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Original Article

Effects of Ketamine on Neuronal Spontaneous Excitatory Postsynaptic Currents and Miniature Excitatory Postsynaptic Currents in the Somatosensory Cortex of Rats

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Abstract

Background: Ketamine is a commonly used intravenous anesthetic which produces dissociation anesthesia, analgesia, and amnesia. The mechanism of ketamine-induced synaptic inhibition in high-level cortical areas is still unknown. We aimed to elucidate the effects of different concentrations of ketamine on the glutamatergic synaptic transmission of the neurons in the primary somatosensory cortex by using the whole-cell patch-clamp method.

Methods: Sprague-Dawley rats (11–19 postnatal days, n=36) were used to obtain brain slices (300 µM). Spontaneous excitatory postsynaptic currents (data from 40 neurons) were recorded at a command potential of -70 mV in the presence of bicuculline (a competitive antagonist of $GABA_A$ receptors, 30 µM) and strychnine (glycine receptor antagonist, 30 µM). Miniature excitatory postsynaptic currents (data from 40 neurons) were also recorded when 1 µM of tetrodotoxin was added into the artificial cerebrospinal fluid. We used GraphPad Prism5for statistical analysis. Significant differences in the mean amplitude and frequency were tested using the Student paired 2-tailed *t* test. Values of $P<0.05$ were considered significant.

Results: Different concentrations of ketamine inhibited the frequency and amplitude of the spontaneous excitatory postsynaptic currents as well as the amplitude of the miniature excitatory postsynaptic currents in a concentration-dependent manner, but they exerted no significant effect on the frequency of the miniature excitatory postsynaptic currents.

Conclusion: Ketamine inhibited the excitatory synaptic transmission of the neurons in the primary somatosensory cortex. The inhibition may have been mediated by a reduction in the sensitivity of the postsynaptic glutamatergic receptors.

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Keywords ● Ketamine ● Patch-clamp techniques ● Excitatory postsynaptic potentials ● Synaptic transmission

What's Known

- Ketamine is a commonly used intravenous anesthetic which produces dissociation anesthesia, analgesia, and amnesia. The mechanism whereby ketamine affects synaptic transmission is still unknown.

What's New

- Ketamine inhibited the excitatory synaptic transmission of neurons in the primary somatosensory cortex, which may be mediated by reducing the sensitivity of the postsynaptic glutamatergic receptors.
- Postsynaptic glutamatergic receptors in the primary somatosensory cortex played a role in the pharmacological mechanism of ketamine.

Introduction

With the development of anesthesiology, millions of patients have received surgical operations under general anesthesia. However, how the general anesthetics produce analgesia,

unconsciousness, immobility, and amnesia has yet to be defined. The primary somatosensory cortex modulates somatosensory information and receives projections from the thalamus ventral posteromedial nucleus and facial sensory information integration itself.[1,2] Our previous studies showed that anesthetics were able to interrupt the thalamocortical pathway during general anesthesia.[3,4]

Ketamine is the only intravenous anesthetic with an analgesic effect, and it is widely used for general anesthesia. Moreover, ketamine is associated with a special phenomenon, known as "dissociation anesthesia". Research indicates that ketamine acts on the N-methyl-D-aspartate (NMDA) receptors in neurons. According to are view by Alkire et al.,[5] aside from a close relationship with the excitatory NMDA receptors in the central nervous system, ketamine also interacts with the voltage-gated ion channels, including voltage-gated Ca^{2+} channels, delayed rectifier potassium channel, and hyperpolarization-activated cyclic nucleotide-gatechannel,[6] which eventually leads to interference on the synaptic transmission.

Nonetheless, the mechanism whereby ketamine specifically influences the electrophysiological activities in the cortical neurons has not been extensively explored. We chose the neurons of the primary somatosensory cortex as the target area to investigate the effects of ketamine on the glutamate receptors-related spontaneous excitatory postsynaptic currents (sEPSCs) and miniature excitatory postsynaptic currents (mEPSCs) in rats.

Materials and Methods

Animals

Sprague-Dawley rats (male and female, 11–19 postnatal days, 20–30 g, n=36) were purchased from the Animal Center of the Third Military Medical University (Chongqing, China) and housed in 12-hour light/dark controlled rooms at 22±2 °C and humidity of 50%, with *ad libitum* access to food and water. The protocols of animal experiments were approved by the Committees on Investigations Involving Animals in Zunyi Medical College and complied with the Guide for the Care and Use of Laboratory Animals in China (#14924, 2001).

