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RESEARCH ARTICLE

Memantine prevents acute stress-induced memory deficits by reversing sex-dependent pathophysiological glutamatergic alterations in the dorsal hippocampus

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Abstract

Background and Purpose: The neural mechanisms underlying effects of acute stress on memory are poorly understood. We demonstrated previously that acute stress produces identical spatial memory deficits in male and female mice but through distinct molecular mechanisms, with females exhibiting up-regulation of N-methyl-D-aspartate (NMDA) receptor subunits in the dorsal hippocampus (dHPC). Here, we tested whether pharmacological manipulation of NMDA receptors prevents stress-induced memory deficits and hippocampal glutamatergic dysfunction in a sex-dependent manner.

Experimental Approach: Male and female mice were exposed to 2 hours of restraint stress. We performed electrophysiological and molecular analysis of the glutamatergic synapse in the dHPC. We evaluated two non-competitive NMDA receptor antagonists administered pre-stress: MK-801, which blocks synaptic and extrasynaptic NMDA receptors, and memantine, which preferentially blocks extrasynaptic NMDA receptors. We evaluated effects of both drugs on stress-induced spatial memory impairment and the effect of memantine on hippocampal glutamatergic neurotransmission.

Key Results: Acute stress induces sex- and time-dependent alterations in glutamate–glutamine metabolism, with a striking increase in stressed females. This outcome was associated with female glutamatergic neurotransmission impairment, indicated by reduced miniature EPSC amplitude and AMPA/NMDA ratio at CA3–CA1 synapses. MK-801 and memantine rescued stress-induced spatial memory impairment in male and female mice, respectively. Memantine counteracted stress-induced reduction in

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF, brain-derived neurotrophic factor; C, control mice; dHPC, dorsal hippocampus; DI, discrimination index; EPSC, excitatory postsynaptic current; HPLC, high-performance liquid chromatography; LTD, long-term depression; mEPSC, miniature excitatory postsynaptic current; NMDA, N-methyl-D-aspartate; NOL, novel object location; RS, restraint stressed mice; SC, Schaffer collateral; UHPLC, ultra-high-performance liquid chromatography.

AMPA/NMDA ratio in female mice, normalising synaptic strength at CA3–CA1 synapses.

Conclusions and Implications: Acute stress disrupts hippocampal glutamatergic homeostasis through sex-dependent pathophysiological mechanisms. The study of sex-dependent mechanisms may lead to targeted pharmacological treatments for cognitive deficits in stress-related disorders.

KEYWORDS
glutamate, memantine, memory, NMDA, sex, stress

1 | INTRODUCTION

Cognitive impairments are a central feature of several neuropsychiatric disorders, substantially contributing to disability and decreased quality of life (Rock et al., 2014). These deficits often involve disturbances in memory and executive functions, which are significantly influenced by stress exposure throughout life (Millan et al., 2012). Understanding the neural mechanisms underlying stress-induced cognitive dysfunction is therefore critical for developing effective interventions. Cognitive functions can be differently affected by exposure to acute or chronic stress. In particular, acute stress influences cognitive functions in a complex manner, with a predominant negative impact on episodic memory (Shields et al., 2017). Compelling clinical and preclinical evidence indicates that acute stress affects hippocampal-dependent memory, particularly spatial memory, in a sex-dependent manner. In human studies, acute stress has been shown to impair spatial memory performance in women, whereas men are less affected or can even outperform women under similar conditions. For example, a study found that acute stress before learning significantly reduced spatial memory retention in women but not in men (Guenzel et al., 2014), suggesting an increased vulnerability of female hippocampal circuits to acute stress during spatial memory tasks. However, other human studies suggest that, under certain conditions, acute stress can affect spatial memory similarly in men and women (Richardson & VanderKaay Tomasulo, 2011). Preclinical studies shed further light on human findings. Acute stress influenced spatial memory performance differently in male and female rats, when assessed in a Morris water maze, with females showing more pronounced impairment compared to males (Beiko et al., 2004). Moreover, acute predator-odour stress administered 30 min before radial-arm water maze training produced comparable spatial memory impairment in rats of both sexes (Park et al., 2008).

The dorsal hippocampus (dHPC) plays a key role in spatial and episodic memory formation, with **glutamate** acting as the primary excitatory neurotransmitter modulating synaptic transmission within this region. Activation of hippocampal **N-methyl-D-aspartate (NMDA) receptors** requires both presynaptic glutamate release and sufficient postsynaptic membrane depolarisation; this dual gating mechanism allows calcium influx, which in turn activates molecular pathways underlying synaptic strengthening necessary for memory formation (Paoletti et al., 2013). In general, exposure to acute or chronic stress robustly alters the hippocampal glutamate synapse (Musazzi et al., 2022; Sanacora et al., 2022). Acute stress rapidly increases extracellular glutamate in the hippocampus, as detected by in vivo microdialysis immediately following a single immobilisation stress session (Lowy et al., 1993). This acute stress-induced increase of glutamate release triggers a complex cascade of molecular and cellular events, which profoundly alter glutamatergic synaptic function within the dHPC. Studies have shown that acute stress facilitates the induction of NMDA receptor-dependent long-term depression (LTD) in the CA1 region, which leads to spatial memory retrieval impairment (Wong et al., 2007). This acute stress-enabled LTD is mediated by **corticosterone**-induced activation of the **glucocorticoid receptor** and involves enhanced glutamate spillover, which activates extrasynaptic **NR2B**-containing NMDA receptors (C. H. Yang et al., 2005). It is important to underline that the vast majority of these findings

What is already known?

- Acute stress impairs spatial memory via hippocampal glutamatergic alterations.
- Memantine is a clinically used NMDA receptor antagonist.

What does this study add?

- Acute stress triggers sex-specific pathophysiological alterations in the dorsal hippocampus.
- MK-801 and memantine prevent these deficits via distinct sex-dependent mechanisms.

What is the clinical significance?

- Sex-specific pharmacological strategies are required for stress-related disorders.
- Memantine may be more effective for stress-related cognitive dysfunction in women.

regarding acute stress–induced alterations of glutamatergic synaptic function in the dHPC have been obtained mostly using male rodents. Consequently, the potential influence of biological sex on these molecular and synaptic dysfunctions subserving spatial memory impairment remains largely unexplored. We recently demonstrated that male and female C57BL/6J mice, exposed to 2 h of restraint stress immediately after the training session of the novel object location (NOL) task, equally exhibited impaired long-term spatial memory (Torrisi et al., 2023). Notably, this memory impairment was associated with significant up-regulation of major NMDA and **α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor** subunit mRNAs in the dHPC of only stressed female mice. Overall, our findings indicate that acute stress can trigger similar spatial memory impairments across sexes, but through distinct molecular mechanisms. The present study tested the hypothesis that pharmacological modulation of NMDA receptors may prevent acute stress–induced impairments in spatial memory and related glutamatergic synaptic alterations in the dHPC in a sex-dependent manner. To address this hypothesis, we employed a multidisciplinary approach combining electrophysiological recordings and molecular analyses of the glutamatergic synapse in the dHPC of male and female mice exposed to 2 h of restraint stress. We further examined the effects of **memantine**, a clinically approved non-competitive NMDA receptor antagonist, administered systemically prior to stress exposure, on spatial memory deficits and associated alterations in hippocampal glutamatergic synapses.

2 | METHODS

2.1 | Experimental subjects and housing

Male and female C57BL6/J mice (10–16 weeks old, Charles River Laboratories Italia, Italy) were group-housed three to five per cage under controlled conditions (12-h light/dark cycle, 22 ± 2°C, food and water ad libitum). The experimenter handled animals on alternate days during the week preceding the beginning of each experiment. Animals were acclimatised to the testing room 1 h before the beginning of the tests. All experiments were carried out according to EU Directive 2010/63/EU, the Institutional Animal Care and Use Committees of Catania and the Italian Ministry of Health (authorization n.497/2022 PR). Moreover, all animal experiments comply with ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (Percie du Sert et al., 2020) and with the recommendations provided by the *British Journal of Pharmacology* (Lilley et al., 2020).

