et al., 2016; Aleksandrova et al., 2020; Kavalali and Monteggia, 2020; Sun et al., 2020; Grieco et al., 2022). 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 (Li et al., 2011; Kallarackal et al., 2013; Fischell et al., 2015).

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 induces CP-AMPAR expression 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 the expression of CP-AMPARs. Our findings thus suggest that subanesthetic low-dose ketamine rapidly triggers the expression 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.

Results

Subanesthetic low-dose ketamine induces the expression 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 et al., 2016; Schumacher et al., 2016; Phillips et al., 2019; Kim and Cho, 2020; Sun et al., 2020; Watarai et al., 2021; Yoshida et al., 2021; Hong and Kaang, 2022; Tao et al., 2022; Wang and Zhan, 2022). An increase in hippocampal activity reverses stress-induced memory impairment, social dysfunction, and mood disorder-associated behaviors (Bird and Burgess, 2008; Tovote et al., 2015; Miller et al., 2016; Okuyama et al., 2016; Aleksandrova et al., 2020; Kavalali and Monteggia, 2020; Sun et al., 2020; Grieco et al., 2022; Su et al., 2023). More importantly, the hippocampus is selectively targeted by ketamine at the low doses (Davoudian et al., 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 CP-AMPAR expression in hippocampal neurons using whole-cell patch clamping. 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 amplitude and frequency of spontaneous AMPAR-mediated EPSCs (sEPSCs) (V_{hold} = -70 mV) in CA1 pyramidal cells. We found that ketamine treatment significantly elevated the amplitude of sEPSCs in these cells when compared to controls (FEMALE; Saline, 24.67 ± 3.17 pA and Ketamine (Ket), 27.79 ± 3.03 pA, p = 0.0384. MALE; Saline, 23.69 ± 1.82 pA and Ket, 26.80 ± 2.66 pA, p = 0.0034) (**Fig. 1 and Table 1**). To examine whether the ketamine-induced increase in the amplitude of sEPSCs was dependent on CP-AMPARs, we added 20 μM NASPM, a CP-AMPAR antagonist, to the internal solution and measured the amplitude of sEPSCs in CA1 pyramidal cells from both female and male animals in the presence or absence of ketamine. We found that NASPM treatment reversed the ketamine's effects on the amplitude of sEPSCs in these cells (FEMALE; Ket + NASPM, 23.08 ± 3.17 pA, p = 0.0014. MALE; Ket + NASPM, 23.51 ± 3.18 pA, p = 0.0031) (**Fig. 1 and Table 1**). However, NASPM had no

effect on the amplitude of sEPSCs in the absence of ketamine (FEMALE; Saline + NASPM, 23.80 ± 2.02 pA, p = 0.8692. MALE; Saline + NASPM, 22.50 ± 1.26 pA, p = 0.6295) (**Fig. 1 and Table 1**). Interestingly, when compared to controls, we found that ketamine treatment significantly increased the frequency of sEPSCs in hippocampal CA1 pyramidal neurons from only female mice (FEMALE; Saline, 0.79 ± 0.40 Hz and Ket, 1.18 ± 0.51 Hz, p = 0.0455. MALE; Saline, 1.01 ± 0.63 Hz and Ket, 0.87 ± 0.68 Hz, p = 0.9122) (**Fig. 1 and Table 2**). To test whether CP-AMPARs were involved in increased frequency of sEPSCs in ketamine-treated cells of the female hippocampus, 20 μM NASPM was added to block CP-AMAPRs, and we analyzed the frequency of sEPSCs in the presence of ketamine. We found that NASPM treatment significantly decreased the frequency of sEPSCs in these neurons of the female hippocampus (Ket + NASPM, 0.44 ± 0.19 Hz, p < 0.0001) (**Fig. 1and Table 2**). In contrast, NASPM had no effect on the frequency of sEPSCs in neurons from the male hippocampus although there was a trend of a decrease (**Fig. 1 and Table 2**). The lack of statistical significance was largely driven by the lack of an increase in sEPSC frequency with ketamine in males. These findings demonstrated that subanesthetic low-dose ketamine induced the expression of CP-AMPARs to increase synaptic strength in hippocampal CA1 pyramidal neurons.

CRS-induced social dysfunction is reversed by ketamine via the expression of 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 whether ketamine reversed this via the expression of 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 (Yun et al., 2010; Huang et al., 2015; Park et al., 2018). After restraint stress, mice were returned to the home cage. Following 2-week CRS, conducted the reciprocal social interaction test was carried out as shown in the experimental timeline in **Fig. 2**. 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 hr 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-AMPAR expression, 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 the expression of CP-AMPARs.

Repeated stress can impair learning and memory (Kim and 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 the expression of CP-AMPARs. To measure hippocampus-dependent fear memory, contextual fear conditioning 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-AMPAR expression, 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 the expression of CP-AMPARs.

Repeated stress is a known trigger for depression in humans and depression-like behavior in animals (Tafet and Nemeroff, 2016; Richter-Levin and Xu, 2018). Indeed, CRS is known to induce depression-like behavior in rodents (Mao et al., 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 the tail suspension test to examine whether low-dose ketamine reversed CRS-induced depression-like behavior in mice via the expression of 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 the expression of CP-AMPARs.

No abnormality in the open field test

As animals' locomotor capacities can influence behavior, we carried out the open field test 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 the open field test 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 the expression of CP-AMPARs.

Chronic stress activates the hypothalamic-pituitary-adrenal axis, resulting in the release of glucocorticoid class stress hormones (Phillips et al., 2006; McEwen, 2007). Cortisol is the primary endogenous adrenal steroid in most mammals, including humans, whereas corticosterone is the primary adrenal corticosteroid in rodents (Raff et al., 1989; Usa et al., 2007; Raff et al., 2014; Yu et al., 2015). Chronic stress can keep these hormone levels high, which in turn alters brain functions (McEwen, 2007; Burke et al., 2024). In fact, CRS is known to increase corticosterone levels in animals, which is associated with stress-induced behavioral changes (Marin et al., 2007). We thus examined whether low-dose ketamine reversed the CRS-induced elevation of corticosterone levels via the expression of CP-AMPARs. As shown previously (Tan et al., 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 $\pm$ 376.8 pg/ml and CRS, 2374 $\pm$ 1101 pg/ml, $p = 0.0001$. MALE; Naïve, 756.1 $\pm$ 210.6 pg/ml and CRS, 2140 $\pm$ 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 $\pm$ 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 the expression of CP-AMPARs.

Discussion

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 (Miller et al., 2016; Aleksandrova et al., 2020; Kavalali and Monteggia, 2020). 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.

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 (Li et al., 2011; Fischell et al., 2015). 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 (Thiagarajan et al., 2005; Goel and Lee, 2007; Aoto et al., 2008; Goel et al., 2011; Lee, 2012; Kim and Ziff, 2014; Kim et al., 2015a; Sanderson et al., 2016; Sanderson et al., 2018; Purkey and Dell'Acqua, 2020). We have indeed shown that ketamine-induced CP-AMPAR expression enhances glutamatergic synaptic strength in cultured neurons (Zaytseva et al., 2023). 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 (Bird and Burgess, 2008; Tovote et al., 2015; Miller et al., 2016; Okuyama et al.,

2016; Aleksandrova et al., 2020; Kavalali and Monteggia, 2020; Sun et al., 2020; Grieco et al., 2022), 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 CP-AMPAR expression 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-AMPAR expression (**Fig.8**). This suggests that low-dose ketamine induces the expression of CP-AMPARs in the hippocampus, which in turn enhances synaptic strength to reverse hippocampus-dependent behavioral dysfunctions in chronically stressed animals.

