

Effects of Propofol and Ketamine on The Neural Oscillations in CA1 of Rat Intact Hippocampus

Zhang Yu

Department of Anesthesiology, Huashan Hospital, Fudan University <https://orcid.org/0000-0002-0811-4032>

Ping Chen

Huashan Hospital Fudan University Department of Anesthesiology

Zhi-yi Tu

Shanghai Tenth People's Hospital

Yi-heng Liu

Huashan Hospital Fudan University Department of Anesthesiology

Zhi-ru Wang (✉ zrwang@brain.ecnu.edu.cn)

ECNU: East China Normal University

Feng-yan Shen

Huashan Hospital Fudan University Department of Anesthesiology

Ying-wei Wang

Huashan Hospital Fudan University Department of Anesthesiology

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Effects of propofol and ketamine on the neural oscillations in CA1 of rat intact hippocampus

Yu Zhang,¹ Ping Chen,¹ Zhi-yi Tu,² Yi-heng Liu,¹ Zhi-ru Wang,^{3*} Feng-yan Shen^{1*} and Ying-wei Wang^{1*}

¹Department of Anesthesiology, Huashan Hospital, Fudan University, Shanghai 200040, China

²Department of Anesthesiology, Shanghai 10th people's hospital, Tongji University, Shanghai, 200070, China

³Institute of Brain Functional Genomics, East China Normal University, Shanghai 200062, China

*Response may be addressed to:

Ying-wei Wang, MD, PhD

Department of Anesthesiology, Huashan Hospital, Fudan University, Shanghai 200040, China; Phone & Fax number: +86-21-5288-7689; E-mail: wangyingwei@yahoo.com

Zhi-ru Wang, PhD

Institute of Brain Functional Genomics, East China Normal University, Shanghai 200062, China; Phone & Fax number: +86-21-6223-8231; E-mail: zrwang@brain.ecnu.edu.cn

Feng-yan Shen, MD

Department of Anesthesiology, Huashan Hospital, Fudan University, Shanghai 200040, China; Phone & Fax number: +86-21-5288-7689; E-mail: charlesmagic007@hotmail.com

Running title: Effects of general anesthetics on hippocampus

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ABSTRACT

In this study, *in vitro* intact hippocampal preparation model was utilized to observe the effects of propofol and ketamine on the neural oscillations in CA1 of rat hippocampus. The intact hippocampi were dissected from the brain tissues of rats aged 14-16 days postnatal. Local field potential (LFP) recordings were performed with propofol and ketamine bath application at different concentrations. The power spectrum intensity of LFP in all the frequency bands, including delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz) and gamma (30-80 Hz), were inhibited in a concentration-dependent manner by both general anesthetics. In order to further investigate the underlying mechanisms, the major binding site of propofol and ketamine were blocked respectively by picrotoxin and (2R)-amino-5-phosphonopentanoate when bath applying the general anesthetics. It revealed that the inhibitory effect of propofol on hippocampal oscillations might be via γ -aminobutyric acid A receptor, while the inhibitory effect of ketamine might be unconcerned with N-methyl-D-aspartic acid receptor.

INTRODUCTION

Propofol and ketamine are common intravenous anesthetics those have been studied intensively both in clinic and laboratory. It has been well documented that the major target receptors for propofol and ketamine are γ -aminobutyric acid A (GABA_A) receptor and N-methyl-D-aspartic acid (NMDA) receptor, respectively.^[1, 2] However, studying those receptors in neuronal level is very limited in explaining the general anesthesia effect because of the intricate network connections in the brain. Thus, the influence of general anesthetics on neural networks has become a research hotspot in recent years.

Electroencephalogram (EEG) studies have shown that an increase in the concentration of propofol in the human body will shift the activity of the cerebral cortex from high-frequency/low-amplitude oscillations to high-amplitude/low-frequency oscillations.^[3] On the other hand, ketamine suppresses alpha oscillation in a low dose administration, while it increases delta, theta and gamma oscillations in a high dose.^[4] However, the effects of propofol and ketamine on neural oscillations in hippocortical brain regions are not fully understood.

Hippocampus is the key brain area for acquisition and early storage of memory.^[5] Neural oscillation is rhythmic or repetitive neural activity in the central nervous system that is usually generated by oscillatory activity of neuronal ensembles, reflecting regular and synchronized activities within these cell populations. Neural oscillation in different frequency bands can be detected in different brain regions of human and animal, and plays an essential role in cognition, learning and memory process.^[6, 7] Most of general anesthetics, including propofol and ketamine, are proved to induce

neurotoxicity and long-term cognitive dysfunction in the mammalian.^[8] Therefore, we were wondering whether the general anesthetics interfere the hippocampal oscillations, and how they work.

In this study, an *in vitro* intact hippocampal preparation model was used to observe the effects of propofol and ketamine on the activity of single neurons and local field potential (LFP) in the hippocampus by electrophysiological techniques. In current study, we aimed to compare the identical or distinct effect of two different general anesthetics on hippocampal oscillations.

METHODS

Experimental animals

This experimental study has passed the relevant regulations formulated by the Ethics Committee of Fudan University (ethical examination batch number: 20180213S). Male Sprague-Dawley rats aged 14-16 days and weighing 30-40 g were provided from the Animal Center of Brain Functional Genomics Institute of East China Normal University. The experimental animals had an illumination period of 12 h, an ambient temperature of 19-25 °C, and a relative humidity of 70%.

