

Original Research

Astrocytes Are Involved in the Effects of Ketamine on Synaptic Transmission in Rat Primary Somatosensory Cortex

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Abstract

Background: Ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, is widely used as a general anaesthetic. However, the mechanisms of analgesic/anaesthetic effects induced by ketamine are only partially understood. Previously, studies have demonstrated that various general anaesthetics affect the primary somatosensory cortex (S1), a potential target of general anaesthetics in the central nervous system. However, it is unknown if astrocyte activities affect ketamine's effects on information transmission in S1 pyramidal neurons. **Methods:** The whole-cell patch-clamp technique was employed to study the role of astrocytes in ketamine-induced anaesthetic actions. The whole-cell patch-clamp method was used to record the spontaneous postsynaptic currents (SPSCs) of rat S1 pyramidal neurons. We used the glia-selective inhibitor of the aconitase enzyme fluorocitrate (FC), to test if astrocyte activities alter the effects of ketamine on S1 pyramidal neurons. **Results:** Ketamine lowered the SPSCs of rat S1 pyramidal neurons in a concentration-dependent manner at clinically relevant doses. The concentration-effect curve revealed that ketamine had an EC₅₀ value of 462.1 M for suppressing SPSCs. In rat S1 pyramidal neurons, the glia-selective metabolic inhibitor fluorocitrate (FC), which inhibits the aconitase enzyme, lowered the amplitude and frequency of SPSCs. The inhibitory impact of ketamine on the amplitude and frequency of SPSCs was significantly amplified in the presence of FC. **Conclusions:** Astrocytes impact the effects of ketamine on pre- and postsynaptic components and play a role in synaptic transmission.

Keywords: ketamine; astrocytes; primary somatosensory cortex; patch-clamp; spontaneous postsynaptic currents

1. Background

Ketamine, a racemic combination of (S)- and (R)-ketamine, has been used in therapeutic trials since 1970. Because of its short half-life and lack of clinically significant respiratory depression, ketamine has proven to be a popular anaesthetic [1]. Ketamine has analgesic [2], anti-inflammatory [3], and antidepressant properties [4] in addition to its well-known anaesthetic action in adults, children, and obstetric patients. Numerous placebo-controlled trials have shown that ketamine, when administered intravenously (IV) at subanesthetic dosages (0.5 mg/kg over 40 min), can produce rapid (within hours), transitory antidepressant effects [5–7]. In contrast to the majority of other anaesthetics with sedative or hypnotic effects, ketamine does not predominantly operate through gamma-aminobutyric acid (GABA) receptors [8]. The direct ketamine-induced inhibition of N-methyl-D-aspartate receptors (NMDARs) is thought to be responsible for ketamine's anaesthetic and analgesic characteristics [9]. The sensation of being aware while being drawn away from sensory perceptions can be used to describe the dissociative effects of ketamine. Because NMDARs are crucial for ex-

citatory neurotransmission, long-term potentiation (LTP), and memory formation, a high dose of ketamine induces a condition of deep dissociation accompanied by amnesia and loss of consciousness. Notably, the rise in abuse of ketamine as a drug from the 1970s to the present shows that its psychedelic properties are also desired by recreational users [2]. GABA, dopamine, serotonin, sigma, opioid, and cholinergic receptors, as well as voltage-gated sodium and hyperpolarization-activated cyclic nucleotide-gated channels, are all potential therapeutic targets of ketamine [10, 11]. However, the underlying neurological mechanisms of unconsciousness induced by ketamine remain unknown. Previous research has suggested that the thalamocortical system is linked to the loss of consciousness caused by general anaesthetics [12–14]. The primary sensory cortex (S1) is the principal area of the brain that receives face sensory sensation information, and may play a significant role in general anaesthetic-induced unconsciousness in the central nervous system [15, 16]. In previous studies, synaptic transmission modulation has been found to be the primary mechanism of action for general anaesthetics [17, 18].