Pipette Solution

The recording pipettes, pulled with a P-97 Micropipette Puller (Sutter Instruments, Novato, CA), with a tip diameter <1 μM, were borosilicate glass capillaries(Sutter Instruments, Novato, CA), and the other regents of the artificial cerebrospinal fluid (ACSF) and the pipette solution were analytical grade and were purchased from a domestic company. The formulation of the pipette solution varied with the target current or voltage, whereas the components, osmotic pressure, and pH value were similar to those of the intracellular fluid. The pipette solution for recording mEPSCs (in mM) comprised 140KCL, 10EGTA, 10HEPES, and $2Na_2ATP$; pH=7.4; adjusted with 9.2mM of KOH. Next, sEPSCs were measured (in mM) with a special pipette solution containing high Cs, 140CsCl, 10EGTA, 10HEPES, and $2Na_2ATP$; pH=7.4; adjusted with CsOH. The osmotic pressure was 310mOsmol.

Artificial Cerebrospinal Fluid (ACSF)

The ACSF, the extracellular fluid, was used to irrigate slices in the period of preparation, incubation, and recording. The formulation of the ACSF also varied with the purpose of recording and was different for different uses slightly. The standard ACSF (in mM) was comprised of 126NaCl, 2.5KCl, $2CaC1_2$, $2MgSO_4{\cdot}7H_2O$, $25NaHCO_3$, $1.5NaH_2PO_4{\cdot}2H_2O$, and 10 glucose·H_2O. The ACSF (in mM) for recording sEPSCs consisted of 126NaCl, 2.5KCl, $2CaC1_2$, $2MgSO_4{\cdot}7H_2O$, $1.5NaH_2PO_4{\cdot}2H_2O$, $25NaHCO_3$, 10 glucose·H_2O, and 0.03 bicuculline (BIC, Sigma-Aldrich). The ACSF (in mM) form EPSCs was comprised of 126NaCl, 4KCl, $2CaC1_2$, $1MgSO_4{\cdot}7H_2O$, $1.25NaH_2PO_4{\cdot}2H_2O$, $25NaHCO_3$10 glucose·H_2O, 0.03BIC, 0.03 strychnine (Str, Sigma-Aldrich), and 0.001 tetrodotoxin (TTX, Sigma-Aldrich). Additionally, pH was adjusted to 7.34–7.45 and osmotic pressure to 315–320 mOsmol. All the ACSFs were bubbled with a mixture of 95% O_2+5% CO_2 before all the procedures.

Brain Slices

The rats were decapitated after being anesthetized with 1.5% isoflurane. Craniotomy was performed rapidly to make sure that the total brain tissue could be separated quickly with a spatula. The brain was then immersed in cold (0 °C) standard ACSF bubbled with 95% O_2+5% CO_2, as was mentioned before, and was refrigerated for 5 minutes. A mass tissue containing the primary somatosensory cortex was then cut, separated from the brain, affixed with cyanoacrylate, and placed in a cutting chamber. The tissue was again immersed in the ACSF and sectioned into about 6 to 8300-μM thick slices with the HM 650 V Vibratome (Thermo Instruments, USA). The slices were incubated in the ACSF for about 1 hour at 32 °C and 1 hour at 26 °C.

Drug Administration

A routine flow at a rate of 2 mL/min was adjusted on the perfusion system. Ketamine (GuTian Pharma, Fujian, China) was diluted into the ACSF with final concentrations of 30, 100, 300, and 1000 µM. In each concentration, sEPSCs and mEPSCs were recorded continuously within 5minutes. Subsequently, the normal ACSF was re-perfused in order to record the washout data. Data were excluded when the neuronal activity showed unstable signal or the continuous recording was interrupted by any reason (such as vibration and electric noise). For each concentration of ketamine, 8 continuous data were obtained for the statistics of the frequency and amplitude of sEPSCs and mEPSCs.