In vivo intact hippocampus preparation

After the experimental animals were given anesthesia with pentobarbital sodium 80 mg/kg, they were quickly decapitated, the skull was dissected, the whole brain was taken and transferred to the frozen artificial cerebrospinal fluid (aCSF: NaCl 119mM, KCl 2.5mM, CaCl₂·2H₂O 2.5mM, MgSO₄·7H₂O 1.3 mM, D-Glucose 11 mM, NaH₂PO₄·2H₂O 1mM, NaHCO₃ 26.2mM, osmotic pressure 290~300 mOsm/L, pH 7.24~7.26). At 4°C, the left hemisphere was separated from the right hemisphere on the filter paper soaked with aCSF, half was placed in the aCSF to continue freezing, and the other half was placed on the filter paper. The forebrain and brainstem are partially excised first, and the intact hippocampus is separated from the cortex. The residual blood vessels in the hippocampus are removed and placed in the aCSF, and incubated at room temperature. The other side of the hippocampus was removed and placed in the aCSF using the same method. After incubation for 1-2 h, it was used for the experiment.

Electrophysiological recording

The intact hippocampus was placed in a recording tank and fixed with a compression frame, and aCSF-containing mixed gas (95% O₂ + 5% CO₂) was continuously perfused, and recording was started after stabilization. In the experiment, two glass recording electrodes were placed at the same time, the left electrode entered the strata radiatum to record the local field potential (LFP), and the right electrode entered the CA1 pyramidal cell layer for whole cell patch recording (Fig.1A).

1) LFP recording

The recording electrode was formed by drawing a borosilicate glass tube having an outer diameter of 1.8 mm and an inner diameter of 1.5 mm on a horizontal electrode drawing apparatus, and the outer diameter of the electrode tip was 3 μm. The aCSF is injected into the electrode, and the impedance of the electrode after entering the liquid is 3 to 4 MΩ. The glass electrode is slowly pushed into the hippocampal CA1 brain area driven by the microelectrode manipulator. Using the Clampex current clamp mode, when the recording electrode enters the strata radiatum (about 200 μm downward from the surface of the hippocampal CA1 area), the low-frequency high-amplitude field potential spontaneous oscillation activity can be recorded.

2) Perforated whole cell patch recording

The electrode used in the perforated whole-cell recording was also a glass electrode with an electrode impedance of about 3.5-4.5 MΩ, with a conventional intracellular fluid infused (The internal solution contained: K-gluconate 136.5 mM, KCl 17.5 mM,

NaCl 9.0mM, MgCl₂ 1.0 mM, HEPES 10.0mM, EGTA 0.2 mM, and amphotericin B 0.3 mg/mL). The electrode is slowly pushed into the CA1 pyramidal cell layer of the hippocampus driven by the microelectrode manipulator. When the electrode contacts the pyramidal cells, the electrode resistance becomes larger and an inward current occurs. At this time, the negative air pressure in the electrode is applied to attract the cell membrane and form a high-impedance seal, whole-cell recording by perforation of Amphotericin B or by manual pipetting of the cell membrane.

Thereby, both LFP and whole cell recording signals can be obtained simultaneously. The data from 10 to 15 min after the start of the recording was taken as the base value for analysis. From the 15th min, different concentrations of propofol or ketamine were added to the circulating artificial cerebrospinal fluid for perfusion, and the perfusion time was 30 min. The last 5 min of the drug perfusion phase was taken to analyze the power spectrum of the local field potential and compared with the baseline values (Fig.1B).

Signal acquisition

The amplifier is (Axonpatch 700B) and converted by a digital-to-analog converter (Digidata 1440), which is then acquired and stored at 5 kHz by Clampex 10.2 software. The data was collected and analyzed by the power spectrum of the field potential by the NeuroExplorer software.

Data analysis

Numerical data were expressed as the mean \pm S.E.M. The data obtained were statistically processed by Sigma Stat 3.5 statistical software. The results before and after drug treatment were analyzed by paired *t*-test. The two groups of different concentrations were treated with two-way ANOVA test. The frequency band comparison was analyzed by one-way ANOVA test, and the student's *t*-test was used to compare the anesthetic with the anesthetic plus receptor blocker. $P < 0.05$ was considered statistically significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

RESULTS

The inhibitory effects of propofol on single neuron and the LFP in hippocampus

In this study, we performed a dual recording for simultaneous observing the effects of propofol or ketamine on single neuron and the LFP in intact hippocampus. As our previous study reported, the action potential fired spontaneously with normal aCSF in the intact hippocampal neuron^[9] (Fig.1C). The action potential was dose-dependently inhibited by propofol application (Fig.2A, Table1). On the other hand, the power spectrum densities of all the frequency bands were significantly suppressed by high concentration of propofol, while delta and gamma bands were less sensitive to the low-dose propofol (Fig.2B-F, Table2).

The inhibitory effect of propofol on hippocampal LFP via GABA_A receptor

In order to explore the possible mechanism of the inhibitory effect of propofol on hippocampal LFP, we first attempted to find out the half-effect concentration of propofol on action potential in single neuron. We calculated the half-effect concentration, which was 29.38 μ M, by fitting dose-response equation (Fig.3A). After that, we confirmed the half-effect concentration by additional single cell recording (Propofol/Normal aCSF = $0.63 \pm 0.04 / 1.30 \pm 0.04 \approx 48.5\%$, Fig.3B).