2.2 | Materials

Memantine hydrochloride (cat. no. 5.04905; Sigma-Aldrich; St. Louis, MO, USA) and **(+)-MK-801** (dizocilpine hydrogen maleate, cat. no. M107; Sigma-Aldrich; St. Louis, MO, USA) were dissolved in 0.9% saline. Both drugs were administered via intraperitoneal (i.p.) injections before the onset of the acute restraint stress procedure. Memantine was administered at a dose of 5 $mg \cdot kg^{-1}$ (Costa et al., 2008), whereas MK-801 was administered at a dose of 0.1 $mg \cdot kg^{-1}$ (Torrisi et al., 2017; B. Yang et al., 2016). Both drugs were injected in a volume of 10 $ml \cdot kg^{-1}$.

Ortho-phthalaldehyde, sodium hydrogen phosphate, sodium hydroxide, 3-mercaptopropionic acid, glutamate and **glutamine** standards were supplied at the highest purity from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile, methanol and tetrahydrofuran were purchased from Merck. All chemicals for electrophysiological procedures were provided by Sigma-Aldrich (St. Louis, MO, USA). **Tetrodotoxin** and **picrotoxin** were purchased from Tocris Bioscience (Bristol, UK).

2.3 | Acute restraint stress

Acute restraint stress was carried out as previously reported (Torrisi et al., 2023). Restraint-stressed (RS) mice were gently put in Falcon 50 ml conical centrifuge tubes for 2 h (Chen et al., 2024; Satoh & Shimeki, 2010). During this stressful procedure, three 0.5-mm holes drilled along the sidewall and at the end of the tube ensured adequate air flow. Another hole was drilled in each cup to keep the tails of the mice out of the tube. About five tubes containing mice, randomly chosen, were placed in conventional cages (Tecniplast, 425 × 266 × 185 mm) with a level of illumination of 400 lux. At the end of the restraint, mice were immediately put back to their home cages, with free access to food and water. Control (C) mice remained in their home cages in a different room during the stressful procedure.

2.4 | NOL test

The NOL test was performed as previously described with minor modifications (Torrisi et al., 2023). The test was carried out in evenly illuminated (40 ± 1 lx) grey open fields (44 × 44 × 40 cm, Ugo Basile, Gemonio, Italy). The objects used, which were different in shape, colour and size (4 × 4 × 4 cm to 6 × 6 × 6 cm), were fixed to the floor of the apparatus to avoid displacement during the test. The behavioural test started after 1 week of handling. A 1-day pretest procedure was carried out to acclimatise mice to the apparatus. On Day 1, mice were placed into the empty apparatus and allowed to freely explore for 15 min. The test consisted of one training session and one test session interspersed with a 24-h delay (assessment of long-term memory). During the training session (Day 2), animals were placed in the centre of the apparatus and then allowed to explore two copies of an identical object for a total of 10 min. The objects were located in two corners of the apparatus, 10 cm from the side walls. During the test session (Day 3), mice were placed again in the centre of the apparatus and allowed to explore two copies of the same familiar objects explored in the training session but with one placed in different location. One of these objects was placed in the same position (familiar object) occupied in the training session, whereas the other object

(displaced object) was placed in a new location on the opposite side of the apparatus (these two objects were diagonal from each other; see Figures 3, 4). Exploratory behaviour was defined as a mouse directing its nose towards the object at a distance of ≤2 cm. Looking around whilst sitting, climbing the objects and rearing against the objects were not considered exploratory behaviour. Mice that failed to complete a minimum of 5 s of total exploration in each session of the task were excluded from the analysis. If the object location memory is intact, animals should explore the displaced object more instead of the familiar one. Objects and locations were counterbalanced between animals. Discrimination between the objects during the test session was calculated using a discrimination index (DI), obtained through the following formula: [(time spent exploring the displaced object − time spent exploring the familiar object)/total exploration time]. The higher the DI, the better is the cognitive performance. The total exploration as well as the percentage of exploration of each object during the test session were further calculated.

2.5 | Experimental design

2.5.1 | Experiment 1: Effect of acute stress on glutamate homeostasis in the dHPC

Male and female mice were exposed to 2 h of restraint stress and were sacrificed either immediately or 24 h after the end of the acute stressful procedure. The dHPC was micro-dissected to assess glutamate and glutamine as markers of glutamate–glutamine cycle and glutamatergic homeostasis. This was performed by ultra-high-performance liquid chromatography (UHPLC) analysis.

2.5.2 | Experiment 2: Effect of acute stress on glutamatergic neurotransmission in the dHPC

Male and female mice were exposed to 2 h of restraint stress and sacrificed 24 h after the end of the stress procedure for electrophysiological assessment. Whole-cell patch-clamp recordings in voltage-clamp mode were obtained to evaluate glutamatergic synaptic function. Two complementary electrophysiological approaches were employed: (i) analysis of spontaneous miniature excitatory post-synaptic currents (mEPSCs) in CA1 pyramidal neurons and (ii) evaluation of synaptic strength at CA3–CA1 glutamatergic synapses by calculating the AMPA/NMDA ratio.

2.5.3 | Experiment 3: Effects of the non-competitive NMDA antagonists MK-801 and memantine on acute stress–induced spatial memory impairment

Non-competitive NMDA antagonism can induce pro-cognitive effects both in humans and rodents under pathological conditions (Karimi Tari et al., 2024). In this study, we used MK-801 (dizocilpine) and memantine, two non-competitive NMDA antagonists with distinct pharmacodynamic profiles. Specifically, MK-801 acts as a high-affinity, long-lasting non-competitive NMDA antagonist that non-selectively blocks both synaptic and extrasynaptic NMDA receptors (Song et al., 2018), whereas memantine exhibits low-to-moderate affinity with rapid off-rate kinetics, preferentially blocking extrasynaptic NMDA receptors (Lipton, 2006). Different cohorts of mice of both sexes were intraperitoneally administered with MK-801 or memantine immediately before the onset of the acute stressful procedure, which was performed after the training session of the NOL test. Their long-term object location memory, which is a form of spatial memory, was assessed in the test session after a delay of 24 h.

2.5.4 | Experiment 4: Effects of the non-competitive NMDA antagonist memantine on acute stress–induced electrophysiological alterations in glutamatergic neurotransmission in the dHPC

Female C57BL/6 mice were assigned to control, stress, or stress plus memantine treatment groups. Animals underwent 2 h of restraint stress and were sacrificed 24 h later for electrophysiological assessment. Whole-cell patch-clamp recordings in voltage-clamp mode were obtained from pyramidal neurons in the dHPC. Synaptic strength at CA3–CA1 glutamatergic synapses was evaluated by calculating the AMPA/NMDA ratio.

2.6 | Tissue preparation for glutamate and glutamine determination

Tissue preparation was performed using the organic solvent deproteinising procedure to give clear aqueous protein-free tissue extracts suitable for the HPLC analysis (Lazzarino et al., 2023). Specifically, freeze-clamped tissue samples were mixed (5% ratio) with a precipitating solution (75% acetonitrile, 25% KH_2PO_4, 10 mM, pH 7,4), strongly vortexed and blended through a RSHO-250-001 homogeniser for 60 s at 10000 rpm. Following a high-speed centrifugation (20.690 × g for 10 min at 4°C), a double volume of chloroform was added to the supernatant, centrifuged again, to finally obtain the aqueous phase, ready for chromatographic analysis.

2.7 | UHPLC separation of glutamate and glutamine

The chromatographic analyses were performed using a UHPLC system (Ultimate 3000, Thermo Fisher Scientific Italia, Rodano, Milan, Italy) coupled with a fluorescence detector (FLD-3400RS, Thermo Fisher Scientific Italia, Rodano, Milan, Italy). The autosampler also was used for the in-needle derivatisation of the amino acid content. Briefly, 1 µl of the sample was mixed with 1 µl of the derivatisation

reagent (6.5-mM ortho-phthalaldehyde, 0.25% 3-mercaptopropionic acid, 62.5-mM boric acid) and 5 μl of borate buffer (250 mM, pH 10.0) for 1 min. After the addition of 3 μl of 1 M acetic acid, the solution was injected into a Hypersil Gold C18 column (50 × 2.1 mm, 2.1-μm particle size) with a matching guard column. The separation of the amino acids was performed in isocratic mode, flowing buffer A (24-mM CH_3COONa, 24-mM NaH_2PO_4, 2% tetrahydrofuran, 0.1% trifluoroacetic acid, pH 6.5) at 0.1 ml·min^{-1}. Eluent B (CH_3OH:CH_3CN:H_2O 4:3:3) was used in final washing runs. Data collection and analysis were carried out using the Chromeleon® software provided by the manufacturer. Excitation and emission wavelengths were 340 and 455 nm, respectively. Identification and quantification of glutamate and glutamine in cell samples were based on retention times and peak areas.