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 and 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 et al., 2020), how ketamine enhances synaptic Ca^{2+} signaling is not fully understood. 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 expression 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.

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 (Miller et al., 2016; Yang et al., 2018; Kavalali and Monteggia, 2020; Ma et

al., 2023; Zaytseva et al., 2023; Chen et al., 2024); *2)* NMDAR inhibition-independent, ketamine metabolite-dependent mechanism (Carrier and Kabbaj, 2013; Franceschelli et al., 2015; Zanos et al., 2016); and *3)* NMDAR inhibition-induced disinhibition- and BDNF-induced protein synthesis-dependent mechanism (Deyama and 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 et al., 2013; Yang et al., 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 and 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.

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 et al., 2016; Zanos et al., 2016; Moda-Sava et al., 2019). More recently, other areas of the brain have been investigated, including the lateral habenula (Yang et al., 2018; Ma et al., 2023; Chen et al., 2024). 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 primarily targets the hippocampus (Davoudian et al., 2022). The hippocampus is a crucial brain area that governs learning, memory, social interaction, and depression (Jarrard, 1993; Fortin et al., 2002; Campbell and Macqueen, 2004; Bird and Burgess, 2008; Xu et al., 2021; Ghasemi et al., 2022). Research also demonstrates that an increase in hippocampal activity can be protective against stress (Bird and Burgess, 2008; Tovote et al., 2015; Miller et al., 2016; Okuyama et al., 2016; Aleksandrova et al., 2020; Kavalali and Monteggia, 2020; Sun et al., 2020; Grieco et al., 2022). Importantly, one of the most vulnerable targets of stress is hippocampus, because it abundantly expresses both glucocorticoid (stress hormone) and mineralocorticoid receptors (stress hormone receptors) (Uno et al., 1989; Smith, 1996). Moreover, stress changes hippocampal neural activity and synaptic plasticity, activating hippocampal glucocorticoid receptors, and decreasing neuronal cell survival and neurogenesis (Kim

and Diamond, 2002; Egeland et al., 2015; 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 cross-talk from the hippocampus to other brain regions including the lateral habenula for better understanding of the ketamine's therapeutic effects.

Chronic stress is known to increase the release of corticosterone in animals (Phillips et al., 2006; McEwen, 2007). 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 et al., 2017). Moreover, chronic inflammatory responses directly affect brain structure and function, resulting in pathological behaviors in animals (McEwen et al., 2016). Importantly, ketamine is known to be associated with anti-inflammatory effects in humans (Dale et al., 2012; Nikkheslat, 2021) and animals (Wang et al., 2015; Spencer et al., 2022; Ommati et al., 2023), 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 (Zhang et al., 2017; Getachew et al., 2018) and priming of Type 1 T helper (Th1)-type immune response (Ohta et al., 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.

In summary, 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 the expression of CP-AMPARs. Therefore, our work advances current understandings on the mechanisms of ketamine's antistress effects to guide the developments of new therapies.

Materials and 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. CSU's Institutional Animal Care and Use Committee (IACUC) reviewed and approved the animal care and protocol (3408).

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. 2**.

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). 20 μM intracellular 1-Naphthyl acetyl spermine trihydrochloride (NASPM, Tocris Bioscience) was used to block CP-AMPARs in hippocampal neurons (Kim and Ziff, 2014; Zaytseva et al., 2023). 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 (Wiltgen et al., 2010; Szczurowska and Mares, 2015; Adotevi et al., 2020). 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 the expression of CP-AMPARs.

Brain slice preparation

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.

Electrophysiology

Whole-cell patch-clamp recording procedures used in the present study were similar to those described previously (Govindaiah et al., 2018; Kang et al., 2018; Kang et al., 2020). 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 the amplitude and frequency of spontaneous AMPAR-mediated EPSCs (sEPSCs) (V_{hold} = -70 mV) in CA1 pyramidal cells and analyzed them using the Mini Analysis software (Synaptosoft) (Kim and Ziff, 2014; Kim et al., 2015b; Kim et al., 2015a).

Reciprocal social interaction

We performed the reciprocal social interaction test to examine social interaction between two freely moving mice (Bolivar et al., 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 open field 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.

Open field test (OFT)

We measured locomotor activity and anxiety-like behavior using the open field test as carried out previously (Shou et al., 2018; Kang et al., 2021; Zaytseva et al., 2023). The 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).

Contextual fear conditioning (CFC)

Contextual fear conditioning (Habitest Modular System, Coulbourn Instrument) was carried out as described previously (Kim et al., 2016; Shou et al., 2018; Lee et al., 2024). On Day 1 (encoding), the 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 for overnight (consolidation). A contextual memory test was conducted the next day (Day 2 - retrieval) 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).

Tail suspension test (TST)

Studies suggest that the forced swim test and the tail suspension test are based on the same principle: measurement of the duration of immobility when rodents are exposed to an inescapable situation (Castagne et al., 2011). Importantly, it has been suggested that the tail suspension test is more sensitive to antidepressant agents than the forced swim test because the animals remain immobile longer in the tail suspension test than the forced swim test (Cryan et al., 2005). We thus used the tail suspension test to examine depression-like behavior as described previously (Kim et al., 2016; Kim et al., 2018; Zaytseva et al., 2023). The 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. Mice that successfully climbed their tails to escape were excluded from the analysis. 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).

Corticosterone

Corticosterone levels in mice were measured as described previously (Luo et al., 2016; McCosh et al., 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).

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.

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

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

Aleksandrova LR, Phillips AG, Wang YT (2017) Antidepressant effects of ketamine and the roles of AMPA glutamate receptors and other mechanisms beyond NMDA receptor antagonism. J Psychiatry Neurosci 42:222-229.

Aleksandrova LR, Wang YT, Phillips AG (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 13:92.

Anis NA, Berry SC, Burton NR, Lodge D (1983) The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. British journal of pharmacology 79:565-575.

Aoto J, Nam CI, Poon MM, Ting P, Chen L (2008) Synaptic signaling by all-trans retinoic acid in homeostatic synaptic plasticity. Neuron 60:308-320.

Bird CM, Burgess N (2008) The hippocampus and memory: insights from spatial processing. Nat Rev Neurosci 9:182-194.

Bolivar VJ, Walters SR, Phoenix JL (2007) Assessing autism-like behavior in mice: variations in social interactions among inbred strains. Behavioural brain research 176:21-26.

Brachman RA, McGowan JC, Perusini JN, Lim SC, Pham TH, Faye C, Gardier AM, Mendez-David I, David DJ, Hen R, Denny CA (2016) Ketamine as a Prophylactic Against Stress-Induced Depressive-like Behavior. Biological psychiatry 79:776-786.

Burke MR, Sotiropoulos I, Waites CL (2024) The multiple roles of chronic stress and glucocorticoids in Alzheimer's disease pathogenesis. Trends Neurosci.

Campbell S, Macqueen G (2004) The role of the hippocampus in the pathophysiology of major depression. J Psychiatry Neurosci 29:417-426.

Carreno FR, Donegan JJ, Boley AM, Shah A, DeGuzman M, Frazer A, Lodge DJ (2016) Activation of a ventral hippocampus-medial prefrontal cortex pathway is both necessary and sufficient for an antidepressant response to ketamine. Molecular psychiatry 21:1298-1308.