When astrocytes were first described, they were thought to serve just as a structural framework to support



and fill in the spaces between neurons. However, evidence indicates that astrocytes protect neuronal networks in ways that go well beyond providing nutrients or acting as a structural support system [19]. They also play a minor role in information representation and processing. Previous studies have demonstrated that astrocytes play a role in the physiological control of synaptic transmission and that synapses and astrocytes communicate bidirectionally [11,20–22]. By using *in vivo* two-photon Ca^{2+} imaging, Winship *et al.* [23] demonstrated that astrocytes in mouse primary somatosensory cortex exhibit short-latency (peak amplitude ~ 0.5 s after stimulus onset), contralateral hindlimb-selective sensory-evoked Ca^{2+} signals that operate on a time scale similar to neuronal activity and correlate with the onset of the hemodynamic response as measured by intrinsic signal imaging. Since more recent research has suggested that astrocytes may also produce their own group of gliotransmitters [24,25], astrocytes are highly secretory cells that play a role in rapid brain communication via modulating neurotransmission [26,27]. Neurotransmitters released from the synapse, such as glutamate, Ach, and others, could stimulate astrocytes [28–30]. For instance, the neuromodulator norepinephrine can activate astrocyte networks in the mouse visual cortex in response to dynamic shifts in the animal's level of arousal. This elevated astrocyte activity may also improve astrocytes' capacity to recognise changes in neuronal activity [31]. It has also been shown that astrocytes may regulate circuit activity in *Drosophila*, where octopaminergic neurons communicate with astrocytes to influence downstream neurons and behaviors [32]. Gliotransmitters, which include purines, D-serine, glutamate, adenosine, and ATP, are released by astrocytes and can alter synaptic transmission and neuronal activity [33,34]. Consciousness is thought to be the result of these interactions between astrocytes and neurons [11]. Poskanzer [31] suggested that astrocytes may control sleep state more than a century ago, and now, there is a number of studies to support this theory.

However, it is unclear whether the activity of astrocytes plays a role in the effect of ketamine on synaptic transmission. To address this issue, we investigated how ketamine affected spontaneous postsynaptic currents (SPSCs) in rat S1 pyramidal neurons before and after the astrocytes were suppressed by fluorocitrate (FC), an inhibitor of glioselective metabolism that inhibits the aconitase enzyme and has been demonstrated to be efficient in reducing astrocytic function both *in vivo* [35,36], to determine the role of astrocytes in the analgesic/anaesthetic actions of ketamine.

2. Methods

2.1 Experimental Animals

Forty Sprague–Dawley rats were obtained from the third military medical university's animal centre (Chongqing, China). All experimental procedures were approved by the Animal Care and Use Committees of Zunyi

Medical University in Guizhou, China, and carried out in accordance with the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals [37]. Experiments were performed following the “Guide for the Care and Use of Laboratory Animals” in China (No. 14924, 2001). All rats were housed in an environment with an ambient temperature of 23 ± 0.5 °C, a relative humidity of 52%, a 12-hour light/12-hour dark cycle (light on at 8:00 AM), and free access to food and water. All the Sprague-Dawley rats were anaesthetized with 2% isoflurane (R510-22-10, RWD, Shenzhen, China) and then sacrificed.

2.2 Preparation of Brain Slices

The process of brain slice preparation was performed as previously described [10,38]. Male Sprague-Dawley rats (10–20 days after birth) were anaesthetized with 2% isoflurane and assessed by the toe pinch reflex to ensure adequate depth of anaesthesia. Then rats were decapitated using super-cut scissors. The scissors were used to cut away the skin around the skull cap and create minor incisions at the caudal/ventral base of the skull on either side. Shallow cuts were made starting at the caudal/dorsal aspect of the skull and working up the dorsal midline in a rostral direction, being careful not to injure the underlying brain. A final “T” cut was made at the level of the olfactory bulbs, perpendicular to the midline, and the complete brain mass was rapidly separated with a metal spatula. The brain was submerged in an ice-cold artificial cerebrospinal fluid (ACSF) consisting of 126 mM NaCl, 3 mM KCl, 2 mM CaCl_2 , 2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.5 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 25 mM NaHCO_3 , and 10 mM Glucose H_2O (pH = 7.3, 290–300 mOsm). The ACSF was constantly bubbling with 95 percent oxygen and 5 percent carbon dioxide. The S1-containing tissue block was then separated from the brain, and coronal slices (350 μm thick) were cut using an HM 650V vibroslicer (Thermo Instruments, Waltham, MA, USA). The slices were incubated in ACSF for one hour prior to whole-cell recording at 32 °C.