Since propofol inhibits the neuronal activity by potentiating the GABAergic hypolarizing,^[10, 11] we were wondering whether the GABA_A receptor mediated the inhibitory effect of propofol on hippocampal oscillation. Therefore, we added the picrotoxin(100 μ M), a GABA_A receptor antagonist,^[12] with propofol for bath

application. We found that the picrotoxin significantly reversed the inhibitory effect of propofol on hippocampal LFP (delta: propofol vs propofol+picrotoxin = 0.61 ± 0.47 vs 2.33 ± 0.97 , $P=0.038$; theta: propofol vs propofol+picrotoxin = 0.13 ± 0.12 vs 2.99 ± 0.63 , $P=0.001$; alpha: propofol vs propofol+picrotoxin = 0.16 ± 0.15 vs 2.76 ± 0.84 , $P<0.001$; beta: propofol vs propofol+picrotoxin = 0.26 ± 0.22 vs 1.58 ± 0.76 , $P=0.005$; gamma: propofol vs propofol+picrotoxin = 0.60 ± 0.47 vs 2.06 ± 0.51 , $P=0.01$; $n=8$ per group, Mann-Whitney Rank Sum Test, Fig.4A, B). These results indicates that the propofol may inhibit the hippocampal LFP via GABA_A receptor.

The inhibitory effects of ketamine on single neuron and the LFP in hippocampus

In this study we also investigated the effect of ketamine, another common general anesthetic, on single neuron and the LFP in hippocampus. Similar to propofol, ketamine inhibited the action potentials of hippocampal neurons in a dose-dependent manner (Fig.5A, Table3). Moreover, the power spectrum densities of all the frequency bands were significantly suppressed by high concentration of ketamine as well (Fig.5B-F, Table4).

NMDA receptor may be not essential for ketamine inhibiting the hippocampal LFP

Same as propofol experiment, we first calculated the half-effect concentration of ketamine on the action potential in single neuron, that is $136.46 \mu\text{M}$ (Fig. 6A), and the effect was also confirmed ($\text{Ketamine/Normal aCSF} = 0.70 \pm 0.10 / 1.36 \pm 0.13 \approx 51.5\%$,

Fig. 6B).

Since ketamine inhibits the neuronal activity by interfering the NMDA receptor,^{[2] [13]} we were wondering whether interfering the NMDA receptor mediated the inhibitory effect of ketamine on hippocampal oscillation. Therefore, we added the (2R)-amino-5-phosphonopentanoate (APV, 50 μ M), an NMDA receptor antagonist,^[14] with ketamine for bath application. We found that all the frequency bands were not affected by the ketamine with the concentration we chose (Fig.7A, B), and the most of LFP bands, except the delta band, were not changed when coapplying with APV (delta: ketamine vs ketamine+APV = 1.34 ± 0.43 vs 0.63 ± 0.58 , $P=0.021$; theta: ketamine vs ketamine+APV = 0.89 ± 0.46 vs 0.70 ± 0.38 , $P=0.39$; alpha: ketamine vs ketamine+APV = 0.86 ± 0.46 vs 0.71 ± 0.29 , $P=0.46$; beta: ketamine vs ketamine+APV = 0.76 ± 0.37 vs 0.65 ± 0.24 , $P=0.49$; gamma: ketamine vs ketamine+APV = 0.87 ± 0.513 vs 1.14 ± 0.6 , $P=0.38$; $n=8$ per group, student's *t*-test, Fig.7B). These results indicate that NMDA receptor may be not essential for ketamine inhibiting the hippocampal oscillation.

DISCUSSION

In this study, *in vitro* intact hippocampus was used to investigate the effects of propofol and ketamine on the neural oscillations in CA1. The current study found that propofol and ketamine inhibited the hippocampal CA1 pyramidal neuronal activity and LFP in a concentration-dependent manner. The inhibitory effect of propofol on hippocampal oscillation might be via GABA_A receptor, while the inhibitory effect of ketamine might be unconcerned with NMDA receptor.

The standpoint of anesthetic-induced cognitive dysfunction is still controversial. Especially, the recent clinical researches revealed that the general anesthesia almost had nothing to do with the cognitive dysfunction in pediatric short operation.^{[15] [16]} However, there is no doubt that many general anesthetics induce neurotoxicity and cognitive dysfunction at high doses, which is validated by a great deal of laboratory studies. Previous studies have found that administration of a sedative dose of propofol in rodent animals produces anterograde amnesia, and the degree of amnesia increases with increasing dose.^[17] Previous studies have shown that repeated treatment of ketamine can lead to neurotoxicity/apoptosis of the neonatal central nervous system.^[18] Wang et al. found that even a single treatment of ketamine could induce cortical apoptosis and it was dose dependent.^[19]

The activity of neural networks is often accompanied by the occurrence of neural rhythms, so there is often an important connection between the rhythmic oscillations generated by synchronized activities with cognition and memory.^[20] Different external information received by neurons is then expressed by different discharge patterns. In

the hippocampus, different modes of neuron oscillations occur depending on the active state. They can be divided into five categories according to different frequencies: delta oscillation (1-4Hz), theta oscillation (4-8 Hz), alpha oscillation (8-13 Hz), beta oscillation (13-30 Hz), and gamma oscillation (30-80 Hz). These rhythmic oscillations of the hippocampus are closely related to the behavior and physiological state of the hippocampus, are produced by specific mechanisms, and are related to the distribution characteristics of neurons, and participate in different brain functions independently or synergistically. The hippocampus plays a key role in the formation and maintenance of learning and memory.^[21] Grastyan et al. first found theta oscillation in the hippocampus in the discrimination learning task, confirming that theta oscillation is related to cognitive behavior.^[22] Since then, much experimental evidences have suggested that theta oscillation in the hippocampus is closely related to the neural function of learning and memory.^[23, 24] These oscillations of neurons not only perform their respective functions independently, but also regulate and influence each other. For example, gamma oscillation is often accompanied by theta oscillation to accompany advanced cognitive behaviors such as learning and memory.^[25, 26]

Propofol and ketamine, as general anesthetics, have a different mechanism of action, but they inhibit the discharge of hippocampal neurons in a concentration-dependent manner, and can completely inhibit the release of action potentials at high concentrations. And at high concentrations, both of them can significantly inhibit the hippocampal oscillation, including theta and gamma bands. Therefore, the general anesthetics may induce cognitive dysfunction by inhibiting hippocampal oscillation

activity. However, through detailed analysis of different concentrations of the two anesthetics, it is found that the effects of the two on local field potential are different. We have found through experiments that the two anesthetics have different effects on the field potential when they have the same 50% inhibitory effect on hippocampal neuronal discharge. Propofol is more sensitive to the effects of field potentials. This phenomenon may be related to the different mechanisms of action of the two anesthetics.