Carrier N, Kabbaj M (2013) Sex differences in the antidepressant-like effects of ketamine. Neuropharmacology 70:27-34.

Castagne V, Moser P, Roux S, Porsolt RD (2011) Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Current protocols in neuroscience / editorial board, Jacqueline N Crawley [et al] Chapter 8:Unit 8 10A.

Chen M, Ma S, Liu H, Dong Y, Tang J, Ni Z, Tan Y, Duan C, Li H, Huang H, Li Y, Cao X, Lingle CJ, Yang Y, Hu H (2024) Brain region-specific action of ketamine as a rapid antidepressant. Science 385:eado7010.

Cryan JF, Mombereau C, Vassout A (2005) The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neuroscience and biobehavioral reviews 29:571-625.

Dale O, Somogyi AA, Li Y, Sullivan T, Shavit Y (2012) Does intraoperative ketamine attenuate inflammatory reactivity following surgery? A systematic review and meta-analysis. Anesth Analg 115:934-943.

daSilva AW, Huckins JF, Wang W, Wang R, Campbell AT, Meyer ML (2021) Daily perceived stress predicts less next day social interaction: Evidence from a naturalistic mobile sensing study. Emotion 21:1760-1770.

Davoudian PA, Shao L-X, Kwan AC (2022) Shared and distinct brain regions targeted for immediate early gene expression by ketamine and psilocybin. bioRxiv:2022.2003.2018.484437.

Davoudian PA, Shao LX, Kwan AC (2023) Shared and Distinct Brain Regions Targeted for Immediate Early Gene Expression by Ketamine and Psilocybin. ACS Chem Neurosci 14:468-480.

Deyama S, Duman RS (2020) Neurotrophic mechanisms underlying the rapid and sustained antidepressant actions of ketamine. Pharmacol Biochem Behav 188:172837.

Diering GH, Huganir RL (2018) The AMPA Receptor Code of Synaptic Plasticity. Neuron 100:314-329.

Duman RS, Aghajanian GK, Sanacora G, Krystal JH (2016) Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nature medicine 22:238-249.

Egeland M, Zunszain PA, Pariante CM (2015) Molecular mechanisms in the regulation of adult neurogenesis during stress. Nat Rev Neurosci 16:189-200.

Feder A, Parides MK, Murrough JW, Perez AM, Morgan JE, Saxena S, Kirkwood K, Aan Het Rot M, Lapidus KA, Wan LB, Iosifescu D, Charney DS (2014) Efficacy of intravenous ketamine for treatment of chronic posttraumatic stress disorder: a randomized clinical trial. JAMA Psychiatry 71:681-688.

Felix-Ortiz AC, Burgos-Robles A, Bhagat ND, Leppla CA, Tye KM (2016) Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex. Neuroscience 321:197-209.

Fischell J, Van Dyke AM, Kvarta MD, LeGates TA, Thompson SM (2015) Rapid Antidepressant Action and Restoration of Excitatory Synaptic Strength After Chronic Stress by Negative Modulators of Alpha5-Containing GABAA Receptors. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 40:2499-2509.

Fortin NJ, Agster KL, Eichenbaum HB (2002) Critical role of the hippocampus in memory for sequences of events. Nat Neurosci 5:458-462.

Franceschelli A, Sens J, Herchick S, Thelen C, Pitychoutis PM (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.

Gerhard DM, Pothula S, Liu RJ, Wu M, Li XY, Girgenti MJ, Taylor SR, Duman CH, Delpire E, Picciotto M, Wohleb ES, Duman RS (2020) GABA interneurons are the cellular trigger for ketamine's rapid antidepressant actions. J Clin Invest 130:1336-1349.

Getachew B, Aubee JI, Schottenfeld RS, Csoka AB, Thompson KM, Tizabi Y (2018) Ketamine interactions with gut-microbiota in rats: relevance to its antidepressant and anti-inflammatory properties. BMC Microbiol 18:222.

Ghasemi M, Navidhamidi M, Rezaei F, Azizikia A, Mehranfard N (2022) Anxiety and hippocampal neuronal activity: Relationship and potential mechanisms. Cogn Affect Behav Neurosci 22:431-449.

Gill H, Gill B, Rodrigues NB, Lipsitz O, Rosenblat JD, El-Halabi S, Nasri F, Mansur RB, Lee Y, McIntyre RS (2021) The Effects of Ketamine on Cognition in Treatment-Resistant Depression: A Systematic Review and Priority Avenues for Future Research. Neuroscience and biobehavioral reviews 120:78-85.

Goel A, Lee HK (2007) Persistence of experience-induced homeostatic synaptic plasticity through adulthood in superficial layers of mouse visual cortex. J Neurosci 27:6692-6700.

Goel A, Xu LW, Snyder KP, Song L, Goenaga-Vazquez Y, Megill A, Takamiya K, Huganir RL, Lee HK (2011) Phosphorylation of AMPA receptors is required for sensory deprivation-induced homeostatic synaptic plasticity. PLoS One 6:e18264.

Govindaiah G, Kang YJ, Lewis HES, Chung L, Clement EM, Greenfield LJ, Jr., Garcia-Rill E, Lee SH (2018) Group I metabotropic glutamate receptors generate two types of intrinsic membrane oscillations in hippocampal oriens/alveus interneurons. Neuropharmacology 139:150-162.

Grieco SF, Castren E, Knudsen GM, Kwan AC, Olson DE, Zuo Y, Holmes TC, Xu X (2022) Psychedelics and Neural Plasticity: Therapeutic Implications. J Neurosci 42:8439-8449.

Hare BD, Shinohara R, Liu RJ, Pothula S, DiLeone RJ, Duman RS (2019) Optogenetic stimulation of medial prefrontal cortex Drd1 neurons produces rapid and long-lasting antidepressant effects. Nat Commun 10:223.

He JG, Zhou HY, Wang F, Chen JG (2023) Dysfunction of Glutamatergic Synaptic Transmission in Depression: Focus on AMPA Receptor Trafficking. Biol Psychiatry Glob Open Sci 3:187-196.

Hong I, Kaang BK (2022) The complexity of ventral CA1 and its multiple functionalities. Genes Brain Behav 21:e12826.

Huang P, Li C, Fu T, Zhao D, Yi Z, Lu Q, Guo L, Xu X (2015) Flupirtine attenuates chronic restraint stress-induced cognitive deficits and hippocampal apoptosis in male mice. Behavioural brain research 288:1-10.

Jarrard LE (1993) On the role of the hippocampus in learning and memory in the rat. Behav Neural Biol 60:9-26.

Kallarackal AJ, Kvarta MD, Cammarata E, Jaberi L, Cai X, Bailey AM, Thompson SM (2013) Chronic stress induces a selective decrease in AMPA receptor-mediated synaptic excitation at hippocampal temporoammonic-CA1 synapses. J Neurosci 33:15669-15674.

Kang YJ, Clement EM, Park IH, Greenfield LJ, Jr., Smith BN, Lee SH (2021) Vulnerability of cholecystokinin-expressing GABAergic interneurons in the unilateral intrahippocampal kainate mouse model of temporal lobe epilepsy. Experimental neurology 342:113724.

Kang YJ, Lewis HES, Young MW, Govindaiah G, Greenfield LJ, Jr., Garcia-Rill E, Lee SH (2018) Cell Type-specific Intrinsic Perithreshold Oscillations in Hippocampal GABAergic Interneurons. Neuroscience 376:80-93.