Whole-Cell Recording

The slices were transferred to a recording plate after incubation and perfused with ACSF at a flow rate of 2 mL/min. According to the stereotaxic coordinates of the primary somatosensory cortex (Bregma coordinates: 1.32–3.72 mm posterior, 5.0–5.5 mm lateral, and 1.8–3.4 mm depth; Charles and Watson George 6th, figure 1), the pyramidal neurons in layer IV of the primary somatosensory cortex were selected with a BX51WI microscope (Olympus, Japan). The cells were required to form a complete dendritic and axonal structure and smooth cell wall, with high light transmittance and a diameter of 10–20 µM. Electrodes with a tip resistance of 3–5 MΩ were used to perform whole-cell recording. The rupture of the plasma membrane was created by a slight suction through a polyethylene tube after the patch resistance was ≥2 GΩ. The laboratory temperature was controlled at 23-25°C. A short-term synaptic alteration was studied in the voltage clamp mode, at a holding voltage of -70 mV. Data were recorded at the concentration gradient of ketamine (µM): 30, 100, 300, and 1000 (n=8). Capacitance and 60–80% series resistance were compensated routinely. Finally, sEPSCs and mEPSCs were filtered at 2.9 kHz and sampled at 10 kHz using PATCHMASTER software (HEKA Instruments, Lambrecht, DE) and an HEKA EPC10 amplifier.

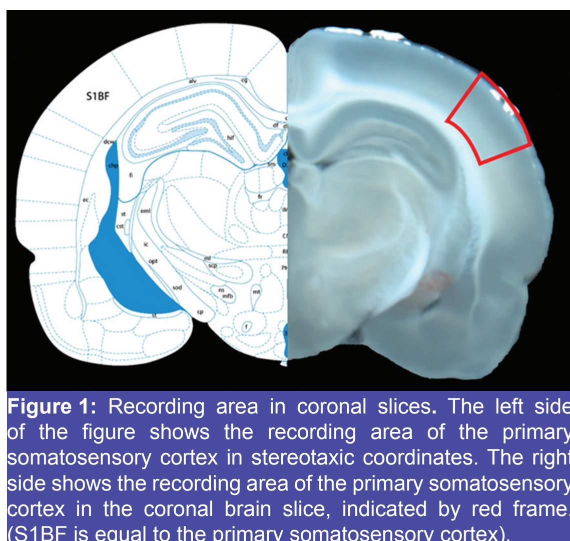

Figure 1: Recording area in coronal slices. The left side of the figure shows the recording area of the primary somatosensory cortex in stereotaxic coordinates. The right side shows the recording area of the primary somatosensory cortex in the coronal brain slice, indicated by red frame. (S1BF is equal to the primary somatosensory cortex).

Data Analysis

Currents were acquired with PATCHMASTER software, v2x53 (HEKA), and the original images were stored on the computer. The average values of both the frequency and amplitude of mEPSCs and sEPSCs during the 5-minute recording period were calculated in Mini Analysis 6.0 (Synaptosoft, Inc.). The frequency and amplitude of mEPSCs and sEPSCs before ketamine administration were normalized as 100% to these values during ketamine infusion. The root mean square of each group of data noise was calculated. In order to exclude false positive data, we visually filtered the original signals to delete electrical interference before analysis. The numerical values are expressed as mean±standard error of the mean using values normalized to the control. Cumulative probability plots for the frequency and amplitude of mEPSCs and sEPSCs were made using Mini Analysis 6.0 (Synaptosoft, Inc.).

Results

1. Effects of Ketamine on Spontaneous Excitatory Postsynaptic Currents in the Primary Somatosensory Cortex

When high resistance (≥2 GΩ) between the electrode and cell membrane was built, the cell membrane was ruptured to establish the whole-cell recording mode. The voltage was clamped at -70 mV. Thereafter, 30 µM of BIC was added into the ACSF and the GABA receptors were blocked. Then, sEPSC was performed. The control data were operated after 5 minutes' perfusion of the normal ACSF, and the amplitude and frequency of the currents were considered the data statistics (100%). Next, the different concentrations of ketamine were perfused, and independent data were obtained at a 5-minute interval. The original data are illustrated in figure 2A. A significant observation was made inasmuch as the frequency and amplitude of sEPSCs were decreased in a concentration-dependent reduction pattern (figure 2B, table 1). The finding indicated that the cumulative probability plots for frequency and amplitude

were shifted to the left and that they increased with the increasing concentration of ketamine (figure 2C). In conclusion, sEPSCs in the primary somatosensory cortex were concentration-dependent and were restrained by ketamine.