In order to investigate whether this phenomenon is related to their respective major receptors, we observed inhibitors of their respective receptors in the experiment. It showed that the picrotoxin significantly reversed the inhibitory effect of propofol on hippocampal oscillation, while most of frequency bands were not changed when coapplying with APV. Studies have shown that local field potentials are mainly regulated by GABA receptors.^[27, 28] Although it is well known that ketamine inhibits NMDA receptor, it selectively enhances the activity of extracellular GABA_A receptors at high concentrations.^[29] That may be the reason why both propofol and ketamine have same effect on hippocampal oscillation at high concentrations.

Although brain block and brain slice are both *in vitro* studies, since the integrity of hippocampal circuits fibers is preserved, we can still study hippocampal oscillation *in vitro*, which is an ideal model for studying the mechanism of hippocampal internal circuits. This study was only limited to the effect of general anesthesia on CA1, and did not observe the situation of other hippocampal regions or their mutual effects. We will continue to explore along this research idea in our future work. We believe that understanding the mechanism of action of general anesthetics on the neural circuits in

the hippocampus will be helpful for the study of general anesthetics in the complex *in vivo* neural network.

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Availability of data and material

Please contact author for data requests.

AUTHOR INFORMATION

Affiliations

Department of Anesthesiology, Huashan Hospital, Fudan University, Shanghai, China

Yu Zhang, Feng-yan Shen, Ping Chen, Yi-heng Liu, and Ying-wei Wang

Department of Anesthesiology, Shanghai 10th people's hospital, Tongji University, Shanghai, China

Zhi-yi Tu

Institute of Brain Functional Genomics, East China Normal University, Shanghai, China

Zhi-ru Wang

Contributions

Ying-wei Wang is responsible for overall design of the research and experiment. Ying-wei Wang and Zhi-ru Wang supervised the experimental analyses. Yu Zhang and Feng-

yan Shen co-wrote the manuscript. Yu Zhang carried out all experiments. Ping Chen interpreted the data. Yi-heng Liu and Zhi-yi Tu performed preliminary experiments. All authors read and approved this manuscript.

ETHICS DECLARATIONS

Ethics approval

This experimental study has passed the relevant regulations formulated by the Ethics Committee of Fudan University (ethical examination batch number: 20180213S).

Consent for publication

Not applicable.

Competing interests

All authors claim that there are no conflicts of interest.