Kang YJ, Clement EM, Sumsky SL, Xiang Y, Park IH, Santaniello S, Greenfield LJ, Jr., Garcia-Rill E, Smith BN, Lee SH (2020) The critical role of persistent sodium current in hippocampal gamma oscillations. Neuropharmacology 162:107787.

Kavalali ET, Monteggia LM (2020) Targeting Homeostatic Synaptic Plasticity for Treatment of Mood Disorders. Neuron 106:715-726.

Kessler RC (1997) The effects of stressful life events on depression. Annu Rev Psychol 48:191-214.

Kim EJ, Kim JJ (2023) Neurocognitive effects of stress: a metaparadigm perspective. Molecular psychiatry 28:2750-2763.

Kim JJ, Diamond DM (2002) The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 3:453-462.

Kim S, Ziff EB (2014) Calcineurin mediates synaptic scaling via synaptic trafficking of Ca2+-permeable AMPA receptors. PLoS biology 12:e1001900.

Kim S, Violette CJ, Ziff EB (2015a) Reduction of increased calcineurin activity rescues impaired homeostatic synaptic plasticity in presenilin 1 M146V mutant. Neurobiol Aging 36:3239-3246.

Kim S, Shou J, Abera S, Ziff EB (2018) Sucrose withdrawal induces depression and anxiety-like behavior by Kir2.1 upregulation in the nucleus accumbens. Neuropharmacology 130:10-17.

Kim S, Titcombe RF, Zhang H, Khatri L, Girma HK, Hofmann F, Arancio O, Ziff EB (2015b) Network compensation of cyclic GMP-dependent protein kinase II knockout in the hippocampus by Ca2+-permeable AMPA receptors. Proc Natl Acad Sci U S A 112:3122-3127.

Kim S, Pick JE, Abera S, Khatri L, Ferreira DD, Sathler MF, Morison SL, Hofmann F, Ziff EB (2016) Brain region-specific effects of cGMP-dependent kinase II knockout on AMPA receptor trafficking and animal behavior. Learning & memory 23:435-441.

Kim WB, Cho JH (2020) Encoding of contextual fear memory in hippocampal-amygdala circuit. Nat Commun 11:1382.

Kohtala S (2021) Ketamine-50 years in use: from anesthesia to rapid antidepressant effects and neurobiological mechanisms. Pharmacol Rep 73:323-345.

Lee EH, Park JY, Kwon HJ, Han PL (2021) Repeated exposure with short-term behavioral stress resolves pre-existing stress-induced depressive-like behavior in mice. Nat Commun 12:6682.

Lee HK (2012) Ca-permeable AMPA receptors in homeostatic synaptic plasticity. Frontiers in molecular neuroscience 5:17.

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.

Li N, Liu RJ, Dwyer JM, Banasr M, Lee B, Son H, Li XY, Aghajanian G, Duman RS (2011) Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biological psychiatry 69:754-761.

Lisek M, Zylinska L, Boczek T (2020) Ketamine and Calcium Signaling-A Crosstalk for Neuronal Physiology and Pathology. Int J Mol Sci 21.

Liu YZ, Wang YX, Jiang CL (2017) Inflammation: The Common Pathway of Stress-Related Diseases. Front Hum Neurosci 11:316.

Luo E, Stephens SB, Chaing S, Munaganuru N, Kauffman AS, Breen KM (2016) Corticosterone Blocks Ovarian Cyclicity and the LH Surge via Decreased Kisspeptin Neuron Activation in Female Mice. Endocrinology 157:1187-1199.

Lupien SJ, McEwen BS, Gunnar MR, Heim C (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci 10:434-445.

Ma JH, Wang SY, Yu HY, Li DY, Luo SC, Zheng SS, Wan LF, Duan KM (2019) Prophylactic use of ketamine reduces postpartum depression in Chinese women undergoing cesarean section(☆). Psychiatry Res 279:252-258.

Ma L, Wang L, Chang L, Shan J, Qu Y, Wang X, Wan X, Fujita Y, 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 12:417.

Ma S, Chen M, Jiang Y, Xiang X, Wang S, Wu Z, Li S, Cui Y, Wang J, Zhu Y, Zhang Y, Ma H, Duan S, Li H, Yang Y, Lingle CJ, Hu H (2023) Sustained antidepressant effect of ketamine through NMDAR trapping in the LHb. Nature.

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 50:3000605221075816.

Marin MT, Cruz FC, Planeta CS (2007) Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. Physiology & behavior 90:29-35.

Matsuo N, Tanda K, Nakanishi K, Yamasaki N, Toyama K, Takao K, Takeshima H, 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.

McCosh RB, Kreisman MJ, Tian K, Thomas SA, Breen Church KM (2024) Norepinephrine Neurons in the Nucleus of the Solitary Tract Suppress Luteinizing Hormone Secretion in Female Mice. J Neurosci 44.

McEwen BS (2007) Physiology and neurobiology of stress and adaptation: central role of the brain. Physiological reviews 87:873-904.

McEwen BS, Nasca C, Gray JD (2016) Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 41:3-23.

Miller OH, Moran JT, Hall BJ (2016) Two cellular hypotheses explaining the initiation of ketamine's antidepressant actions: Direct inhibition and disinhibition. Neuropharmacology 100:17-26.

Moda-Sava RN, Murdock MH, Parekh PK, Fetcho RN, Huang BS, Huynh TN, Witztum J, Shaver DC, Rosenthal DL, Alway EJ, Lopez K, Meng Y, Nellissen L, Grosenick L, Milner TA, Deisseroth K, Bito H, Kasai H, Liston C (2019) Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation. Science 364.

Ng SH, Tse ML, Ng HW, Lau FL (2010) Emergency department presentation of ketamine abusers in Hong Kong: a review of 233 cases. Hong Kong Med J 16:6-11.

Nikkheslat N (2021) Targeting inflammation in depression: Ketamine as an anti-inflammatory antidepressant in psychiatric emergency. Brain Behav Immun Health 18:100383.

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 109:793-800.

Okuyama T, Kitamura T, Roy DS, Itohara S, Tonegawa S (2016) Ventral CA1 neurons store social memory. Science 353:1536-1541.

Ommati MM, Mobasheri A, Niknahad H, Rezaei M, Alidaee S, Arjmand A, Mazloomi S, Abdoli N, Sadeghian I, Sabouri S, Saeed M, Mousavi K, Najibi 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:e23468.

Park MJ, Seo BA, Lee B, Shin HS, Kang MG (2018) Stress-induced changes in social dominance are scaled by AMPA-type glutamate receptor phosphorylation in the medial prefrontal cortex. Sci Rep 8:15008.

Phillips LJ, McGorry PD, Garner B, Thompson KN, Pantelis C, Wood SJ, Berger G (2006) Stress, the hippocampus and the hypothalamic-pituitary-adrenal axis: implications for the development of psychotic disorders. Aust N Z J Psychiatry 40:725-741.

Phillips ML, Robinson HA, Pozzo-Miller L (2019) Ventral hippocampal projections to the medial prefrontal cortex regulate social memory. Elife 8.

Purkey AM, Dell'Acqua ML (2020) Phosphorylation-Dependent Regulation of Ca(2+)-Permeable AMPA Receptors During Hippocampal Synaptic Plasticity. Front Synaptic Neurosci 12:8.

Raff H, Skelton MM, Cowley AW, Jr. (1989) Feedback control of vasopressin and corticotrophin secretion in conscious dogs: effect of hypertonic saline. J Endocrinol 122:41-48.