2. Effects of Ketamine on Miniature Excitatory Postsynaptic Currents in the Primary Somatosensory Cortex

As the same operation was performed, the whole-cell mode was established and the voltage was clamped at -70 mV. Next, 1 μM of TTX was added into the ACSF, and then Na^+ channels were blocked. Simultaneously, action potential was excluded and a spontaneous current was recorded. The current could be blockaded by the selective NMDA receptor antagonist, 2-amino-5-phosphonovalerate/pharmacology (APV, Sigma-Aldrich) and selective AMPA receptor antagonist, 6,7-dinitro-quinoxaline-2,3(1H,4H)-dione (DNQX, Sigma-Aldrich), indicating that the recorded current was mEPSCs mediated by the glutamatergic receptor (figure 3A). The control data were obtained in a manner described before as the base of the data statistics (100%). Then the different concentrations of ketamine were subjected to perfusion, and independent data were obtained in a 5-minute time window. We found that the amplitude of mEPSCs was decreased in a concentration-dependent pattern (figure 3B, table 1), but the frequency did not show any variety. The cumulative probability plots for frequency were recorded in a concentration of 100 μM; they showed no obvious shift when compared with those of control group. In contrast the plots of cumulative amplitude were shifted to the left by 30 μM of ketamine (figure 3C).

Discussion

In the present study, we found that ketamine significantly inhibited both the frequency and amplitude of sEPSCs of the pyramidal neurons in the primary somatosensory cortex in a concentration-dependent manner, but it failed to decrease the frequency of glutamatergic mEPSCs. However, ketamine decreased the amplitude of mEPSCs in a concentration-dependent manner. Given that the sodium current is a major component of sEPSCs, the inhibited effects of ketamine on sEPSCs were

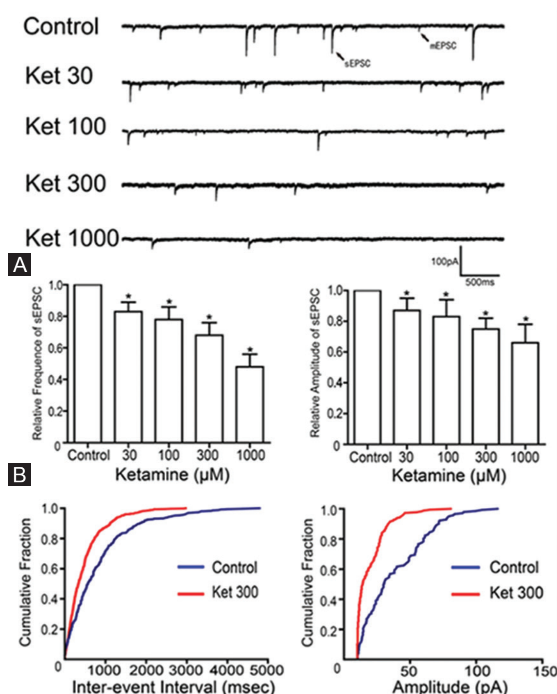

Figure 2: Effects of ketamine on spontaneous excitatory postsynaptic currents (sEPSCs) in the primary somatosensory cortex. (A) Original data of sEPSCs in all the groups. sEPSCs and miniature excitatory postsynaptic currents(mEPSCs) are indicated by arrows. (B) Relative frequency and amplitude from the different concentrations of ketamine (n=8). (C) The cumulative probability plots compared with those of the control group.*P<0.05.