2.3 Electrophysiological Recordings

Individual coronal slices were placed into a thermoregulated (31–32 °C) recording container and continuously perfused with bubbled ACSF (0.52 mL/min) after incubation. S1 stereotaxic coordinates were used to choose electrophysiological recording sites in coronal rat slices (Paul Halasz & Lewis Tsalis 6th). S1 pyramidal neurons were chosen for electrophysiological recordings using an infrared camera on a microscope (BX51WI, Olympus, Japan). Electrical signals were acquired using a HEKA EPC10 amplifier (HEKA Instruments, Ludwigshafen, Germany) and PatchMaster software (v2x80, Heka Instruments, Ludwigshafen, Germany) in whole-cell voltage clamp settings. Data was sampled at 20 kHz and filtered at 3 kHz. When filled with the pipette solution containing 120 mM CsCl, 10 mM NaCl, 10 mM HEPES,

2 mM MgCl₂, 10 mM EGTA, 1 mM CaCl₂, and 10 mM TEA-Cl (pH = 7.3), the electrodes obtained a resistance of 3–5 M. Approximately 70% of the series resistance was corrected. Neurons were voltage-clamped at –70 mV to record SPSCs. FitMaster (v2x73.2, HEKA Instruments, Ludwigshafen, Germany) and the MiniAnalysis Program (6.1, Synaptosoft, Decatur, GA, USA) was used to count and analyse SPSCs. An amplitude threshold of 15 pA was used to automatically filter SPSCs. If the resistance varied by greater than 20% during recording, the neurons were rejected, and events <3 pA were eliminated from data analysis [38].

2.4 Chemicals

D, L-2-amino-5-phosphonovaleric acid (AP5), 6,7-dinitroquinoxaline-2,3-dione (DNQX), tetrodotoxin (TTX), gabazine, and fluorocitrate (FC) were among the substances employed in the analyses. Chemicals were provided by the Sigma-Aldrich Chemical Company (Shanghai, China). Nhwa Pharma. Corp. (Xuzhou, Jiangsu, China) provided the ketamine. All compounds were diluted in ACSF and used at a known concentration in the bath. Ketamine was dissolved into ACSF and the concentrations of ketamine in were 200 μMol/L, 400 μMol/L, 600 μMol/L, 800 μMol/L, 1000 μMol/L, respectively. After impaling, brain slices were given 5 minutes to equilibrate. Brain slices were treated with several drug solutions for 5 minutes prior to whole-cell electrophysiological recording. The whole-cell recording lasted 10 min. During the recording, all brain slices were continuously perfused with regular artificial cerebrospinal fluid or various medication solutions through a gravity-fed device.

2.5 Statistical Analysis

The amplitude and frequency of all events were compared to the mean values observed during the initial control period and during and after the drug application. Cumulative probability plots of the incidence of various amplitudes and intervals, recorded under different conditions from the same neuron, were subjected to the Kolmogorov–Smirnov (K–S) test. StatView software (SAS, Cary, NC, USA). GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA) was used to create the concentration-effect curve. N represents the number of neurons recorded. All results were presented as mean ± SD. One-way ANOVA was used to determine the statistical significance of medication effects, which was then followed by Dunnett's multiple comparison approach (SPSS 25.0, IBM Corp., Chicago, IL, USA). Significant values were defined as $p < 0.05$.

3. Results

3.1 The Spontaneous PSCs of Rat S1 Pyramidal Neurons

The patch electrodes were inserted in the S1 region (Fig. 1a,b). S1 neurons had a multipolar or triangular-

shaped soma, a bright and smooth appearance, and no apparent organelles under the microscope [6] (Fig. 1b). The SPSCs were then recorded by voltage-clamp. In S1 pyramidal neurons, SPSCs were detected before and during ketamine administrations at various doses (Fig. 1c). In the presence of AP5 (N-methyl-D-aspartate (NMDA) glutamate receptors antagonist, 100 μM), DNQX (20 μM, the antagonist of AMPA and kainite glutamate receptors), gabazine (antagonist of GABA_A receptors, 10 μM) or TTX (Na⁺ channel blocker, 1 μM), were recorded. All spontaneous synaptic activities were reversible, indicating that these currents were SPSC (Fig. 1c).

3.2 Effects of Ketamine on SPSCs in S1 Pyramidal Neurons

In brain slices, ketamine (200 μM, 400 μM, 600 μM, 800 μM, 1000 μM) was applied to S1 pyramidal neurons by a gravity-fed system; and decreased the frequency ($n = 12$, $p < 0.05$, Fig. 2a,d) and amplitude ($n = 12$, $p < 0.05$, Fig. 2b,c) of SPSCs, but the decay time of SPSCs was not significantly altered (not shown). Ketamine (200 μM, 400 μM, 600 μM, 800 μM, 1000 μM) decreased the amplitude of SPSCs by $37.8 \pm 4.0\%$, $39.3 \pm 4.8\%$, $46.3 \pm 2.7\%$, $75.6 \pm 4.1\%$ and $83.6 \pm 3.7\%$, respectively ($n = 12$, Fig. 2e). In addition, ketamine reduced the frequency of SPSCs by $29.1 \pm 1.0\%$, $42.3 \pm 1.8\%$, $65.8 \pm 2.2\%$, $72.3 \pm 3.8\%$ and $73.2 \pm 1.9\%$, respectively ($n = 12$, Fig. 2f). These findings showed that ketamine might modify the activity of postsynaptic receptors while inhibiting the release of presynaptic neurotransmitters.