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Figures

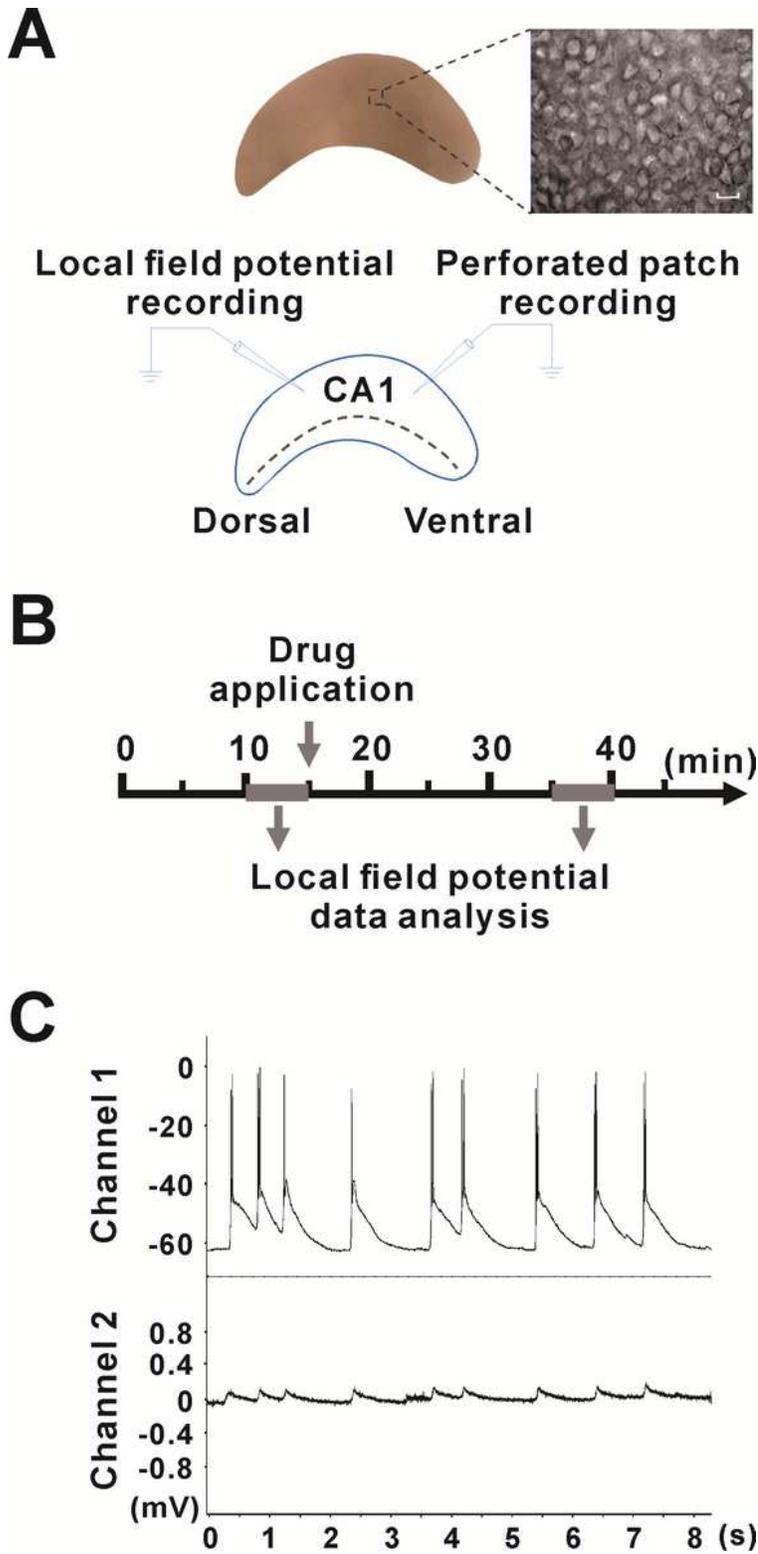


Figure 1

Electrophysiological recording on intact hippocampus. (A) Image of neuronal cell layer in intact hippocampal CA1 region under DIC(differential interference contrast microscope, $\times 400$, scale bar = 25

μm). (B) The experimental flow chart. Grey shadows indicate the time period of data analysis. (C) Simultaneous recording of whole cell patch and local field potential on isolated intact hippocampus.