2.8 | Slice preparation and electrophysiological recordings

For the preparation of brain slices, we followed the protocol previously described (Aceto et al., 2022), with minor modifications. Animals were euthanised by cervical dislocation and decapitated. The brain was rapidly removed and placed in an ice-cold, sucrose-based cutting solution containing (in mM) TRIS-HCl 72, TRIZMA base 18, NaH_2PO_4 1.2, $NaHCO_3$ 30, KCl 2.5, glucose 25, HEPES 20, $MgSO_4$ 10, Na-pyruvate 3, ascorbic acid 5, $CaCl_2$ 0.5 and sucrose 20. Coronal slices (250 μm thick) were cut on a vibratome (VT1200S; Leica Microsystems, Germany) and immediately transferred to an incubation chamber held at 32°C and filled with a recovery solution containing (in mM): TRIS-HCl 72, TRIZMA base 18, NaH_2PO_4 1.2, $NaHCO_3$ 25, KCl 2.5, glucose 25, HEPES 20, $MgSO_4$ 10, Na-pyruvate 3, ascorbic acid 5, $CaCl_2$ 0.5 and sucrose 20. After 30 min, slices were transferred to a second incubation chamber held at 32°C and filled with artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 124, KCl 3.2, NaH_2PO_4 1.2, $MgCl_2$ 1, $CaCl_2$ 2, $NaHCO_3$ 26 and glucose 10, pH 7.4. During incubation, the chambers were continuously oxygenated with (v/v) 95% O_2/(v/v) 5% CO_2. Finally, slices were equilibrated at RT for at least 45 min. For recordings, slices were then transferred to a submerged recording chamber constantly perfused (3 ml·min^{-1}) with oxygenated (v/v) 95% O_2/(v/v) 5% CO_2 and heated aCSF (32°C). Hippocampal neurons from the CA1 region were visualised under DIC infrared illumination using an upright microscope (BX5IWI, Olympus) equipped with a 40× water-immersion objective. Stimulation of the Schaffer collaterals (SC) was obtained by means of a current stimulus isolator (WPI, Worcester, MA, USA), connected to a bipolar concentric stimulating electrode (FHC, Bowdoin, ME, USA), which was positioned in contact with the SC pathway. Borosilicate glass pipettes (4–6 MΩ) were filled with an internal solution containing (in mM): K-gluconate 145, $MgCl_2$ 2, HEPES 10, EGTA 0.1, Na-ATP 2.5, Na-GTP 0.25, phosphocreatine 5, pH adjusted to 7.2 with KOH for mEPSC recordings. For AMPA/NMDA ratio experiments, a caesium-based internal solution containing (in mM): $CsCH_3SO_3$ 135, HEPES 10, NaCl 8, EGTA 0.25, $MgCl_2$ 2, Mg-ATP 4. Na-GTP 0.3, phosphocreatine 5, pH adjusted to 7.3 with CsOH, was used. Standard whole-cell patch-clamp recordings were performed in voltage-clamp mode. After establishing the gigaseal configuration, the breakthrough of the patch was obtained by applying negative pressure to achieve the whole-cell configuration. Series resistance (Rs) was monitored constantly throughout the entire recording and considered acceptable if lower than 15 MΩ. For mEPSC measurements, neurons were held at −70 mV. Tetrodotoxin (TTX; 0.5 μM; Tocris) and picrotoxin (PTX; 50 μM; Tocris) were bath-applied to block Na^+ currents and **$GABA_A$ receptors**, respectively. For evoked EPSCs, electrical stimuli were delivered to the SC. To obtain the AMPA/NMDA current ratios, stimuli of identical intensity were delivered at holding potentials of −70 and +40 mV for each cell with a frequency of 0.05 Hz; 50-μM PTX was present in the bath. The identity of evoked EPSCs was confirmed at the end of the recordings by adding to the bath the selective AMPA receptor blocker **2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide** (NBQX; 10 μM; Tocris). Recordings were performed using a Multiclamp 700B/Digidata 1550A system (Molecular Devices, Sunnyvale, CA, USA) and digitised at a 10-kHz sampling frequency. All the electrophysiological recordings were analysed using the Clampfit 10.6 software (Molecular Devices). To obtain the AMPA/NMDA ratio, the AMPA receptor–mediated EPSC amplitude was calculated as the difference between the peak response and the baseline; within the same cell, NMDA peak amplitude was measured 35 ms after the corresponding peak amplitude obtained at $V_{hold} = -70$ mV (AMPA peak). The AMPA/NMDA ratio was then calculated by dividing the AMPA eEPSC amplitude obtained at −70 mV by the NMDA eEPSC amplitude obtained at +40 mV. For mEPSC frequency analysis, a template was constructed using the 'Event detection/create template' function (Leggio et al., 2019). Thereafter, mEPSCs were detected using the 'Event detection/template search' function, and the result was inspected for false positives. For mEPSC amplitude analysis, all the events detected during a single recording using template analysis were averaged, and the amplitude was calculated.

2.9 | Statistical analysis

Data and statistical analysis complied with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2025). Each experimental group consisted of a minimum of five mice. Data were analysed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). The D'Agostino-Pearson omnibus normality test was carried out to assess data distribution. The Levene's test was used to verify equality of variances. All data assumed a normal distribution, and then, they were subjected to parametric tests. Changes in glutamate and glutamine levels (Experiment 1) were analysed by using two-way analyses of variance (ANOVA; *Sex* × *Stress*). Parameters related to mEPSCs and AMPA/NMDA ratio (Experiment 2) were analysed using two-way ANOVA (*Sex* × *Stress*). DI and total exploration time (Experiment 3) were analysed by using three-way ANOVA (*Sex* × *Stress* × *Treatment*). Specific

exploration of the familiar and displaced object was also analysed using three-way ANOVA (*Object* × *Stress* × *Treatment*). AMPA/NMDA ratio (Experiment 4) after treatment with memantine was analysed using one-way ANOVA (*Treatment*). Detailed statistics, such as *F*- and *P*-values of ANOVA, are reported in the figure legends. For all data analyses, upon confirmation of significant main effects, differences among individual means were assessed using Sidak's multiple comparisons test as recommended. *P*-values of <0.05 were considered significant. The estimate of dispersion is shown as the standard error of the mean (SEM). All data are reported as mean ± SEM.

2.10 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in https://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2023/2024 (Alexander, Fabbro, Kelly, Mathie, Peters, Veale, Armstrong, Faccenda, Harding, Davies, Amarosi, et al., 2023; Alexander, Fabbro, Kelly, Mathie, Peters, Veale, Armstrong, Faccenda, Harding, Davies, Annett, et al., 2023; Alexander, Mathie, Peters, Veale, Striessnig, Kelly, Armstrong, Faccenda, Harding, Davies, Aldrich, & Zhu, 2023).

3 | RESULTS

3.1 | Acute stress induced sex- and time-dependent alterations in glutamate and glutamine levels in the dHPC

We first examined whether acute stress induces sex- and time-dependent alterations in glutamate and glutamine levels in the dHPC. To investigate any differences, we measured glutamate and glutamine levels of C and RS mice of both sexes at two time points: immediately after stress and 24 h after stress (Figure 1a). Immediately after stress, we found a significant increase in either glutamate (Figure 1b) or glutamine (Figure 1c) levels in the dHPC of RS female mice compared with C female mice. We only found a significant increase in glutamate levels, but not glutamine, in the dHPC of C female mice compared to C male mice (Figures 1b–c and 1e–f). We further assessed the total pool (glutamate + glutamine) level, which reflects the overall capacity of the glutamate-glutamine cycle. In line with the previous data, the total pool (glutamate + glutamine) level was significantly increased only in the dHPC of RS female mice in comparison with C female mice (Figure 1d). The total pool (glutamate + glutamine) level also was significantly augmented in the dHPC of C female mice compared to C male mice (Figure 1d,g).