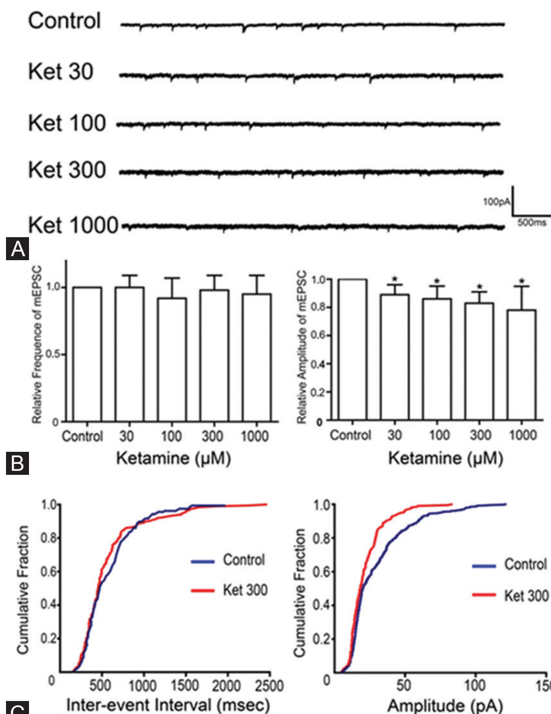

Figure 3: Effects of ketamine on miniature excitatory postsynaptic currents (mEPSCs) in the primary somatosensory cortex. (A) Original data of spontaneous excitatory postsynaptic currents (sEPSCs) in all the groups. (B, n=8) Statistical graph of the relative frequency and amplitude from the different concentrations of ketamine. (C) Cumulative probability plots compared with those of the control group. *P<0.05.

Table 1: Inhibition ratio of the different concentrations of ketamine on the relative frequency and amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) and miniature excitatory postsynaptic currents (mEPSCs)

	sEPSCs				mEPSCs			
	Frequency	P value (95% CI)	Amplitude	P value (95% CI)	Frequency	P value (95% CI)	Amplitude	P value (95% CI)
Ket 30 µM	0.83±0.06	0.014	0.87±0.08	0.042	1.00±0.09	0.897	0.89±0.07	0.031
Ket 100 µM	0.78±0.08	0.006	0.83±0.11	0.031	0.92±0.15	0.515	0.86±0.09	0.045
Ket 300 µM	0.68±0.08	0.001	0.75±0.07	0.001	0.98±0.11	0.802	0.83±0.08	0.022
Ket 1000 µM	0.48±0.10	0.000	0.66±0.12	0.006	0.95±0.14	0.643	0.78±0.17	0.005

invalid when the sodium current channels were blocked by TTX. Our results suggested that the sodium current channels were predominantly involved in the ketamine-induced decrease in the frequency of the sEPSCs of cortical neurons. In addition, according to the electrophysiological role of mEPSCs,[7] ketamine decreased the amplitude of mEPSCs without affecting the frequency, indicating that ketamine acts post-synaptically to decrease the sensitivity of the glutamatergic receptors.

Role of Cortical Neuron in the Anesthetic-Induced Loss of Consciousness

General anesthetics have been used for many years, but the related neurophysiological mechanism has yet to be fully elucidated. A recent research showed that the inhibition of the excitability of the cortical pyramidal neurons might be involved in anesthetic-induced loss of consciousness and that such phenomenon could be secondary to the excitement of the inhibitory interneurons.[8] Previous investigations have demonstrated that the loss of consciousness induced by anesthetics is conducted by the action of anesthetics on the neurons in the brain stem, cerebral cortex, and thalamus.[9-11] Positron emission tomography research has shown that the metabolic activity of the cortex is decreased significantly under general anesthesia.[12,13] Functional magnetic resonance imaging and local field potential recordings have provided additional evidence for the cortical mechanism of unconsciousness induced by general anesthesia.[10,11] Molecular pharmacological studies have determined that the $GABA_A$ and NMDA receptors in the neurons of the cortex, thalamus, striatum, and brain stem are important targets for anesthetics.[14-16]

Effects of Ketamine on the Glutamate Receptors-Related Spontaneous Excitatory Postsynaptic Currents

Synapses are the basic transmission units in the central nervous system and play an important role in the integration of neuralactivity.[17] Synapses have been proven as a vital target of anesthetics.[18,19] Synaptic transmission is triggered by the influx of Ca^{2+} to the presynaptic membrane and then neurotransmitters such as glutamate, acetylcholine, and GABA are released in the form of vesicles.[20,21] Multi-steps in the process of synaptic transmission such as presynaptic neurotransmitter release, postsynaptic neurotransmitter reuptake, and interaction of the postsynaptic receptors are affected by anesthetics.[22]