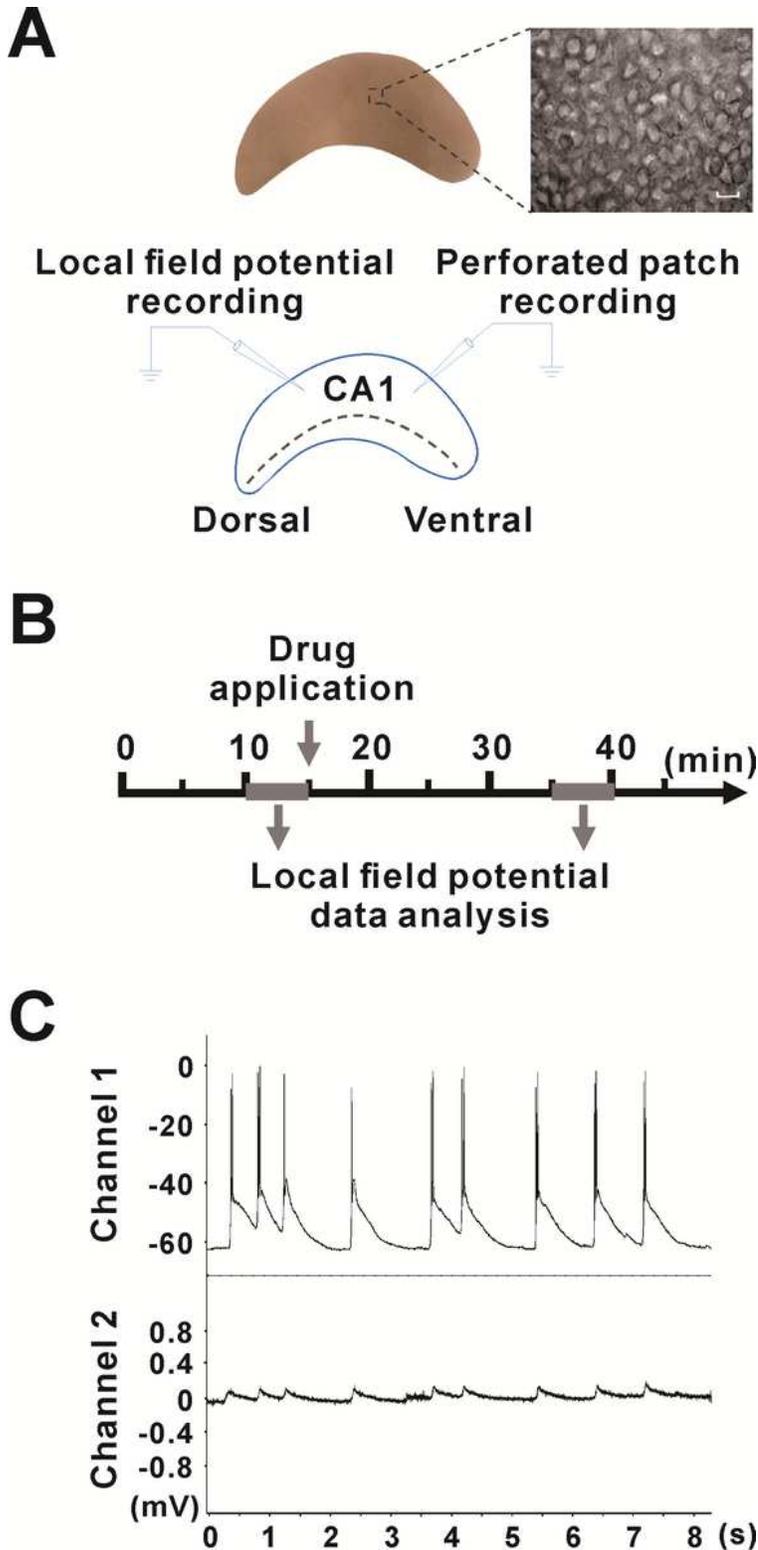


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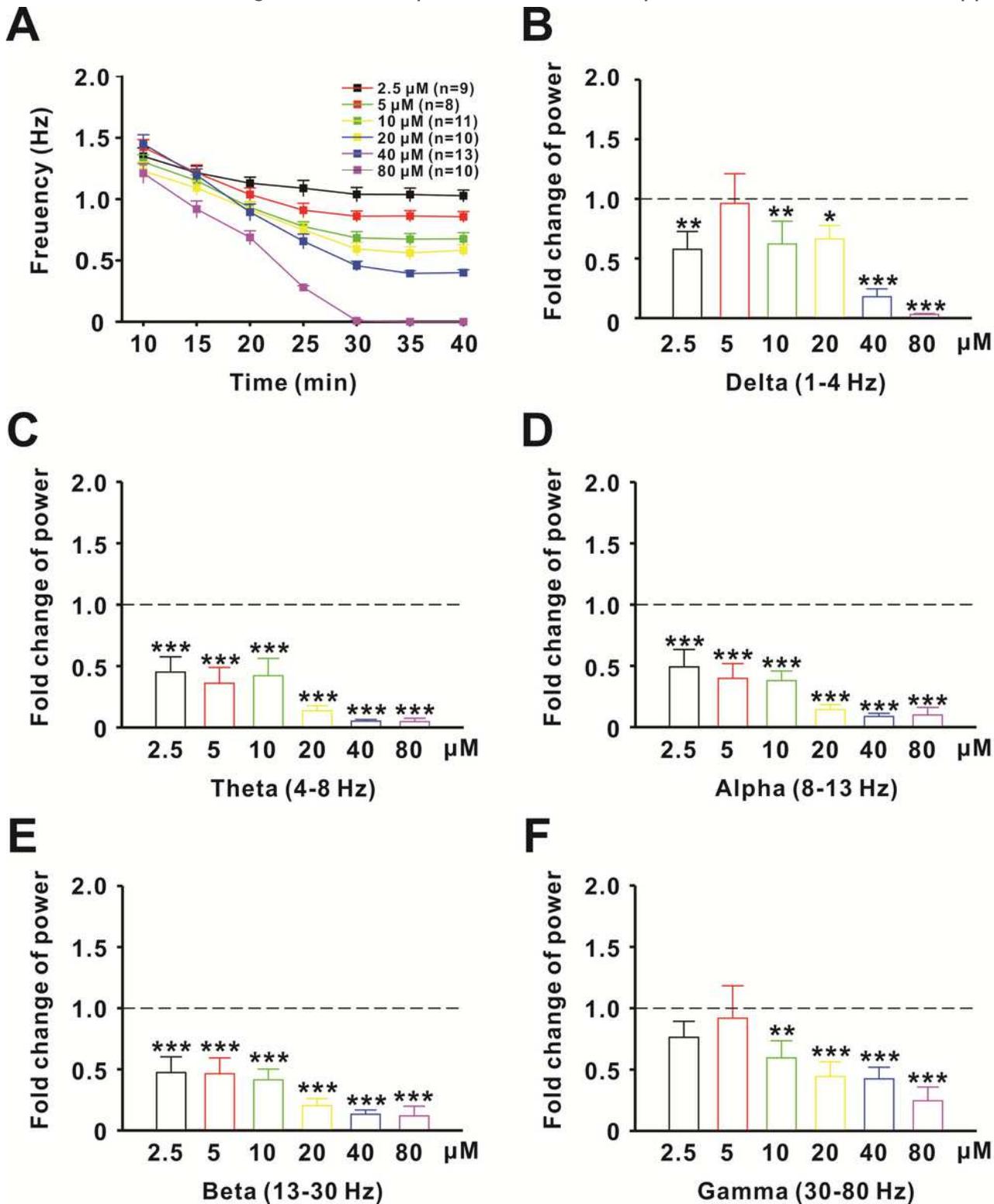


Figure 2

The inhibitory effects of propofol on single neuron and the field potential in hippocampus. (A) Dose-dependent effect of propofol on firing rate of CA1 neuron. (B~F) Dose-dependent effect of propofol on power spectrum of delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (30-80 Hz)

oscillations. All data were normalized and the base value before propofol treatment was set to 1 and the data were compared before propofol treatment. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, twoway ANOVA.