The inhibitory effects of ketamine on the amplitude and frequency of SPSCs were related to its concentration. A concentration-effect curve was drawn (Fig. 2e,f) to calculate the EC₅₀ value of ketamine for inhibiting SPSCs amplitude and frequency in rat S1 pyramidal neurons. The results were 698.4 μM and 462.1 μM, respectively. In a previous study, hippocampus slices were exposed to a wide range of clinically relevant ketamine doses (1–600 M) and it was noted that clinically relevant ketamine dosages reduced long-term potentiation (LTP) in a concentration-dependent way without affecting paired-pulse facilitation (PPF) [39]. In contrast, only high dosages of ketamine (300 and 600 M) had an effect on baseline excitatory synaptic transmission and presynaptic volley amplitude. Therefore, 460 μM ketamine was used in the subsequent experiments.

3.3 Astrocytes Participate in Synaptic Activities in S1 Neurons

Fluorocitrate (FC) is a selective aconitase enzyme inhibitor [40,41], which can effectively inhibit astrocytes' function *in vivo* and *in vitro* [36]. FC solution was prepared as previously described and diluted to 5 μM by adding standard ACSF, which does not appear to alter neuronal function [36]. To completely inhibit the activity of astrocytes, ACSF containing FC was used until the electrophysiological recording was completed.