The glutamatergic receptors are excitatory amino acid receptors in the mammalian central nervous system. It is also a type of ligand-gated ion channel activated by binding glutamate[23] and includes 3 types: NMDA receptor, AMPA receptor, and kainite (KA) receptor. They are extensively distributed in the brain and exhibit the highest density in the cerebral cortex and hippocampus.[5] Excitatory synaptic transmission in the brain is mainly conducted by the NMDA and AMPA receptors. The NMDA and AMPA receptors are both cation channels and coexist in 1 synapse, inducing EPSCs and depolarizing the postsynaptic neurons.[24,25]

In the current study, mEPSCs were recorded when the action potential was blocked by TTX, and the interferences of the other ion channels were eliminated. The frequency of mEPSCs represents the release quantity of glutamate from the presynaptic membrane, and the amplitude represents the amount of opened postsynaptic glutamatergic receptors.[26,27] Accordingly, the inhibition of mEPSCs suggested that the ketamine-induced inhibition of excitatory synaptic transmission was resulted from the blockade of the postsynaptic glutamatergic receptors. Our conclusion is consistent with that in a study by Chau et al.,[28,29] who reported that ketamine inhibited the afferent signal to neurons in the medial solitary tract nucleus and opened the postsynaptic NMDA receptors. According to Schnoebel R et al.,[30] the sodium and voltage-gated potassium currents could be inhibited by ketamine. We, therefore, assumed that the ketamine-induced inhibition of presynaptic glutamate release was due to the blocking effect of ketamine on the influx of calcium ion

and sodium ion, leading to the reduction inaction potential frequency and release of presynaptic vesicles.

On the other hand, in the present study, sEPSCs were recorded in the presence of action potential, which could be used to represent the neural activity in normal state. We found that different concentrations of ketamine were able to inhibit the frequency and amplitude of the sEPSCs of the neurons in the primary somatosensory cortex. Furthermore, the cumulative probability plots of the frequency and amplitude of sEPSCs were also left shifted by ketamine in a concentration-dependent way. These results indicated that ketamine directly inhibited the excitatory transmission of the neurons in the primary somatosensory cortex. Previous investigations have also found that in auditory cortical brain slices, sEPSCs are inhibited by ketamine in a concentration-dependent manner. In addition, the ketamine-induced inhibition of the fast components of the non-NMDA receptors of sEPSCs can be used to explain the decrease in the amplitude of EPSCs.[31,32]

The main limitation of the present study is that we did not look into the effects of ketamine on specific postsynaptic glutamatergic receptors such as the NMDA, AMPA, and KA receptors. In our next stage of experiments, we will be probing into these areas.

Conclusion

Our data demonstrated that the frequency and amplitude of sEPSCs and the amplitude of mEPSCs in the primary somatosensory cortex were inhibited by ketamine. The inhibition may be mediated by the ketamine-induced inhibition of the sensitivity of the postsynaptic glutamatergic receptors. Our results provide some novel evidence indicating that the postsynaptic glutamatergic receptors of the neurons in the primary somatosensory cortex play a role in the pharmacological mechanism of ketamine.

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Conflict of Interest: None declared.