Twenty-four hours after stress, we further found robust sex-specific alterations of glutamatergic metabolism in the dHPC. In particular, we uncovered a robust and significant decrease in both glutamate (Figure 1e) and glutamine (Figure 1f) levels in the dHPC of RS male mice compared with C male mice. According to these data, the total pool (glutamate + glutamine) level was significantly reduced exclusively in the dHPC of RS male mice in comparison with C male mice (Figure 1g).

Collectively, these results highlight a pronounced sexual dimorphism in the temporal dynamics of glutamate–glutamine metabolism under acute stress in the dHPC.

3.2 | Acute restraint stress affected mEPSC amplitude and AMPA/NMDA ratio in dorsal hippocampal pyramidal neurons of female but not male mice

We next assessed whether acute stress induces sex-dependent changes in basal glutamatergic transmission in CA1 pyramidal neurons. To this aim, we examined spontaneous mEPSCs by analysing amplitude, frequency and kinetics in male and female mice 24 h after acute stress (Figure 2a). Mean mEPSC amplitude was found to be similar in both C female and C male mice (Figure 2b). Following acute stress, RS female mice exhibited a significant reduction in mEPSC amplitude compared with C female mice, whereas RS male mice showed no changes compared with C male mice (Figure 2b). In contrast, mEPSC frequency remained comparable between C and RS groups of both sexes (Figure 2c). Similarly, there were no differences among groups regarding mEPSC rise time (Figure 2d) and decay time (Figure 2e).

We further measured the AMPA/NMDA ratio at CA3–CA1 synapses. AMPA/NMDA ratios were similar in C female and C male mice (Figure 2f,g). Acute stress significantly reduced the AMPA/NMDA ratio in RS female mice compared to C female mice, whereas no differences were observed between C male mice and RS male mice (Figure 2f,g). Overall, these electrophysiological findings indicate that acute stress induces measurable alterations in glutamatergic transmission in the dHPC of female mice, affecting both basal synaptic activity and synaptic strength, whereas male mice do not exhibit these stress-induced changes.

3.3 | MK-801 selectively rescued acute stress–induced object location memory impairment in male mice

We investigated whether pre-stress NMDA receptor antagonism could counteract acute stress–induced spatial memory impairment. We first administered the non-competitive NMDA antagonist MK-801 (Figure 3a). We analysed total distance travelled during the NOL test and found no significant differences among groups (Figure 3b), indicating that MK-801 did not differentially affect locomotor activity under our experimental conditions. By assessing the DI, we found that acute stress significantly impaired object location memory in RS vehicle-treated mice of both sexes compared with C vehicle-treated mice (Figure 3c). We also observed a significant sex-dependent effect of MK-801 on this stress-induced memory deficit. Specifically, RS

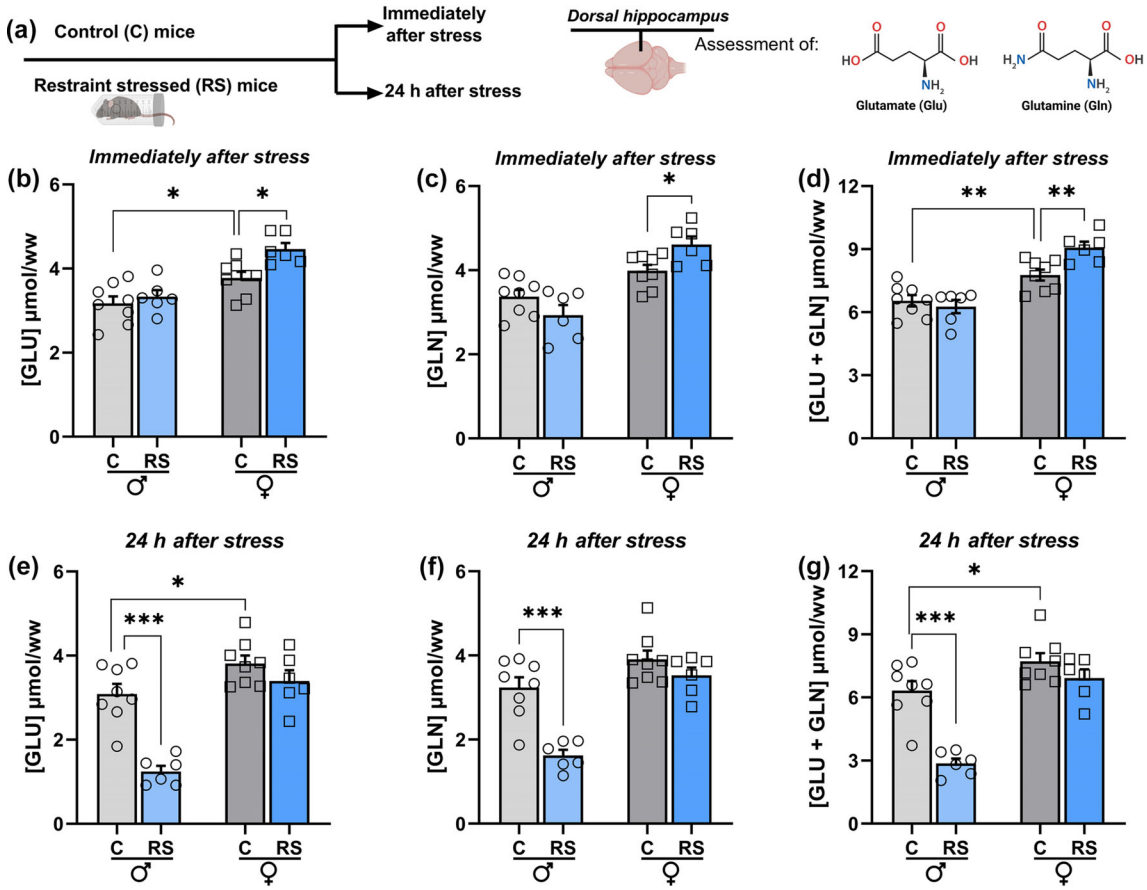

FIGURE 1 **Acute restraint stress triggers sex-specific alterations in glutamate and glutamine levels in the dHPC.** (a) Timeline of the experimental procedure. Male and female mice were exposed to 2 h of restraint stress and sacrificed either immediately or 24 h later for UHPLC analysis. (b–d) Immediate effects of acute stress. (b) Glutamate levels (*Stress*, $F_{(1, 24)} = 7.218$; $P = 0.0129$; *Sex*, $F_{(1, 24)} = 30.33$, $P < 0.0001$), (c) glutamine levels (*Sex*, $F_{(1, 24)} = 40.46$, $P < 0.0001$; *Stress* × *Sex*, $F_{(1, 24)} = 8.747$, $P = 0.0069$) and (d) total glutamate + glutamine pool (*Sex*, $F_{(1, 24)} = 51.02$, $P < 0.0001$; *Stress* × *Sex*, $F_{(1, 24)} = 7.920$, $P = 0.0096$) in the dHPC of C (males, $n = 8$; females $n = 8$) and RS (males, $n = 6$; females $n = 6$) mice immediately after stress. (e–g) Delayed effects of acute stress. (e) Glutamate levels (*Stress*, $F_{(1, 24)} = 27.37$; $P < 0.0001$; *Sex*, $F_{(1, 24)} = 44.20$, $P < 0.0001$; *Stress* × *Sex*, $F_{(1, 24)} = 10.86$, $P = 0.0030$), (f) glutamine levels (*Stress*, $F_{(1, 24)} = 21.74$, $P < 0.0001$; *Sex*, $F_{(1, 24)} = 36.10$, $P < 0.0001$; *Stress* × *Sex*, $F_{(1, 24)} = 8.338$, $P = 0.0081$) and (g) total glutamate + glutamine pool (*Stress*, $F_{(1, 24)} = 28.31$, $P < 0.0001$; *Sex*, $F_{(1, 24)} = 46.32$, $P < 0.0001$; *Stress* × *Sex*, $F_{(1, 24)} = 11.06$, $P = 0.0028$) in the dHPC of C (males, $n = 8$; females $n = 8$) and RS (males, $n = 6$; females $n = 6$) mice 24 h after RS. Statistical significance was analysed by two-way ANOVA followed by Sidak's multiple comparisons test. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. Values are expressed as mean ± SEM.