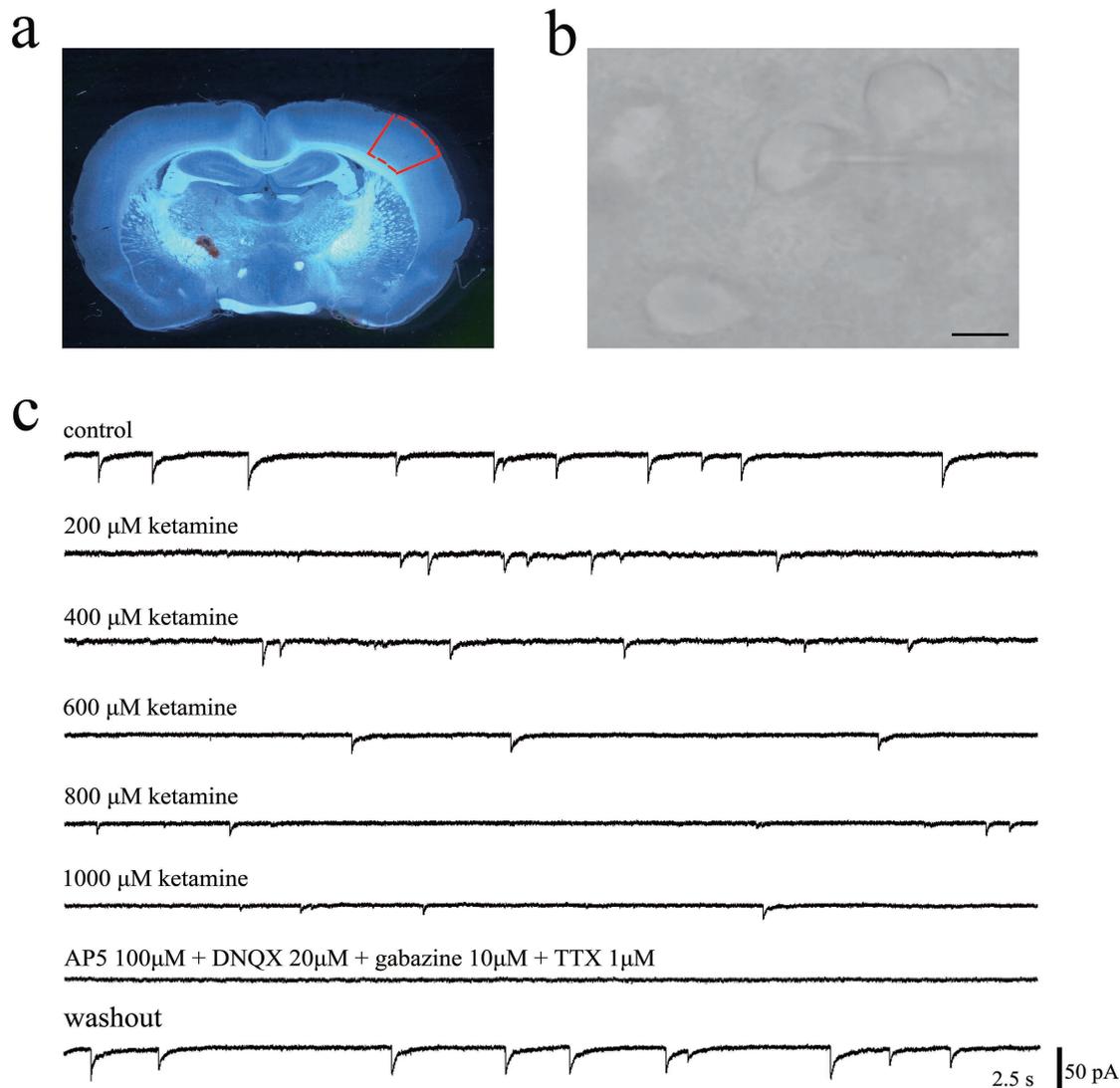


Fig. 1. S1 pyramidal neurons recording location and SPSCs. (a) A coronal brain slice with a thickness of 350 μm and the S1 recording region. (b) S1 pyramidal neuron with whole-cell recording electrode, Scale bar, 5 μm . (c) Typical traces of SPSCs obtained in pyramidal neurons under control circumstances (ACSF), ketamine, AP5 (100 μM) + DNQX (20 μM) + gabazine (10 μM) + TTX (1 μM) application and after washout with ACSF. DNQX, 6,7-dinitroquinoxaline-2,3-dione; TTX, tetrodotoxin; SPSCs, spontaneous postsynaptic currents; ACSF, artificial cerebrospinal fluid.

To inhibit astrocytes, 5 μM ACSF containing FC was perfused after 5 minutes ACSF equilibration. Before and after astrocyte inhibition, SPSCs were measured in S1 pyramidal neurons (Fig. 3a). The amplitude of SPSCs decreased from 46.5 ± 5.1 pA to 38.8 ± 2.6 pA during FC bath treatment ($n = 12$, $p < 0.05$, Fig. 3b), and the frequency of SPSCs decreased from 26.8 ± 4.1 min^{-1} to 21.3 ± 3.1 min^{-1} ($n = 12$, $p < 0.05$, Fig. 3c). FC (5 μM) shifted the distribution of amplitude to the left ($p < 0.05$, K-S test, Fig. 3d) and shifted the interval distribution to the right ($p < 0.05$, K-S test, Fig. 3e). These findings indicate that astrocytes play a role in synaptic activation.

3.4 Astrocyte Activities Are Involved in the Effect of Ketamine on SPSCs in S1 Pyramidal Neurons

Consistent with the method described above, 5 μM ACSF containing FC was perfused after 5 minutes ACSF equilibration. Before and during astrocyte inhibition, we examined the inhibitory effects of ketamine on SPSCs in rat S1 pyramidal neurons. Ketamine (460 M) decreased the amplitude and frequency of SPSCs from 46.5 ± 5.1 pA and 26.8 ± 4.1 min^{-1} to 25.9 ± 2.9 pA and 12.9 ± 2.2 min^{-1} ($n = 12$, $p < 0.05$, Fig. 4a–c), respectively, when only ACSF was perfused ($n = 12$, $p < 0.05$, Fig. 4a–c). Ketamine (460 M) lowered the amplitude of SPSCs from 38.8 ± 2.6 pA to 19.3 ± 2.7 pA ($n = 12$, $p < 0.05$, Fig. 4a,b), and the frequency of SPSCs from 21.3 ± 3.1 min^{-1} to 7.1 ± 1.5