Raff H, Sharma ST, Nieman LK (2014) Physiological basis for the etiology, diagnosis, and treatment of adrenal disorders: Cushing's syndrome, adrenal insufficiency, and congenital adrenal hyperplasia. Compr Physiol 4:739-769.

Richter-Levin G, Xu L (2018) How could stress lead to major depressive disorder? IBRO Rep 4:38-43.

Ryu JR, Hong CJ, Kim JY, Kim EK, Sun W, Yu SW (2016) Control of adult neurogenesis by programmed cell death in the mammalian brain. Mol Brain 9:43.

Sanderson JL, Gorski JA, Dell'Acqua ML (2016) NMDA Receptor-Dependent LTD Requires Transient Synaptic Incorporation of Ca(2)(+)-Permeable AMPARs Mediated by AKAP150-Anchored PKA and Calcineurin. Neuron 89:1000-1015.

Sanderson JL, Scott JD, Dell'Acqua ML (2018) Control of Homeostatic Synaptic Plasticity by AKAP-Anchored Kinase and Phosphatase Regulation of Ca(2+)-Permeable AMPA Receptors. J Neurosci.

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

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.

Silva JCH, Proulx CD (2024) Locking away depression. Science 385:608-609.

Smith MA (1996) Hippocampal vulnerability to stress and aging: possible role of neurotrophic factors. Behavioural brain research 78:25-36.

Spencer HF, Berman RY, Boese M, Zhang M, Kim SY, Radford KD, Choi KH (2022) Effects of an intravenous ketamine infusion on inflammatory cytokine levels in male and female Sprague-Dawley rats. J Neuroinflammation 19:75.

Su T, Lu Y, Fu C, Geng Y, Chen Y (2023) GluN2A mediates ketamine-induced rapid antidepressant-like responses. Nat Neurosci 26:1751-1761.

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 23:100894.

Szczurowska E, Mares P (2015) An antagonist of calcium permeable AMPA receptors, IEM1460: Anticonvulsant action in immature rats? Epilepsy Res 109:106-113.

Tafet GE, Nemeroff CB (2016) The Links Between Stress and Depression: Psychoneuroendocrinological, Genetic, and Environmental Interactions. J Neuropsychiatry Clin Neurosci 28:77-88.

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 40:1260-1267.

Tao K, Chung M, Watarai A, Huang Z, Wang MY, Okuyama T (2022) Disrupted social memory ensembles in the ventral hippocampus underlie social amnesia in autism-associated Shank3 mutant mice. Molecular psychiatry 27:2095-2105.

Taylor JH, Landeros-Weisenberger A, Coughlin C, Mulqueen J, Johnson JA, Gabriel D, Reed MO, Jakubovski E, Bloch MH (2018) Ketamine for Social Anxiety Disorder: A Randomized, Placebo-Controlled

Crossover Trial. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 43:325-333.

Thiagarajan TC, Lindskog M, Tsien RW (2005) Adaptation to synaptic inactivity in hippocampal neurons. Neuron 47:725-737.

Tovote P, Fadok JP, Luthi A (2015) Neuronal circuits for fear and anxiety. Nat Rev Neurosci 16:317-331.

Uno H, Tarara R, Else JG, Suleman MA, Sapolsky RM (1989) Hippocampal damage associated with prolonged and fatal stress in primates. J Neurosci 9:1705-1711.

Usa K, Singh RJ, Netzel BC, Liu Y, Raff H, Liang M (2007) Renal interstitial corticosterone and 11-dehydrocorticosterone in conscious rats. Am J Physiol Renal Physiol 293:F186-192.

Wang N, Yu HY, Shen XF, Gao ZQ, Yang C, Yang JJ, Zhang GF (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 120:241-248.

Wang X, Zhan Y (2022) Regulation of Social Recognition Memory in the Hippocampal Circuits. Frontiers in neural circuits 16:839931.

Watarai A, Tao K, Wang MY, Okuyama T (2021) Distinct functions of ventral CA1 and dorsal CA2 in social memory. Curr Opin Neurobiol 68:29-35.

Wesierska MJ, Duda W, Dockery CA (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.

Wiltgen BJ, Royle GA, Gray EE, Abdipranoto A, Thangthaeng N, Jacobs N, Saab F, Tonegawa S, Heinemann SF, O'Dell TJ, Fanselow MS, Vissel B (2010) A role for calcium-permeable AMPA receptors in synaptic plasticity and learning. PLoS One 5.

Wu H, Savalia NK, Kwan AC (2021) Ketamine for a Boost of Neural Plasticity: How, but Also When? Biological psychiatry 89:1030-1032.

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. Frontiers in neural circuits 15:768294.

Yang B, Ren Q, Ma M, Chen QX, Hashimoto K (2016) Antidepressant Effects of (+)-MK-801 and (-)-MK-801 in the Social Defeat Stress Model. Int J Neuropsychopharmacol 19.

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 554:317-322.

Yoshida K, Drew MR, Kono A, Mimura M, Takata N, Tanaka KF (2021) Chronic social defeat stress impairs goal-directed behavior through dysregulation of ventral hippocampal activity in male mice. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 46:1606-1616.

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.

Yun J, Koike H, Ibi D, Toth E, Mizoguchi H, Nitta A, Yoneyama M, Ogita K, Yoneda Y, Nabeshima T, Nagai T, Yamada K (2010) Chronic restraint stress impairs neurogenesis and hippocampus-dependent fear memory in mice: possible involvement of a brain-specific transcription factor Npas4. Journal of neurochemistry 114:1840-1851.

Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, Alkondon M, Yuan P, Pribut HJ, Singh NS, Dossou KS, Fang Y, Huang XP, Mayo CL, Wainer IW, Albuquerque EX, Thompson SM, Thomas CJ, Zarate CA, Jr., Gould TD (2016) NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature 533:481-486.

Zaytseva A, Bouckova E, Wiles MJ, Wustrau MH, Schmidt IG, Mendez-Vazquez H, Khatri L, Kim S (2023) Ketamine's rapid antidepressant effects are mediated by Ca(2+)-permeable AMPA receptors. Elife 12.

Zhang JC, 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:e1138.

Zhou Y, Wang C, Lan X, Zheng W, Li H, Chao Z, Wu K, McIntyre RS, 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.

Zucker RS (1999) Calcium- and activity-dependent synaptic plasticity. Curr Opin Neurobiol 9:305-313.