MK-801-treated male mice exhibited a DI similar to that of C vehicle-treated mice and significantly higher than that of RS vehicle-treated male mice (Figure 3c). By contrast, RS MK-801-treated female mice exhibited the same stress-induced memory deficit as RS vehicle-treated female mice (Figure 3c). As expected, MK-801 significantly impaired object location memory in C mice of both sexes (Figure 3c). Regarding the total exploration time of the objects, there were no differences among all groups (Figure 3d). This further suggests that differences in cognitive performance were not ascribable to changed motivation or exploratory activity. The sex-dependent pro-cognitive effect of MK-801 also was substantiated by analysing the exploration time of the familiar object and the displaced object. Indeed, only RS MK-801-treated male mice, similar to C vehicle-treated male mice, were capable of discriminating between the two objects, exploring significantly more the displaced object than the familiar object (Figure 3e). In contrast, RS MK-801-treated female mice were unable to recognise and discriminate the familiar object from the displaced object. They spent approximately the same amount of time exploring both objects as RS vehicle-treated female mice (Figure 3f).

3.4 | Memantine selectively reversed acute stress–induced object location memory impairment in female mice

To further investigate the sex-specific role of NMDA receptor antagonism in mitigating acute stress–induced object location memory deficits, we next evaluated the effect of memantine (Figure 4a), a clinically approved noncompetitive NMDA receptor antagonist with different pharmacological properties compared to MK-801. Assessment of the total distance travelled during the NOL test revealed no significant differences among groups, suggesting that memantine also did not

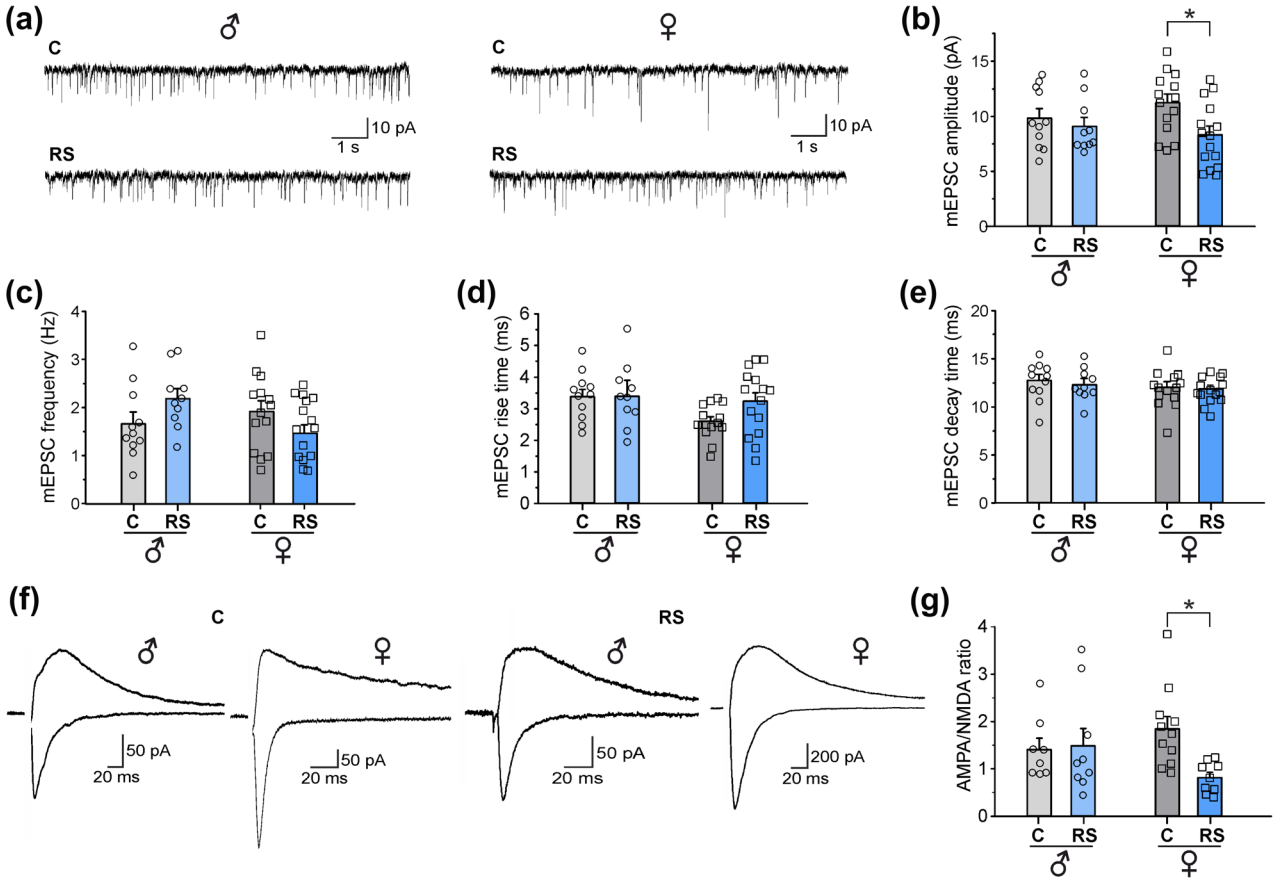

FIGURE 2 **Acute stress decreases AMPA receptor–mediated mEPSC amplitude and AMPA/NMDA ratio in dorsal hippocampal pyramidal neurons of female but not male mice.** (a) Representative traces of mEPSCs recorded from CA1 pyramidal neurons in dorsal hippocampal slices obtained from C and RS mice 24 h after stress exposure (males: C, $n/m = 11$ cells/5 mice; RS, $n/m = 10$ cells/5 mice; females: C, $n/m = 14$ cells/6 mice; RS, $n/m = 15$ cells/6 mice). (b) mEPSC amplitude (*Stress*; $F_{(1, 46)} = 5.247$, $P = 0.0266$), (c) mEPSC frequency, (d) mEPSC rise time and (e) mEPSC decay. (f) Representative traces of AMPA receptor– and NMDA receptor–mediated excitatory postsynaptic currents (EPSCs) recorded from dHPC CA1 pyramidal neurons of C and RS mice 24 h after restraint stress (males: C, $n/m = 8$ cells/5 mice; RS, $n/m = 9$ cells/5 mice; females: C, $n/m = 11$ cells/5 mice; RS, $n/m = 9$ cells/5 mice). (g) Quantification of the AMPA/NMDA ratio (*Stress* × *Sex*; $F_{(1, 33)} = 4.449$, $P = 0.0426$) for the experimental conditions shown in (f). The ratio was calculated as the peak AMPA receptor–mediated EPSC recorded at −70 mV divided by the peak NMDA receptor–mediated EPSC recorded at +40 mV (see Section 2 for details). Statistical significance was analysed by two-way ANOVA followed by Sidak's multiple comparisons test. $^*P < 0.05$. Data are expressed as mean ± SEM.; n/m indicates number of recorded cells/number of animals.

differentially affect locomotor activity in this memory test (Figure 4b). In line with our previous data, analysis of the DI revealed again that acute stress triggered a significant impairment of object location memory in RS vehicle-treated mice regardless of sex (Figure 4c). Remarkably, pre-stress administration of memantine induced a sex-specific rescue of object location memory impairment, which was opposite to that observed with MK-801. Indeed, only RS memantine-treated female mice showed a normal object location memory similar to that of C vehicle-treated female mice (Figure 4c), whereas RS memantine-treated male mice exhibited the same cognitive impairment as RS vehicle-treated male mice (Figure 4c). Unlike MK-801, memantine treatment did not impair object location memory in C mice of either sex (Figure 4c). Also in this experiment, there were no differences in the total exploration time of the objects among all groups (Figure 4d). Detailed analysis of exploration time for the familiar and displaced objects confirmed the sex-specific pro-cognitive effect of memantine. RS memantine-treated male mice failed to discriminate between objects and explored both equally, similar to RS vehicle-treated male mice (Figure 4e). In contrast, RS memantine-treated female mice, like C vehicle-treated female mice, significantly explored the displaced object more than the familiar one (Figure 4f).