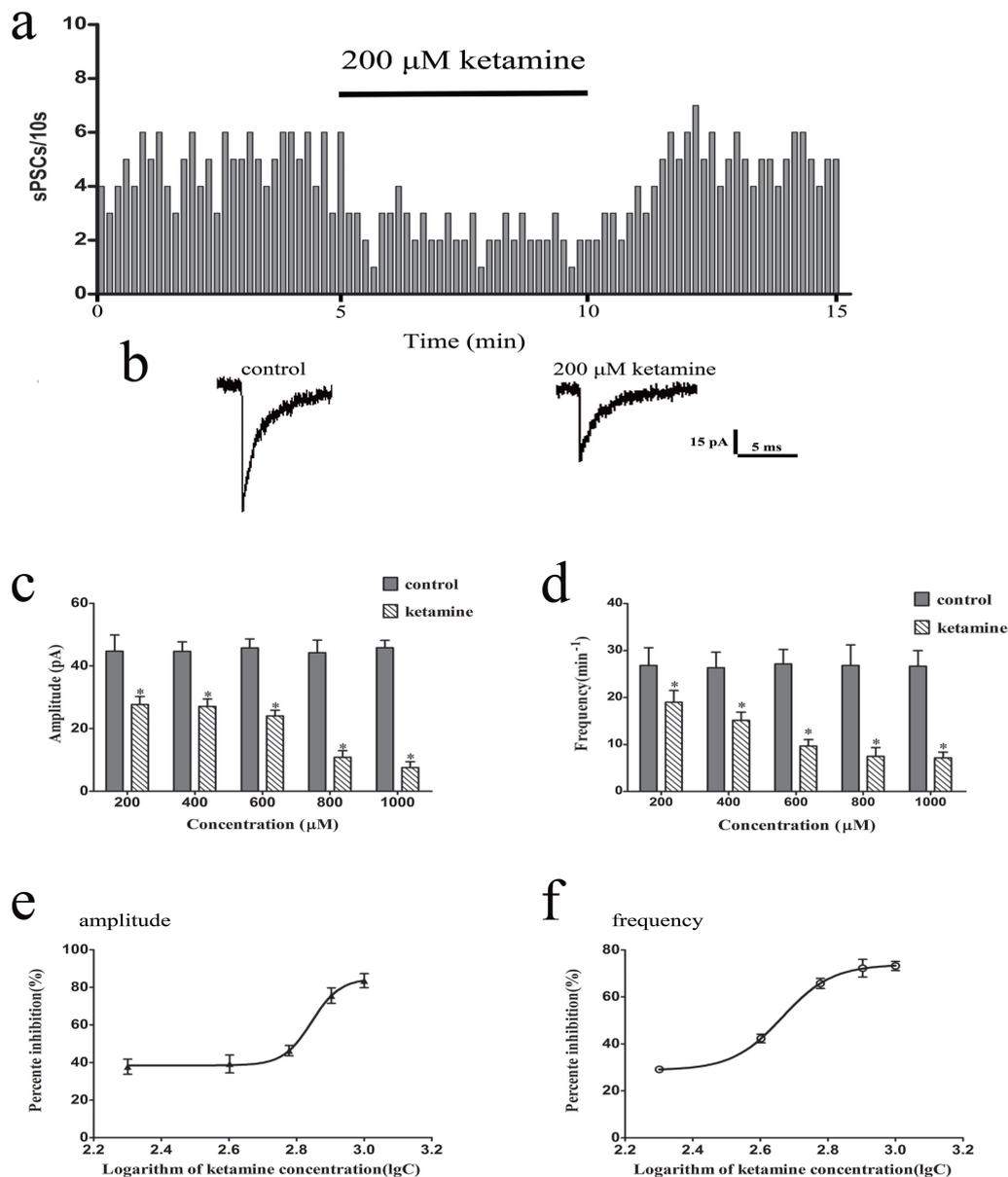


Fig. 2. Ketamine's impact on SPSCs in S1 pyramidal neurons. (a) Ketamine (200 M) reduced SPSC frequency. (b) Ketamine (200 μ M) reduced the amplitude of SPSCs. Summary results for mean SPSCs. (c) amplitude and (d) frequency from pyramidal neurons in control ($n = 12$) and ketamine ($n = 12$) conditions. (e) amplitude and (f) frequency ($n = 12$, each point) concentration-effect curve for ketamine-induced alterations in SPSCs. Data are displayed as mean \pm SD. * $p < 0.05$ by the Student's paired two-tailed t -test for control versus ketamine.

min^{-1} ($n = 12$, $p < 0.05$, Fig. 4a,c) in the presence of FC. Furthermore, the inhibition rate of SPSCs amplitude rose from $44.3 \pm 3.1\%$ in ketamine to $50.5 \pm 4.1\%$ in FC + ketamine ($n = 12$, $p < 0.05$, Fig. 4d), and the inhibition rate of SPSCs frequency increased from $51.9 \pm 6.8\%$ in ketamine to $66.9 \pm 3.7\%$ in FC + ketamine ($n = 12$, $p < 0.05$, Fig. 4e).