References

1. Schubert D, Kotter R, Staiger JF. Mapping functional connectivity in barrel-related columns reveals layer- and cell type-specific microcircuits. Brain Struct Funct. 2007;212:107-19. doi: 10.1007/s00429-007-0147-z. PubMed PMID: 17717691.
2. Tu Y, Yu T, Fu XY, Xie P, Lu S, Huang XQ, et al. Altered thalamocortical functional connectivity by propofol anesthesia in rats. Pharmacology. 2011;88:322-6. doi: 10.1159/000334168. PubMed PMID: 22116025.
3. Zhang Y, Li Z, Dong H, Yu T. Effects of general anesthesia with propofol on thalamocortical sensory processing in rats. J Pharmacol Sci. 2014;126:370-81. doi: 10.1254/jphs.14153FP. PubMed PMID: 25427432.
4. Li Z, Liu X, Zhang Y, Shi J, Zhang Y, Xie P, et al. Connection changes in somatosensory cortex induced by different doses of propofol. PLoS One. 2014;9:e87829. doi: 10.1371/journal.pone.0087829. PubMed PMID: 24516566; PubMed Central PMCID: PMC3917837.
5. Alkire MT, Hudetz AG, Tononi G. Consciousness and anesthesia. Science. 2008;322:876-80. doi: 10.1126/science.1149213. PubMed PMID: 18988836; PubMed Central PMCID: PMC2743249.
6. Chen X, Shu S, Bayliss DA. HCN1 channel subunits are a molecular substrate for hypnotic actions of ketamine. J Neurosci. 2009;29:600-9. doi: 10.1523/JNEUROSCI.3481-08.2009. PubMed PMID: 19158287; PubMed Central PMCID: PMC2744993.
7. Simkus CR, Stricker C. Properties of mEPSCs recorded in layer II neurones of rat barrel cortex. J Physiol. 2002;545:509-20. doi: 10.1113/jphysiol.2002.022095. PubMed PMID: 12456830; PubMed Central PMCID: PMC2290708.
8. He Q, Duguid I, Clark B, Panzanelli P, Patel B, Thomas P, et al. Interneuron- and GABA(A) receptor-specific inhibitory synaptic plasticity in cerebellar Purkinje cells. Nat Commun. 2015;6:7364. doi: 10.1038/ncomms8364. PubMed PMID: 26179122; PubMed Central PMCID: PMC4518301.
9. Ferrarelli F, Massimini M, Sarasso S, Casali A, Riedner BA, Angelini G, et al. Breakdown in cortical effective connectivity during midazolam-induced loss of consciousness. Proc Natl Acad Sci U S A. 2010;107:2681-6. doi: 10.1073/pnas.0913008107. PubMed PMID: 20133802; PubMed Central PMCID: PMC2823915.
10. Purdon PL, Pierce ET, Bonmassar G, Walsh J, Harrell PG, Kwo J, et al.

Simultaneous electroencephalography and functional magnetic resonance imaging of general anesthesia. Ann N Y Acad Sci. 2009;1157:61-70. doi: 10.1111/j.1749-6632.2008.04119.x. PubMed PMID: 19351356; PubMed Central PMCID: PMC2855224.
11. Velly LJ, Rey MF, Bruder NJ, Gouvitsos FA, Witjas T, Regis JM, et al. Differential dynamic of action on cortical and subcortical structures of anesthetic agents during induction of anesthesia. Anesthesiology. 2007;107:202-12. doi: 10.1097/01.anes.0000270734.99298.b4. PubMed PMID: 17667563.
12. Schlunzen L, Juul N, Hansen KV, Cold GE. Regional cerebral blood flow and glucose metabolism during propofol anaesthesia in healthy subjects studied with positron emission tomography. Acta Anaesthesiol Scand. 2012;56:248-55. doi: 10.1111/j.1399-6576.2011.02561.x. PubMed PMID: 22091956.
13. Kaisti KK, Metsahonkala L, Teras M, Oikonen V, Aalto S, Jaaskelainen S, et al. Effects of surgical levels of propofol and sevoflurane anesthesia on cerebral blood flow in healthy subjects studied with positron emission tomography. Anesthesiology. 2002;96:1358-70. doi: 10.1097/00000542-200206000-00015. PubMed PMID: 12170048.
14. Sonner JM, Cantor RS. Molecular mechanisms of drug action: an emerging view. Annu Rev Biophys. 2013;42:143-67. doi: 10.1146/annurev-biophys-083012-130341. PubMed PMID: 23451895.
15. Hemmings HC, Jr., Akabas MH, Goldstein PA, Trudell JR, Orser BA, Harrison NL. Emerging molecular mechanisms of general anesthetic action. Trends Pharmacol Sci. 2005;26:503-10. doi: 10.1016/j.tips.2005.08.006. PubMed PMID: 16126282.
16. Rudolph U, Antkowiak B. Molecular and neuronal substrates for general anaesthetics. Nat Rev Neurosci. 2004;5:709-20. doi: 10.1038/nrn1496. PubMed PMID: 15322529.
17. Satake S, Imoto K. Cav2.1 channels control multivesicular release by relying on their distance from exocytotic Ca2+ sensors at rat cerebellar granule cells. J Neurosci. 2014;34:1462-74. doi: 10.1523/JNEUROSCI.2388-13.2014. PubMed PMID: 24453334.
18. Schiff ND. Central thalamic contributions to arousal regulation and neurological disorders of consciousness. Ann N Y Acad Sci. 2008;1129:105-18. doi: 10.1196/annals.1417.029. PubMed PMID: 18591473.
19. MacIver MB. Anesthetic agent-specific effects on synaptic inhibition. Anesth Analg. 2014;119:558-69. doi: 10.1213/ANE.0000000000000321. PubMed PMID: 24977633; PubMed Central PMCID: PMC4139451.
20. Lewis LD, Weiner VS, Mukamel EA, Donoghue JA, Eskandar EN, Madsen JR, et al. Rapid fragmentation of neuronal networks at the onset of propofol-induced unconsciousness. Proc Natl Acad Sci U S A. 2012;109:E3377-86. doi: 10.1073/pnas.1210907109. PubMed PMID: 23129622; PubMed Central PMCID: PMC3523833.
21. Alkire MT, Asher CD, Franciscus AM, Hahn EL. Thalamic microinfusion of antibody to a voltage-gated potassium channel restores consciousness during anesthesia. Anesthesiology. 2009;110:766-73. doi: 10.1097/ALN.0b013e31819c461c. PubMed PMID: 19322942.
22. Cheng G, Kendig JJ. Enflurane decreases glutamate neurotransmission to spinal cord motor neurons by both pre- and postsynaptic actions. Anesth Analg. 2003;96:1354-9. doi: 10.1213/01.ANE.0000055649.06649.D2. PubMed PMID: 12707133.
23. Stoll L, Hall J, Van Buren N, Hall A, Knight L, Morgan A, et al. Differential regulation of ionotropic glutamate receptors. Biophys J. 2007;92:1343-9. doi: 10.1529/biophysj.106.089896. PubMed PMID: 17114218; PubMed Central PMCID: PMC1783868.
24. Jeun SH, Cho HS, Kim KJ, Li QZ, Sung KW. Electrophysiological Characterization of AMPA and NMDA Receptors in Rat Dorsal Striatum. Korean J Physiol Pharmacol. 2009;13:209-14. doi: 10.4196/kjpp.2009.13.3.209. PubMed PMID: 19885039; PubMed Central PMCID: PMC2766737.
25. Campbell SL, Mathew SS, Hablitz JJ. Pre- and postsynaptic effects of kainate on layer II/III pyramidal cells in rat neocortex. Neuropharmacology. 2007;53:37-47. doi: 10.1016/j.neuropharm.2007.04.008. PubMed PMID: 17543353; PubMed Central PMCID: PMC2033380.
26. Hanell A, Greer JE, Jacobs KM. Increased Network Excitability Due to Altered Synaptic Inputs to Neocortical Layer V Intact and Axotomized Pyramidal Neurons after Mild Traumatic Brain Injury. J Neurotrauma. 2015;32:1590-8. doi: 10.1089/