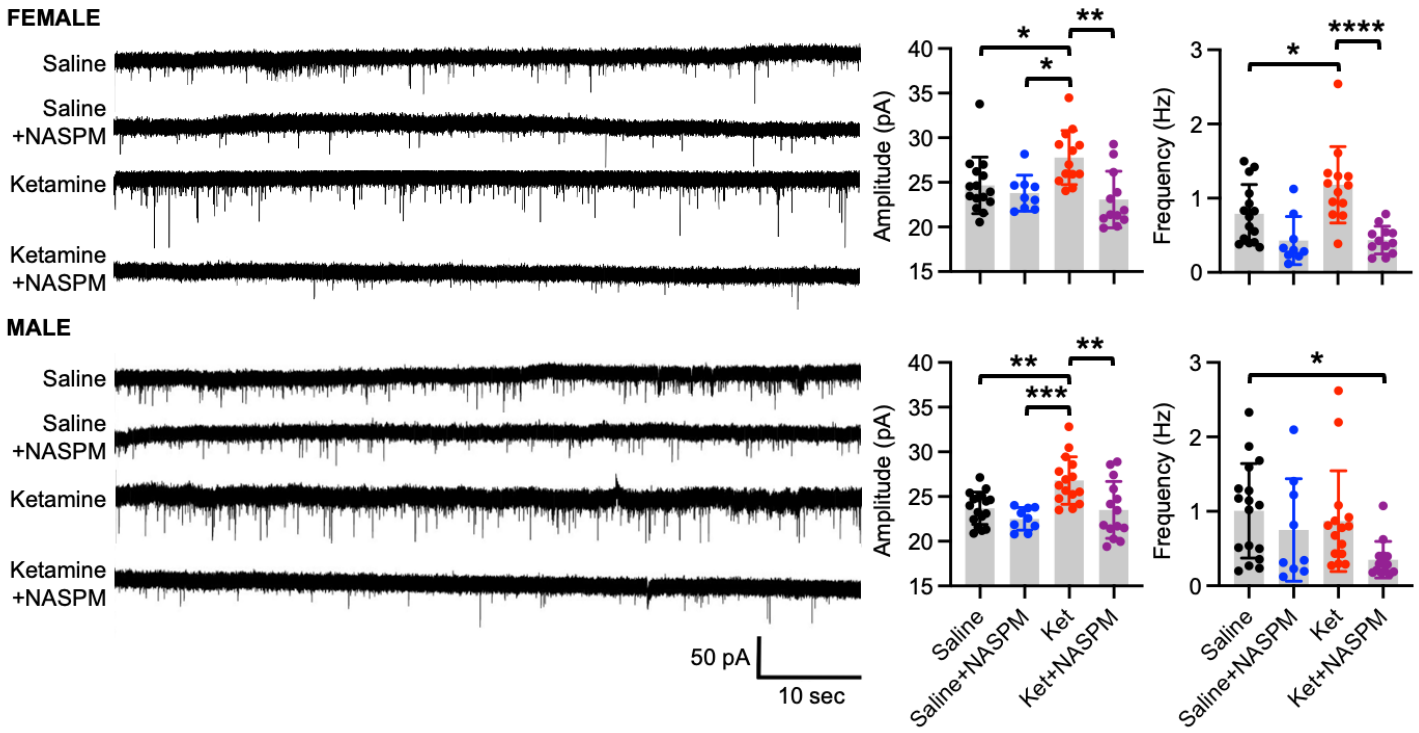

Figure 1. Subanesthetic low-dose ketamine induces the expression of CP-AMPARs in hippocampal CA1 pyramidal neurons. Representative traces and summary of the amplitude and frequency of sEPSCs in each condition (n = number of cells [n = number of animals]. Female; Saline = 15 [5], Saline + NASPM [3] = 9, Ketamine (Ket) [5] = 13, and Ket + NASPM [3] = 12. Male; Saline = 17 [12], Saline + NASPM [3] = 9, Ket [8] = 15, and Ket + NASPM [5] = 14). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$, Two-Way ANOVA, Tukey test.

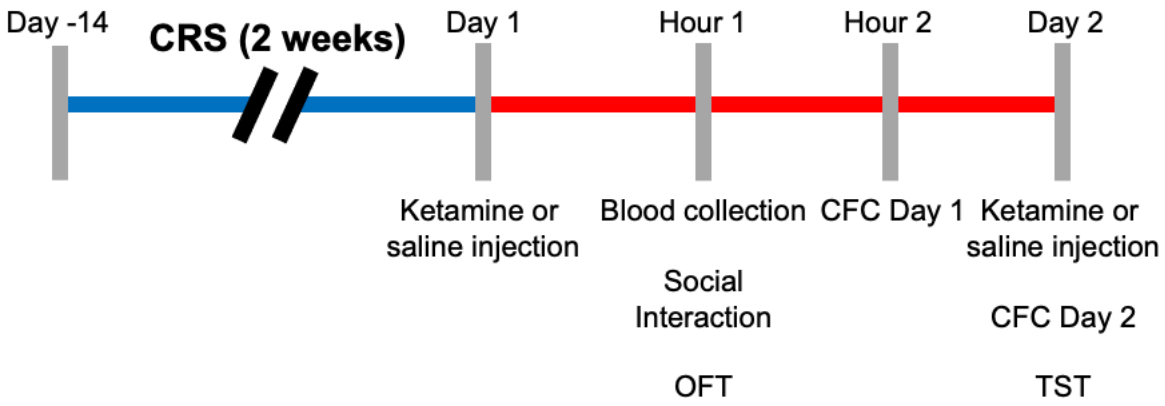

Figure 2. Experimental timeline of chronic restraint stress (CRS) that is applied to mice for 2 weeks, drug treatment, blood collection, and behavioral tests - reciprocal social interaction test, open-field test (OFT), contextual fear conditioning (CFC) day 1 and 2, and tail suspension test (TST). Single intraperitoneal injection of ketamine (5 mg/kg for females and 10 mg/kg for males) or saline in CRS mice.

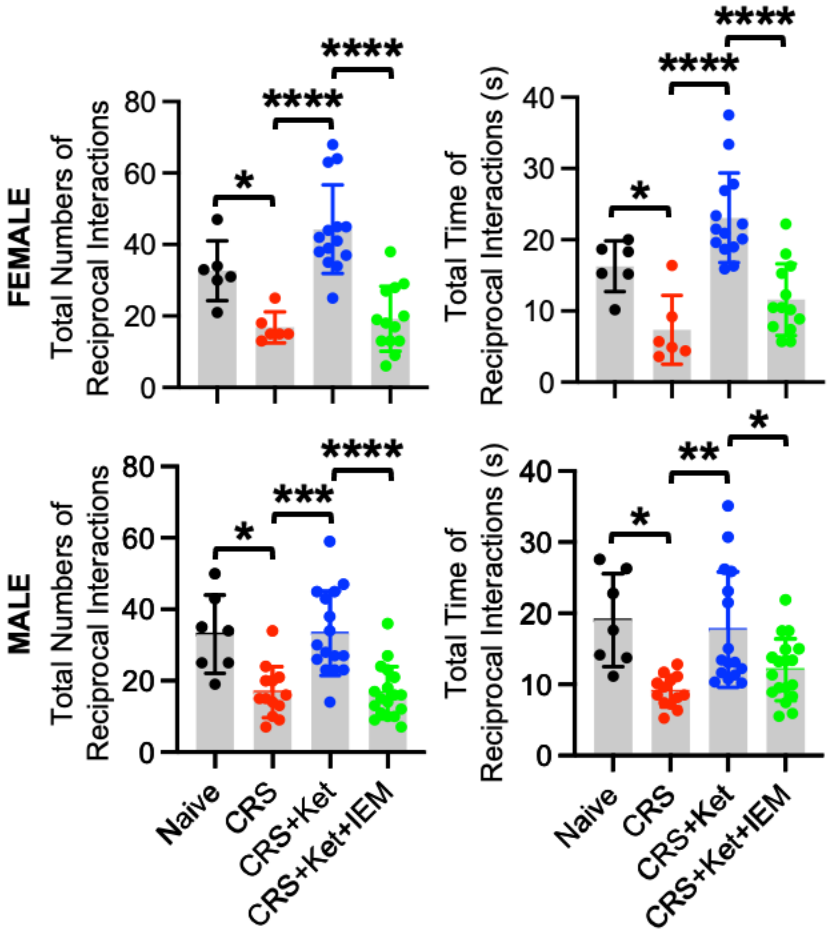

Figure 3. CRS-induced social dysfunction is reversed by ketamine via the expression of CP-AMPARs. Summary of total numbers and time of reciprocal interactions in each condition (n = number of animals. Female; Naïve = 5, CRS = 6, CRS + Ket = 14, and CRS + Ket + IEM = 13. Male; Naïve = 7, CRS = 12, CRS + 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.