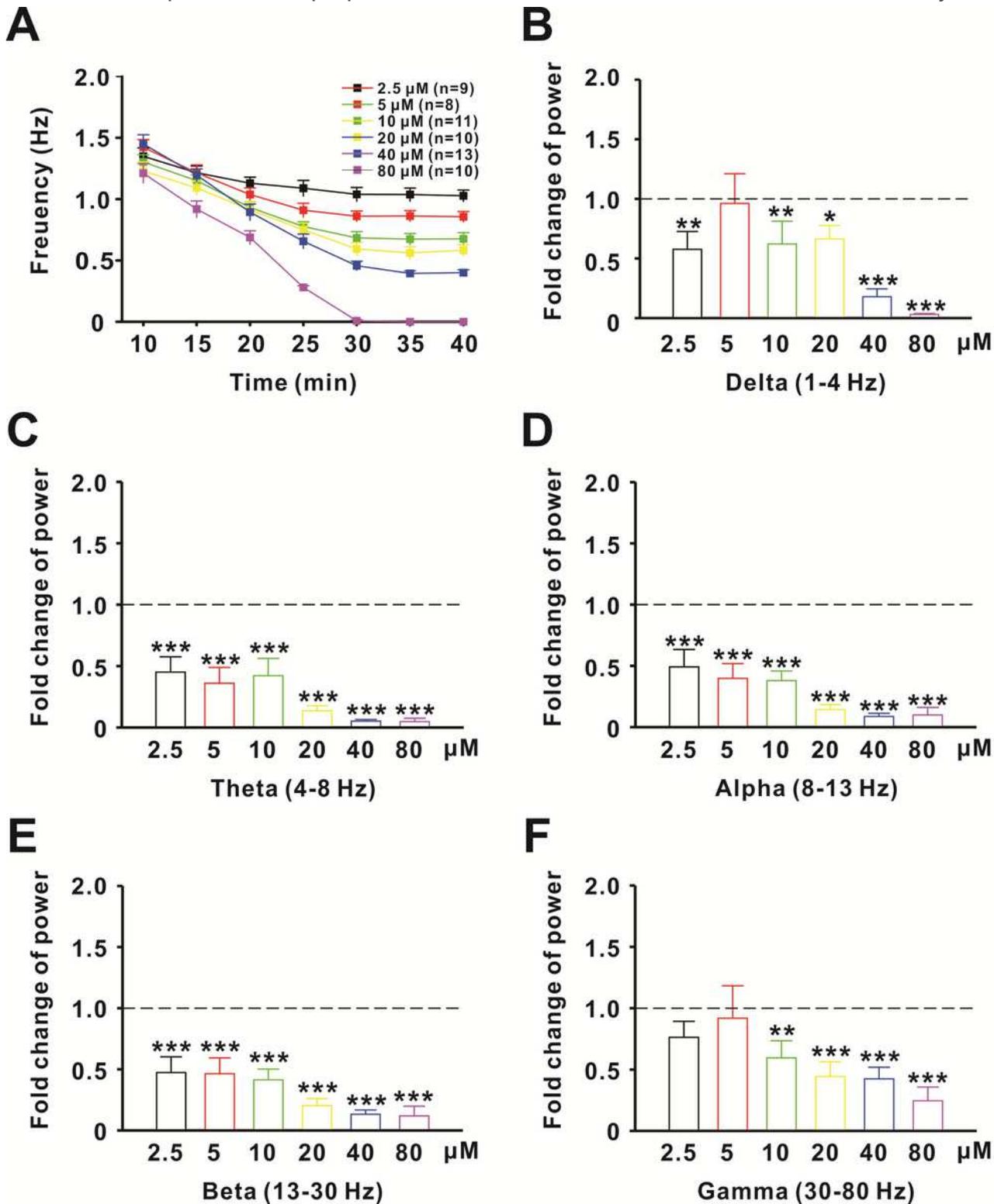


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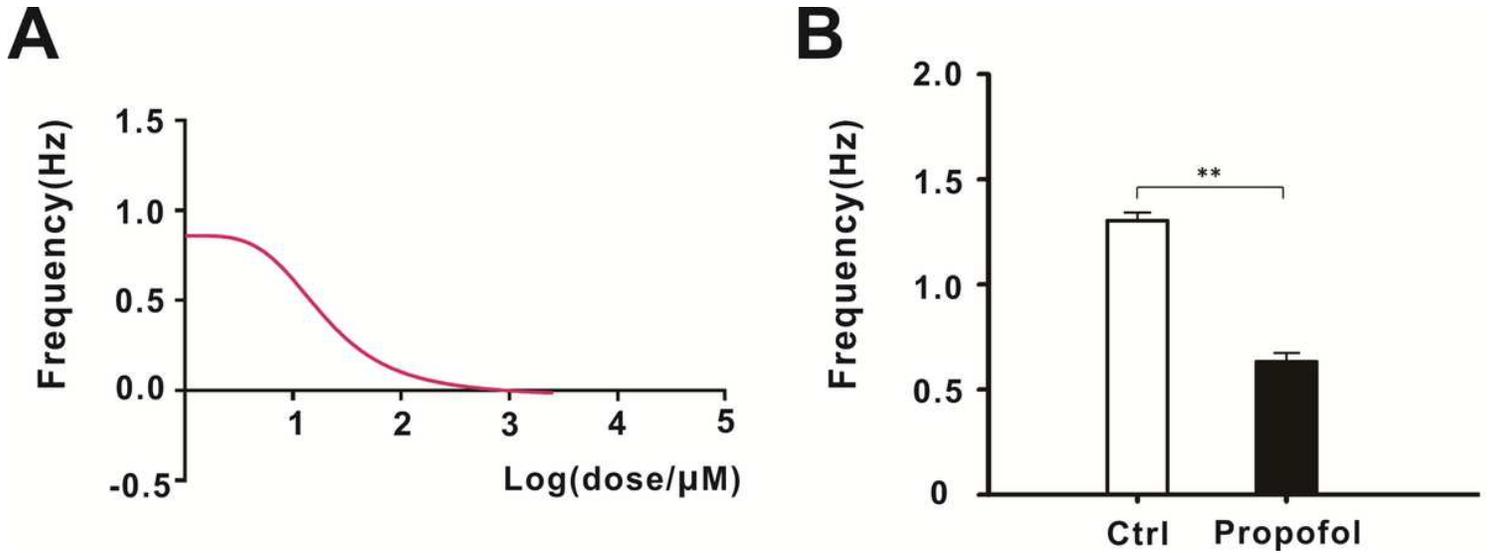


Figure 3

The half-effect concentration of propofol on the spontaneous discharge of hippocampal CA1 neurons. (A) The dose-response curve of propofol was fitted according to the effects of different concentrations of propofol on the frequency of action potential, and the half-effect concentration of propofol on the spontaneous discharge of hippocampal CA1 neurons was 29.38 μM . (B) Confirmation of the effect of propofol with half-effect concentration on the spontaneous discharge of hippocampal CA1 neurons. ** $P < 0.01$, paired t test.