neu.2014.3592. PubMed PMID: 25789412; PubMed Central PMCID: PMC4593977.
27. Bergevin A, Girardot D, Bourque MJ, Trudeau LE. Presynaptic mu-opioid receptors regulate a late step of the secretory process in rat ventral tegmental area GABAergic neurons. Neuropharmacology. 2002;42:1065-78. PubMed PMID: 12128008.
28. Chau PL. New insights into the molecular mechanisms of general anaesthetics. Br J Pharmacol. 2010;161:288-307. doi: 10.1111/j.1476-5381.2010.00891.x. PubMed PMID: 20735416; PubMed Central PMCID: PMC2989583.
29. Tovar KR, Westbrook GL. Mobile NMDA receptors at hippocampal synapses. Neuron. 2002;34:255-64. doi: 10.1016/S0896-6273(02)00658-X. PubMed PMID: 11970867.
30. Schnoebel R, Wolff M, Peters SC, Brau ME, Scholz A, Hempelmann G, et al. Ketamine impairs excitability in superficial dorsal horn neurones by blocking sodium and voltage-gated potassium currents. Br J Pharmacol. 2005;146:826-33. doi: 10.1038/sj.bjp.0706385. PubMed PMID: 16151436; PubMed Central PMCID: PMC1751212.
31. Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci. 2008;9:370-86. doi: 10.1038/nrn2372. PubMed PMID: 18425091.
32. Leong D, Puil E, Schwarz D. Ketamine blocks non-N-methyl-D-aspartate receptor channels attenuating glutamatergic transmission in the auditory cortex. Acta Otolaryngol. 2004;124:454-8. PubMed PMID: 15224874.