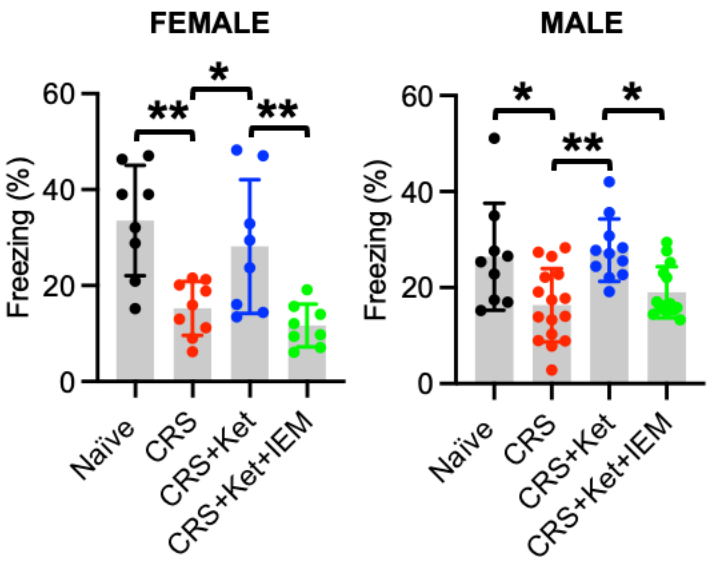

Figure 4. CRS-induced fear memory loss is reversed by ketamine via the expression of CP-AMPARs.

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.

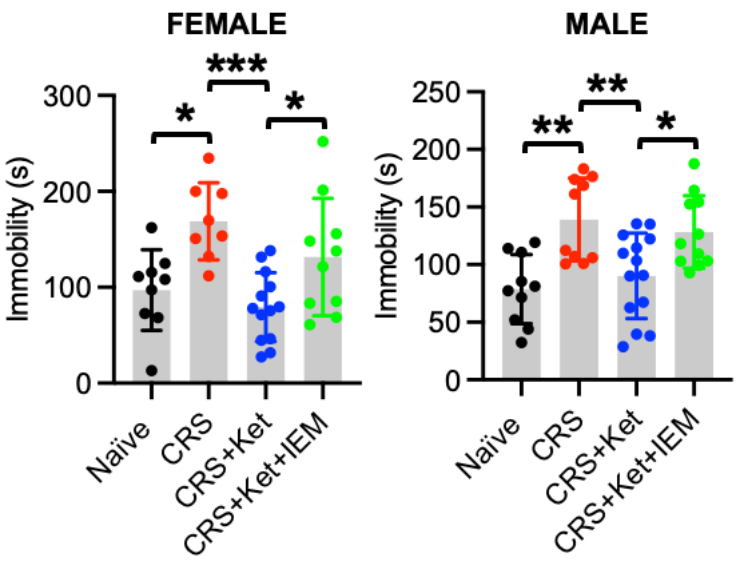

Figure 5. CRS-induced depression-like behavior is reversed by ketamine via the expression of CP-AMPARs. Summary of immobility in each condition (n = number of animals. Female; Naïve = 9, CRS = 8, CRS + Ket = 13, and CRS + Ket + IEM = 10. Male; Naïve = 10, CRS = 10, CRS + Ket = 14, and CRS + Ket + IEM = 11). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, One-Way ANOVA, Tukey test.

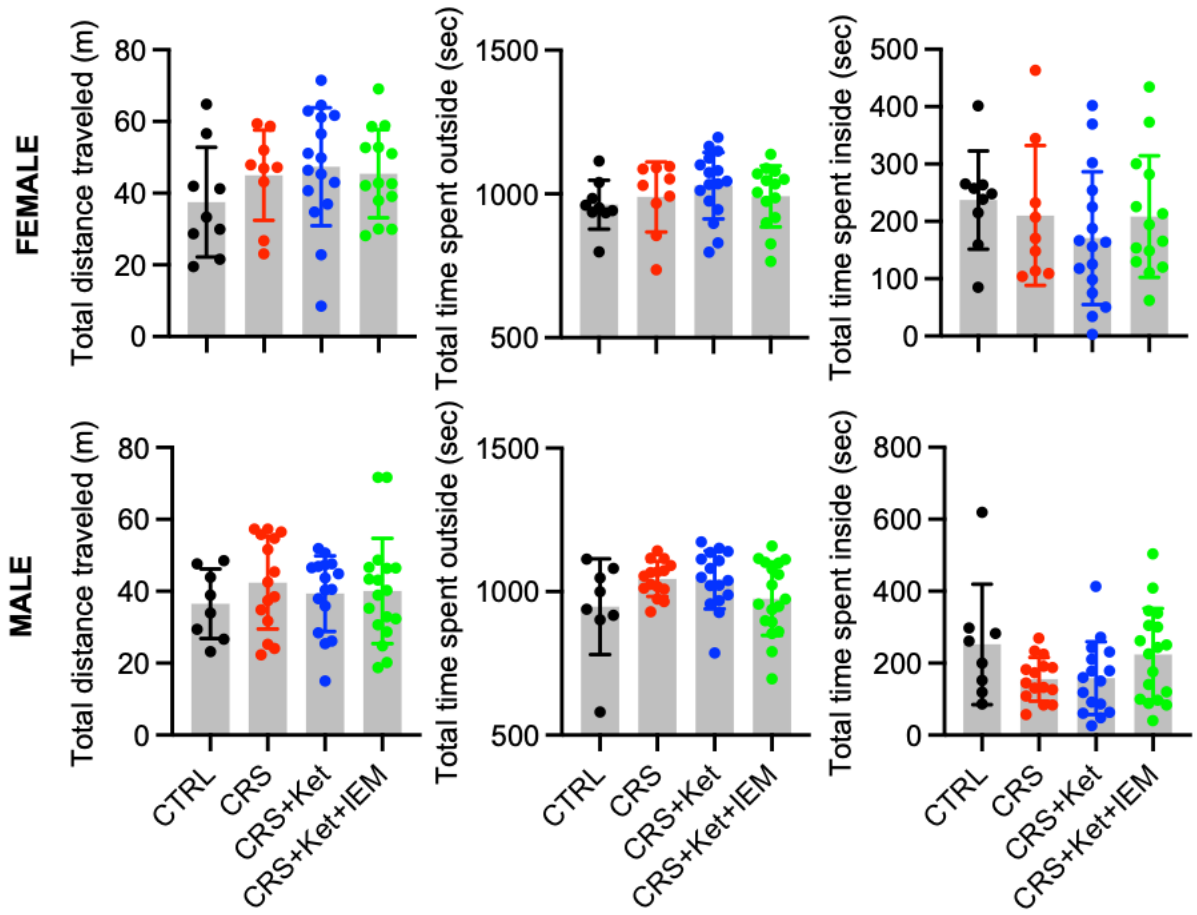

Figure 6. No abnormality in the open field test. Summary of total distance traveled, total time spent outside, and total time spent inside in each condition (n = number of animals. Female; Naïve = 9, CRS = 9, CRS + Ket = 16, and CRS + Ket + IEM = 14. Male; Naïve = 8, CRS = 15, CRS + Ket = 16, and CRS + Ket + IEM = 18).