4. Discussion

In previous studies, the bidirectional connection between astrocytes and neurons was found to be critical for information integration [42,43]. In this study, ketamine

was observed to reduce the amplitude and frequency of SPSCs in rat S1 pyramidal neurons. The activity of astrocytes could determine the effect of ketamine on SPSCs.

We observed that clinically relevant ketamine doses lowered the amplitude and frequency of SPSCs in a concentration-dependent manner without altering the decay time. Changes in the amplitude and frequency of SPSCs suggest that there are changes in the function of post-synaptic receptors. Therefore, our study indicates that ketamine's role on SPSCs is due partly to regulating postsynaptic receptors' function. Ketamine also inhibits several

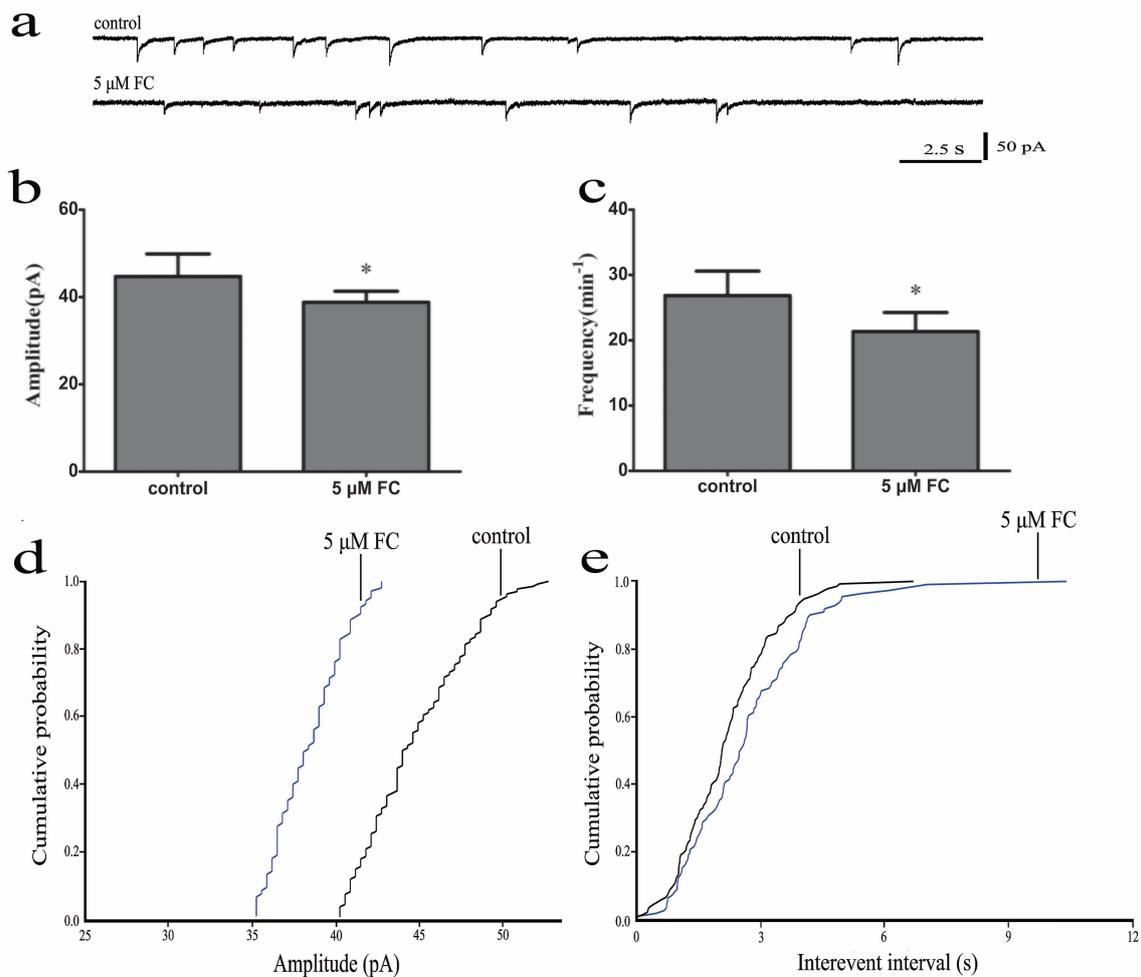


Fig. 3. Astrocytes impact synaptic transmission in rat S1. (a) Typical traces of SPSCs recorded in S1 pyramidal neurons in control circumstances (ACSF) and FC (5 M). Summary results for mean SPSCs (b) amplitude and (c) frequency from pyramidal neurons in control ($n = 12$) and FC ($n = 12$) conditions. Cumulative probability plots for (d) amplitude ($p < 0.05$, K–S test) and (e) interevent interval ($p < 0.05$, K–S test) of SPSCs. The data is presented as mean \pm SD. * $p < 0.05$ by Student’s paired two-tailed t -test for control versus FC. FC, fluorocitrate.

ion channels, such as voltage-gated Na^+ and K^+ channel [44,45], and HCN1 channels [46]. Recent studies found that ketamine also influences the release of neurotransmitters, such as acetylcholine [47], GABA [48], dopamine [49], and glutamate [50].

SPSC frequency and amplitude decreased when astrocytes were suppressed. The current findings suggest that astrocytes play a role in the synaptic information processing of rat S1 by modifying the function of postsynaptic receptors. Our findings are supported by the theory of a “tripartite synapse” [33,51], in which the astrocyte functions as a component of the synapse. The structure is made up of pre- and postsynaptic synapse parts as well as an astrocytic process. A previous study showed that the “tripartite synapse” is present in S1 and participates in sensory information processing [52]. Tripartite synapses are also found in the human brain. Our study helps to further understand astrocyte function in the human central nervous system.

We also examined the inhibitory effect of ketamine on amplitude and frequency and found that the inhibitory effect of ketamine on astrocytes was enhanced. These findings show that astrocyte activity can reduce ketamine’s inhibitory effects on postsynaptic receptor function in rat S1 pyramidal neurons. There is increasing evidence that astrocyte activities are required to enhance synaptic transmission [34,53,54]. Thrane *et al.* [55] also found that ketamine can inhibit astrocytes through modulating its calcium signals. Recent evidence suggests that cortical astrocytes also express functional NMDA receptors [56,57]. These results, suggest that ketamine, an NMDA receptor antagonist, might inhibit astrocytes’ activities at least partially through the NMDA receptor.