3.5 | Memantine counteracted stress-induced deficit in synaptic strength at CA3–CA1 synapses in female mice

To determine whether memantine administration prior to acute restraint stress can prevent the stress-induced reduction in synaptic strength observed in female mice, we measured AMPA/NMDA ratios at CA3–CA1 excitatory synapses in memantine-treated RS animals and compared these values with those from C and RS groups

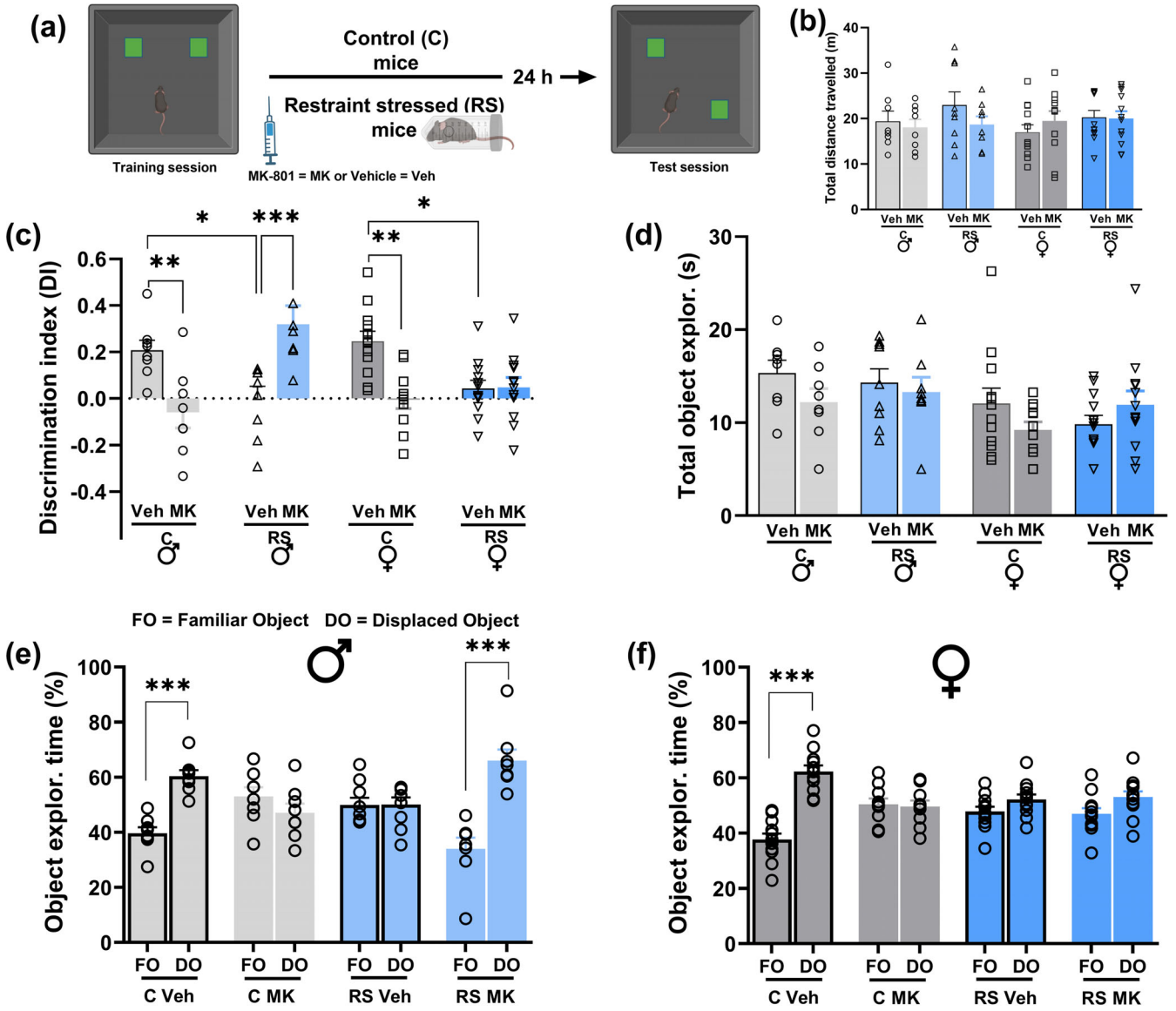

FIGURE 3 **MK-801 rescues acute stress–induced spatial memory impairment in male but not female mice.** (a) Schematic representation of the experimental design. Mice received MK-801 (0.1 mg/kg, i.p.) or vehicle immediately before 2 h of restraint stress and were tested in the NOL task 24 h later. (b) Total distance travelled, (c) DI (*Treatment* × *Stress*, $F_{(1, 72)} = 35.78$ $P < 0.0001$; *Treatment* × *Sex*, $F_{(1, 72)} = 4.571$; $P = 0.0359$; *Stress* × *Sex*, $F_{(1, 72)} = 5.257$; $P = 0.0248$; *Treatment* × *Stress* × *Sex*, $F_{(1, 72)} = 5.520$; $P = 0.0248$) of C male mice (Veh, $n = 8$; MK-801 $n = 8$), C female mice (Veh, $n = 12$; MK-801 $n = 11$), RS male mice (Veh, $n = 9$; MK-801 $n = 8$) and RS female mice (Veh, $n = 12$; MK-801 $n = 12$). (d) Total object exploration time during the test session. (e–f) object exploration time (%) of familiar versus displaced object in male (e; *Object*, $F_{(1, 58)} = 28.72$; $P < 0.0001$; *Object* × *Stress* × *Treatment*, $F_{(1, 58)} = 44.77$; $P < 0.0001$) and female (f; *Object*, $F_{(1, 84)} = 35.93$; $P < 0.0001$; *Object* × *Stress*, $F_{(1, 84)} = 5.472$; $P = 0.0217$; *Object* × *Treatment*, $F_{(1, 84)} = 17.10$; $P < 0.0001$; *Object* × *Stress* × *Treatment*, $F_{(1, 84)} = 22.35$; $P < 0.0001$) mice. Statistical significance was analysed by three-way ANOVA followed by Sidak's multiple comparisons test. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. Values are expressed as mean ± SEM.

(Figure 5a–b). As previously shown (Figure 2g), the AMPA/NMDA ratio was significantly reduced in RS female mice compared with C female mice. Importantly, memantine-treated RS female mice displayed AMPA/NMDA ratios significantly higher than those of RS female mice, indicating a significant effect of the treatment. Taken together, these electrophysiological findings demonstrate that pre-stress memantine administration effectively rescues the stress-induced deficit in synaptic strength at CA3–CA1 synapses in female mice by restoring the AMPA/NMDA ratio to control levels. These results further support the sex-specific protective effect of memantine previously observed at the behavioural level.