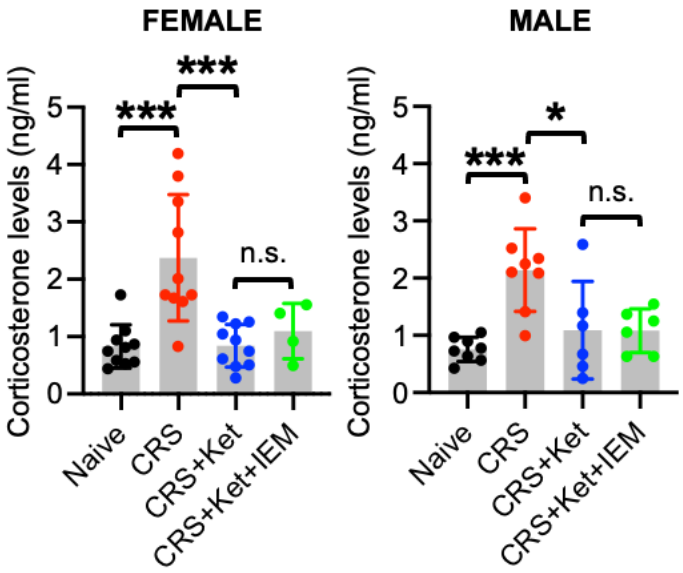

Figure 7. Ketamine reverses the CRS-induced elevation of corticosterone levels, which is not dependent on the expression of CP-AMPARs. Summary of serum corticosterone levels in each condition (n = number of animals with duplication. Female; Naïve = 5, CRS = 5, CRS + Ket = 5, and CRS + Ket + IEM = 2. 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.

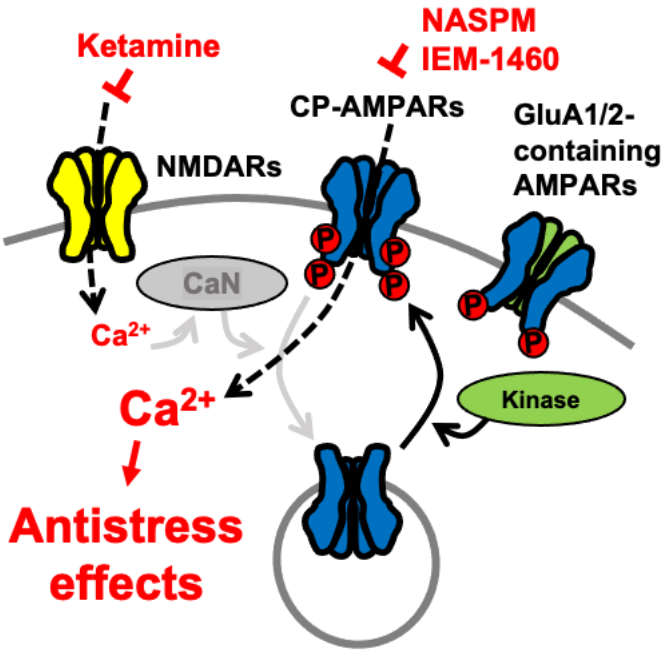

Figure 8. A schematic model of ketamine's antistress effects. Because ketamine is a noncompetitive NMDAR antagonist, a therapeutic dose is enough to block NMDAR-mediated Ca^{2+} influx in excitatory synapses. This can lower the activity of calcineurin (CaN), a Ca^{2+}-dependent phosphatase that dephosphorylates the AMPA receptor (AMPAR) subunit GluA1, and promote GluA1 phosphorylation, resulting in increased synaptic expression 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 amplitude of sEPSCs in hippocampal CA1 pyramidal neurons

Female					
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	43.47	1	43.47	F (1, 45) = 4.966	P=0.0309
Row Factor (Saline vs. Ket)	16.97	1	16.97	F (1, 45) = 1.939	P=0.1706
Column Factor (Saline vs. NASPM)	92.19	1	92.19	F (1, 45) = 10.53	P=0.0022
Residual	393.9	45	8.754		
Tukey's multiple comparisons test	Predicted (LS) mean diff.		95.00% CI of diff.		Adjusted P Value
Saline vs. NASPM	0.8745		-2.453 to 4.202		0.8962
Saline vs. Ket	-3.114		-6.105 to -0.1234		0.0384
Saline vs. Ket + NASPM	1.593		-1.463 to 4.650		0.5117
NASPM vs. Ket	-3.989		-7.411 to -0.5662		0.0165
NASPM vs. Ket + NASPM	0.7190		-2.761 to 4.199		0.9458
Ket vs. Ket + NASPM	4.708		1.548 to 7.868		0.0014

Male					
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	14.30	1	14.30	F (1, 51) = 2.464	P=0.1226
Row Factor (Saline vs. Ket)	54.99	1	54.99	F (1, 51) = 9.473	P=0.0034
Column Factor (Saline vs. NASPM)	65.25	1	65.25	F (1, 51) = 11.24	P=0.0015
Residual	296.0	51	5.804		
Tukey's multiple comparisons test	Predicted (LS) mean diff.		95.00% CI of diff.		Adjusted P Value
Saline vs. NASPM	1.192		-1.446 to 3.830		0.6295
Saline vs. Ket	-3.107		-5.374 to -0.8407		0.0034
Saline vs. Ket + NASPM	0.1839		-2.125 to 2.493		0.9966
NASPM vs. Ket	-4.299		-6.997 to -1.602		0.0005
NASPM vs. Ket + NASPM	-1.008		-3.742 to 1.726		0.7617
Ket vs. Ket + NASPM	3.291		0.9135 to 5.669		0.0031

Table 2. Statistical analysis of the frequency of sEPSCs in hippocampal CA1 pyramidal neurons

Female					
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	0.4365	1	0.4365	F (1, 45) = 2.989	P=0.0907
Row Factor (Saline vs. Ket)	0.4734	1	0.4734	F (1, 45) = 3.242	P=0.0785
Column Factor (Saline vs. NASPM)	3.610	1	3.610	F (1, 45) = 24.72	P<0.0001
Residual	6.571	45	0.1460		

Tukey's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Adjusted P Value
Saline vs. NASPM	0.3603	-0.06953 to 0.7901	0.1291
Saline vs. Ket	-0.3921	-0.7784 to -0.005778	0.0455
Saline vs. Ket + NASPM	0.3523	-0.04249 to 0.7472	0.0955
NASPM vs. Ket	-0.7524	-1.194 to -0.3103	0.0002
NASPM vs. Ket + NASPM	-0.007963	-0.4575 to 0.4416	>0.9999
Ket vs. Ket + NASPM	0.7444	0.3363 to 1.152	<0.0001

Male					
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	0.2244	1	0.2244	F (1, 51) = 0.6562	P=0.4217
Row Factor (Saline vs. Ket)	0.9313	1	0.9313	F (1, 51) = 2.724	P=0.1050
Column Factor (Saline vs. NASPM)	1.954	1	1.954	F (1, 51) = 5.714	P=0.0206
Residual	17.44	51	0.3419		

Tukey's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Adjusted P Value
Saline vs. NASPM	0.2564	-0.3838 to 0.8966	0.7129
Saline vs. Ket	0.1364	-0.4138 to 0.6865	0.9122
Saline vs. Ket + NASPM	0.6557	0.09520 to 1.216	0.0158
NASPM vs. Ket	-0.1201	-0.7749 to 0.5347	0.9616
NASPM vs. Ket + NASPM	0.3993	-0.2643 to 1.063	0.3888
Ket vs. Ket + NASPM	0.5193	-0.05778 to 1.096	0.0919

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