Astrocytes also express other receptors, such as metabotropic glutamate receptors (mGluRs), α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA_Rs), kainate receptors, and GABA_B receptors [58,

Unlike other intravenous anaesthetics, such as propofol and etomidate, ketamine did not affect the permeability of gap junctions between astrocytes [50]. These results strongly suggest that astrocytes play a complex role in the mechanisms of ketamine's analgesic/anaesthetic action. The role of astrocytes in the pharmacological effects of intravenous general anaesthetics requires further study.

This study has a few limitations. Firstly, only neonatal rats' brain slice was examined, whether adult rat astrocytes are involved in synaptic transmission and alter the effects of ketamine on synaptic transmission deserves further study. Secondly, we only observed that astrocytes were engaged in synaptic transmission and altered ketamine's effects on synaptic transmission *in vitro*. It would be interesting to see if the same (or different) alterations might be observed if ketamine were administered *in vivo*.

5. Conclusions

Our findings demonstrate that ketamine inhibits synaptic activity via presynaptic and postsynaptic components. Furthermore, we found that astrocytes engage in synaptic transmission and alter ketamine's effects on synaptic transmission. This work adds to the growing body of evidence indicating astrocytes play a key role in the mechanisms of ketamine's analgesic/anaesthetic actions.

Abbreviations

NMDA, N-methyl-D-aspartate; S1, primary somatosensory cortex; SPSCs, spontaneous postsynaptic currents; FC, fluorocitrate; GABA, γ -aminobutyric acid; ACSF, artificial cerebrospinal fluid; D, AP5, L-2-amino-5-phosphonovaleric acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; TTX, tetrodotoxin; mGluRs, glutamate receptors; AMPARs, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors.

Availability of Data and Materials

On reasonable request, the corresponding author will provide the datasets created and analysed during the current work.

Author Contributions

JY and YZ contributed equally to this work. JY performed the experiment, analyzed the data, and wrote the manuscript. YZ experimented and modified the paper. HY and SC designed and experimented. YL performed the experiment. TY designed and supervised the experiments and modified the paper. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Medical Ethics Committee of the Zunyi Medical University Animal Care and Use Committee (approval code: 2021126).

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Conflict of Interest

The authors declare no conflict of interest.

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