4 | DISCUSSION

The present study shows that acute stress disrupts glutamatergic homeostasis and spatial memory through sex-dependent mechanisms in the dHPC. We demonstrate for the first time that stressed female

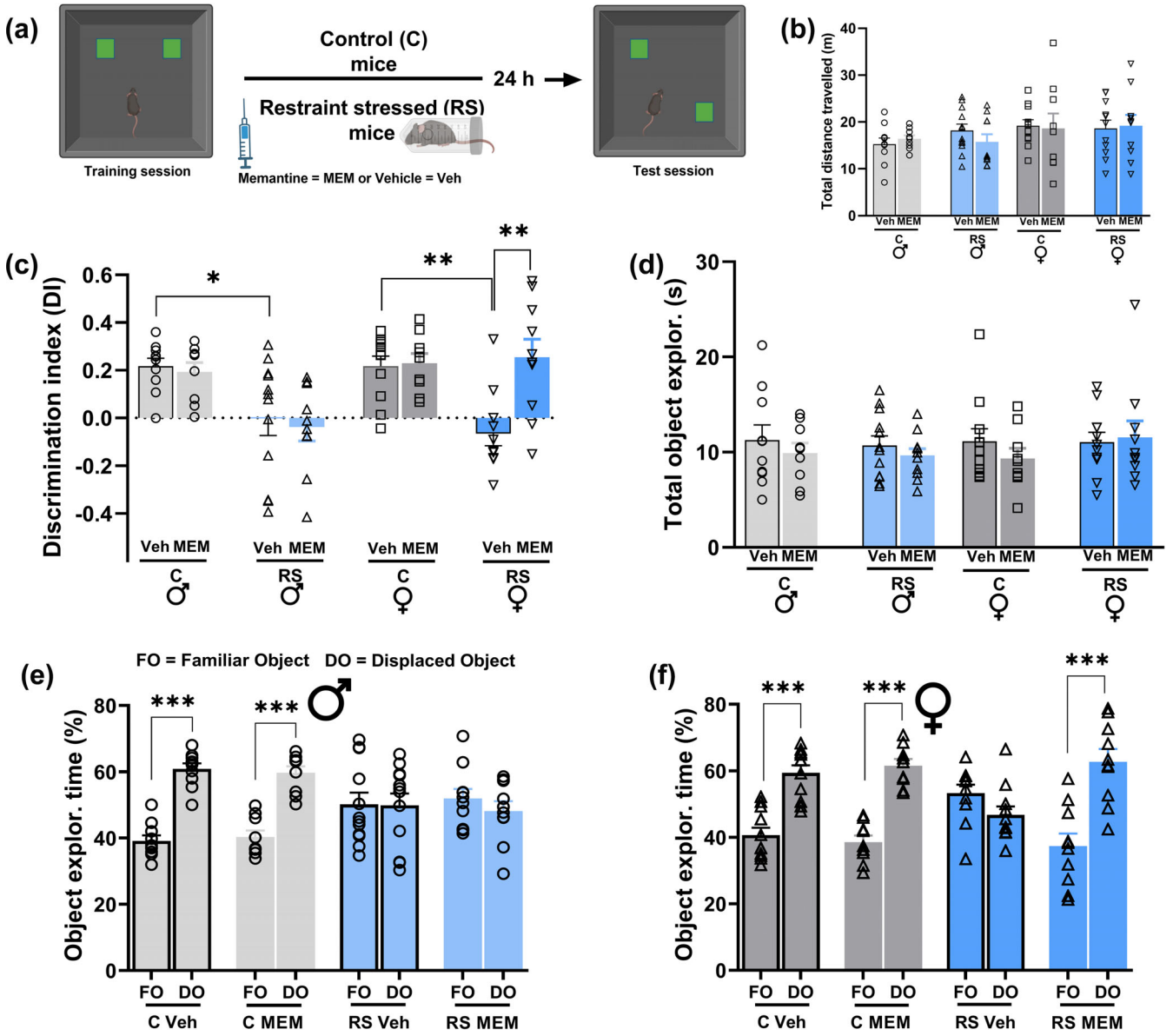

FIGURE 4 **Memantine rescues acute stress–induced spatial memory deficit in female but not male mice** (a) Experimental timeline. Memantine (5 mg/kg, i.p.) or vehicle was administered prior to 2 h of restraint stress. Twenty-four hours later, mice were assessed in the NOL task. (b) Total distance travelled, (c) DI (*Stress*, $F_{(1, 74)} = 20.51$; $P < 0.0001$; *Treatment* × *Sex*, $F_{(1, 74)} = 6.200$; $P = 0.050$; *Treatment* × *Stress* × *Sex*, $F_{(1, 74)} = 4.133$; $P = 0.0456$) of C male mice (Veh, $n = 10$; memantine $n = 9$), C female mice (Veh, $n = 11$; memantine $n = 9$), RS male mice (Veh, $n = 12$; memantine $n = 10$) and RS female mice (Veh, $n = 11$; memantine $n = 10$). (d) Total object exploration time during the test session. (e–f) object exploration time (%) of familiar versus displaced object in male (e; *Object*, $F_{(1, 74)} = 21.65$; $P < 0.0001$; *Object* × *Stress*, $F_{(1, 74)} = 32.00$; $P < 0.0001$) and female (f; *Object*, $F_{(1, 74)} = 59.95$; $P < 0.0001$; *Object* × *Stress*, $F_{(1, 74)} = 8.565$; $P = 0.0045$; *Object* × *Treatment*, $F_{(1, 74)} = 21.43$; $P < 0.0001$; *Object* × *Stress* × *Treatment*, $F_{(1, 74)} = 12.61$; $P = 0.0007$) mice. Statistical significance was analysed by three-way ANOVA followed by Sidak's multiple comparisons test. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. Values are expressed as mean ± SEM.

mice exhibited an immediate increase in dorsal hippocampal glutamate and glutamine, accompanied by selective impairment of glutamatergic neurotransmission, whereas stressed male mice did not exhibit these acute metabolic and synaptic alterations despite developing spatial memory impairment. Memantine rescued spatial memory deficits and normalised the AMPA/NMDA ratio in stressed female mice, whereas MK-801 rescued these memory deficits only in stressed male mice, establishing that the functioning of NMDA receptors depends on sex-specific pathophysiological mechanisms under acute stressful conditions.

This female-specific glutamate/glutamine accumulation in the dHPC is in line with evidence demonstrating sex-dependent alterations in glutamate homeostasis following acute stress. Kokras and colleagues demonstrated that acute stress increases glutamate levels in the prefrontal cortex, but not in the whole hippocampus of female rats, thus revealing a sexual dimorphism in stress-induced glutamatergic

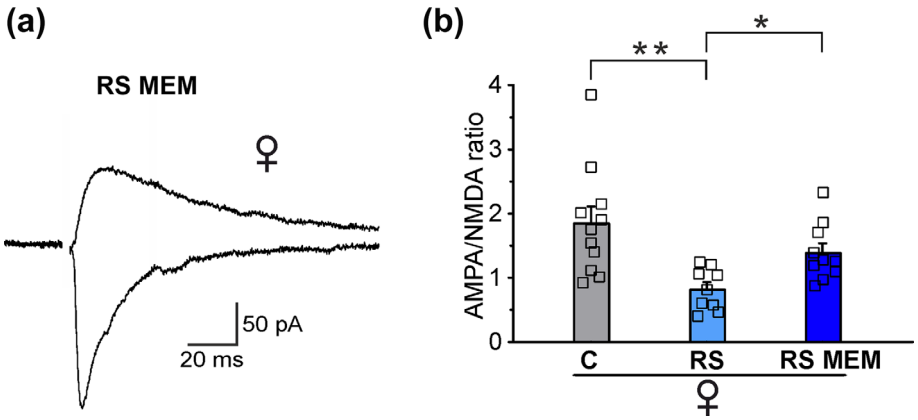

FIGURE 5 **Memantine prevents acute restraint stress-induced decrease in AMPA/NMDA ratio in the dHPC of female mice.** (a) Representative traces of AMPA receptor–mediated and NMDA receptor–mediated EPSCs recorded from CA1 pyramidal neurons in the dHPC of female mice subjected to acute restraint stress and treated with memantine. C ($n = 11$ from 5 mice), RS ($n = 9$ from 5 mice), RS + memantine ($n = 10$ from 5 mice). (b) Quantification of the AMPA/NMDA ratio (*Treatment*, $F_{(2, 27)} = 7.133$, $P = 0.0033$). Statistical significance was analysed by one-way ANOVA followed by Sidak's multiple comparisons test $^{*}P < 0.05$, $^{**}P < 0.01$. Values are expressed as mean ± SEM. *n*/*m* indicates number of recorded cells/number of animals.

responses (Kokras et al., 2018). Our selective focus on the dHPC (Fanselow & Dong, 2010), as opposed to whole hippocampus analysis, may account for the detection of these acute stress–induced metabolic changes, suggesting that regional specificity within the hippocampus is key for understanding sex-specific glutamatergic responses to acute stress. Notably, our temporal analysis revealed a sex-dependent and time-specific pattern: Whereas female mice exhibited immediate hyperglutamatergic responses after stress, male mice showed a significant reduction in both glutamate and glutamine levels at 24 h post-stress, suggesting a delayed hypoglutamatergic state. This male-specific stress-induced metabolic change, in the absence of changes in basal excitatory synaptic transmission, may primarily reflect adaptive modulation of energy metabolism rather than a direct synaptic deficit. Acute stress–induced glutamate release is known to transiently increase glutamate levels in limbic and cortical regions (Moghaddam, 1993; Musazzi et al., 2010), including the hippocampus. In turn, this increase can boost the astrocytic metabolic load and engage the astrocyte–neuron **lactate** shuttle, thereby enhancing oxidative phosphorylation, which sustains glutamate synthesis, vesicular packaging and recycling (Patel et al., 2014). Within this framework, the hypoglutamatergic profile found in stressed male mice at 24 h may represent a delayed, male-specific adjustment of glutamatergic turnover following the initial stress-induced challenge, whereas the sustained increase of the glutamate–glutamine pool in stressed female mice points to a distinct trajectory of glutamatergic adaptation. Together, these findings support fundamental sex differences in the temporal regulation of glutamate homeostasis under acute stressful conditions. The hyperglutamatergic state characterising stressed female mice likely results in glutamate spillover within the dHPC (Kullmann & Asztely, 1998), which could trigger synaptic dysfunctions by excessively activating extrasynaptic NMDA receptors. The concept that overstimulation of extrasynaptic NMDA receptors triggers pro-death pathways and LTD of synaptic transmission (Hardingham & Bading, 2010) provides a mechanistic framework for interpreting our findings. Our electrophysiological findings indeed align with this mechanism: exclusively stressed female mice exhibited a robust reduction in AMPA/NMDA ratio, indicating weakened AMPA receptor–mediated synaptic strength relative to NMDA receptor activity. This shift towards predominant NMDA receptor activation, potentially involving extrasynaptic NMDA receptors, might promote LTD and thereby contribute to memory impairment. These findings thus indicate that the dorsal hippocampal glutamatergic system is more reactive to acute stress in female mice rather than in male mice (Marrocco et al., 2017). This interpretation is consistent with previous studies showing both baseline and stress-induced sex differences in glutamatergic receptor composition and synaptic strength across multiple brain regions (Kniffin & Briand, 2024). For instance, a higher AMPA/NMDA ratio was found in the nucleus accumbens core of female mice and rats compared to males (Knouse et al., 2023), a finding consistent with heightened glutamatergic transmission in females.

Our electrophysiological findings further demonstrate this sex-dependent vulnerability of the dorsal hippocampal glutamatergic system. At the synaptic level, acute stress selectively reduced mEPSC amplitude in CA1 pyramidal neurons of female mice, indicating a weakening of postsynaptic AMPA receptor–mediated responses. This finding is consistent with the mechanism whereby excessive extrasynaptic NMDA receptor activation promotes LTD (Hardingham & Bading, 2010), which is a form of synaptic plasticity involving AMPA receptor internalisation and reduced synaptic strength (Beattie et al., 2000). Importantly, our data further corroborate previous findings demonstrating that exposure to acute stress reduced basal mEPSC amplitude and frequency, which in turn predicted larger magnitudes of LTD (Zhang et al., 2005). Notably, stressed male mice did not exhibit alterations in AMPA/NMDA ratio or mEPSC amplitude despite showing spatial memory deficits. This finding suggests that different mechanistic pathways underlie stress-induced cognitive impairment across sexes.

The selective efficacy of MK-801 in stressed male mice and memantine in stressed female mice reveals sex-dependent interactions between stress-induced pathophysiology and drug pharmacodynamics. In stressed male mice, the rescue of spatial memory impairment induced by MK-801, but not by memantine, suggests that acute stress-induced cognitive impairment in males may be mediated predominantly through synaptic NMDA receptors rather than extrasynaptic NMDA receptors. This is consistent with evidence that acute stress augments synaptic NMDA receptor function, whilst simultaneously suppressing extrasynaptic NMDA receptor activity in CA1 pyramidal neurons (Tse et al., 2021), and consistent with data showing that acute stress increases the binding of MK-801 to NMDA receptors in male but not female mouse forebrain (Akinci & Johnston, 1993). Under these acute stress–induced conditions, memantine would be ineffective given its preferential targeting of extrasynaptic NMDA receptors (Lipton, 2006). Conversely, MK-801, which non-selectively antagonises both synaptic and extrasynaptic NMDA receptors (Song et al., 2018), might effectively counteract the enhanced synaptic NMDA receptor activity. Although we did not find alterations in the AMPA/NMDA ratio in stressed male mice at the 24-h timepoint, the pro-cognitive effect of MK-801 suggests that acute stress can induce earlier transient changes in synaptic NMDA receptor function, consistent with the dynamic time-dependent effects previously reported (Tse et al., 2021).

In contrast, the selective efficacy of memantine in stressed female mice indicates that female-specific spatial memory impairment can be mediated mainly through pathological extrasynaptic NMDA receptor overactivation, consistent with our metabolic and electrophysiological findings. As aforementioned, the glutamate-glutamine accumulation observed exclusively in stressed female mice likely results in tonic activation of extrasynaptic NMDA receptors, creating the pathophysiological conditions that memantine preferentially targets (Lipton, 2006). The normalisation of the AMPA/NMDA ratio by memantine corroborates this mechanism, demonstrating that preferential blockade of extrasynaptic NMDA receptors restores synaptic strength to control levels. MK-801 lacks preferential selectivity for extrasynaptic over synaptic NMDA receptors (Xia et al., 2010), explaining its inefficacy in stressed female mice, where selective extrasynaptic NMDA antagonism is required.

Our findings can have implications for understanding the neurobiological basis of sex-dependent stress responses and their pathophysiological consequences for cognitive function. In our model, we demonstrate that the same spatial memory impairment can arise through different pathophysiological mechanisms in stressed male and female mice. This highlights the critical importance of biological sex as a fundamental variable in the context of stress-induced cognitive impairments. The sex-dependent efficacy of two different NMDA receptor antagonists underscores that sex differences in stress-induced cognitive dysfunction are not merely quantitative but rather qualitatively distinct in their underlying mechanisms. This mechanistic heterogeneity should be considered in the development and testing of cognitive enhancers for stress-related neuropsychiatric disorders. However, our conclusions, which are based on a single preclinical model of acute stress in mice, should be viewed as hypothesis-generating. Future work using additional stress paradigms and behavioural tasks is necessary to determine whether these sex-dependent mechanisms and drug effects extend across models and translate to clinical setting.

AUTHOR CONTRIBUTIONS

Sebastiano A. Torrisi: Conceptualization; investigation; writing—original draft; methodology; writing—review and editing; formal analysis; project administration; data curation; supervision. **Lidia Diolosà:** Investigation; methodology. **Giuseppe Aceto:** Investigation; formal analysis. **Claudia Marchetti:** Investigation; formal analysis. **Federica Geraci:** Investigation. **Konstantinos Partsinevelos:** Investigation. **Silvia Rizzo:** Investigation. **Margherita Grasso:** Investigation. **Filippo Caraci:** Supervision. **Francesco Bellia:** Investigation. **Giovanni Li Volti:** Supervision. **Angela Maria Amorini:** Investigation; formal analysis. **Marcello D'Ascenzo:** Conceptualization; writing—review and editing; formal analysis; data curation; supervision. **Gian Marco Leggio:** Conceptualization; data curation; supervision; writing—review and editing; formal analysis; funding acquisition; writing—original draft; methodology; visualization; project administration; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets underlying this study are available from the corresponding author upon reasonable request. Additional information can be provided upon request.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This declaration confirms that the present paper complies with the principles of transparent reporting and scientific rigour in preclinical research, as outlined in the *British Journal of Pharmacology* guidelines for Design & Analysis and as recommended by funding agencies, publishers and other organizations committed to supporting high-quality research.

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