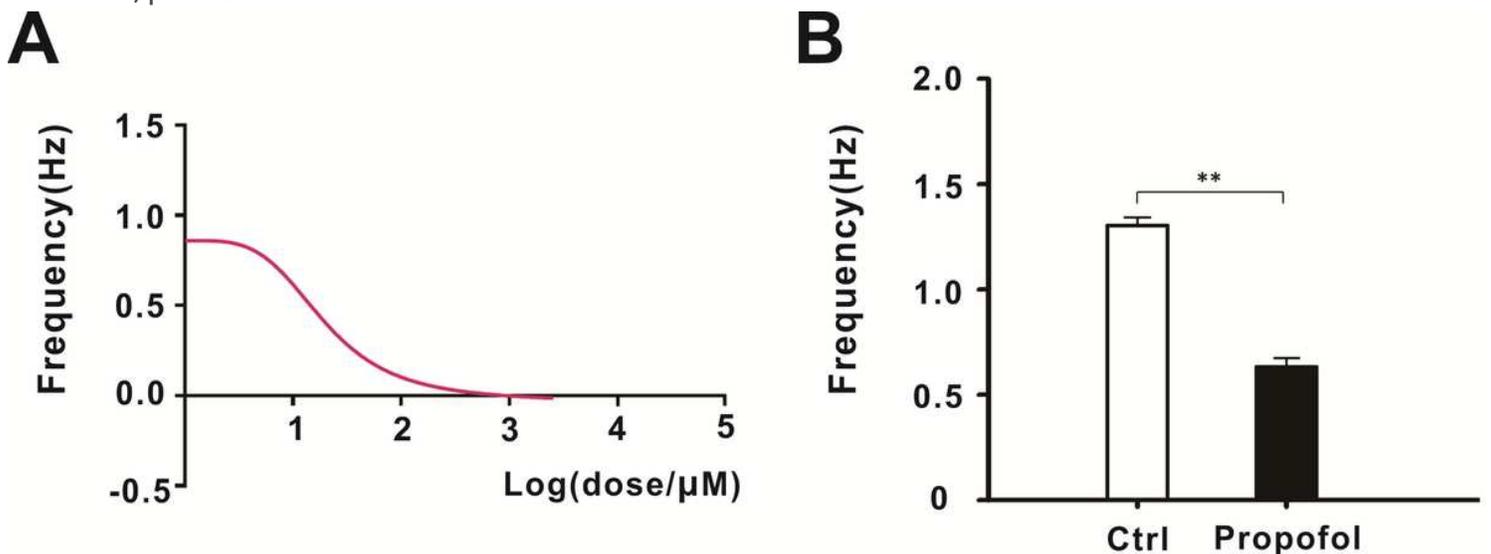


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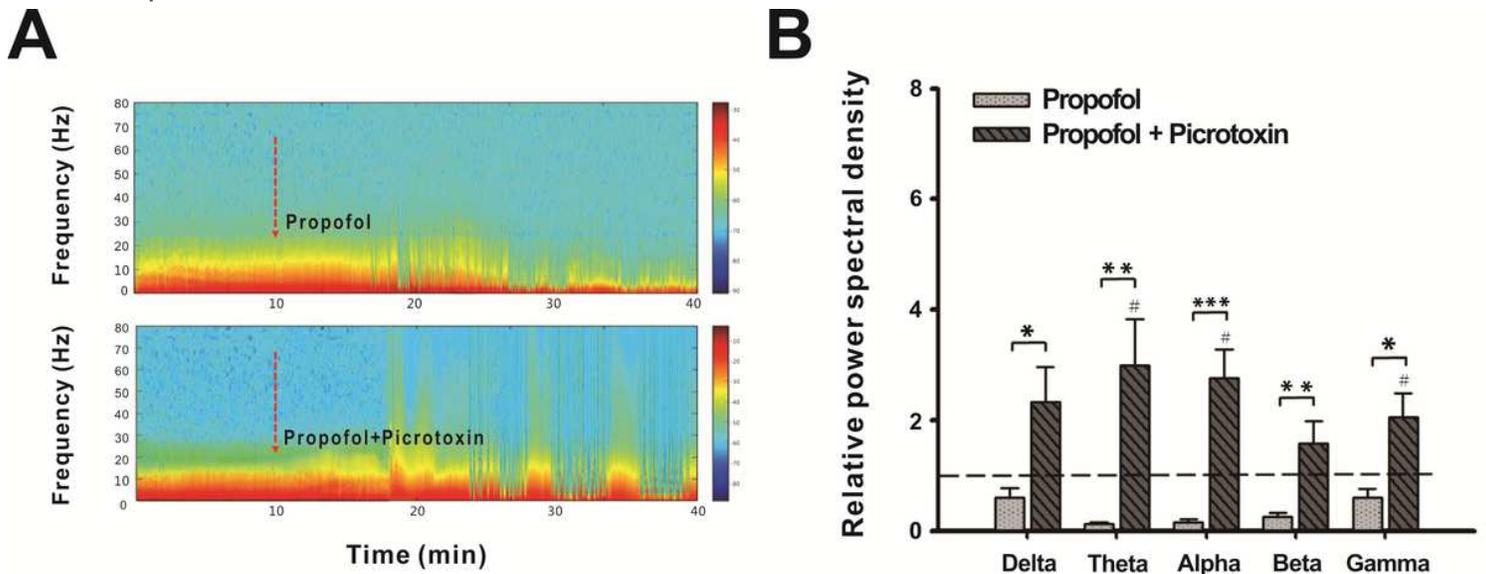


Figure 4

The inhibitory effect of propofol on hippocampal field potential via GABA_A receptor. (A) The power spectrum of full bands varies with time. The red arrow indicates the time point of drug (propofol or propofol plus picrotoxin) application. (B) Comparison of power spectrum of each band between propofol group and propofol plus picrotoxin group. * P<0.05, **P<0.01, ***P<0.001, two-way ANOVA, #P<0.05, paired t test.

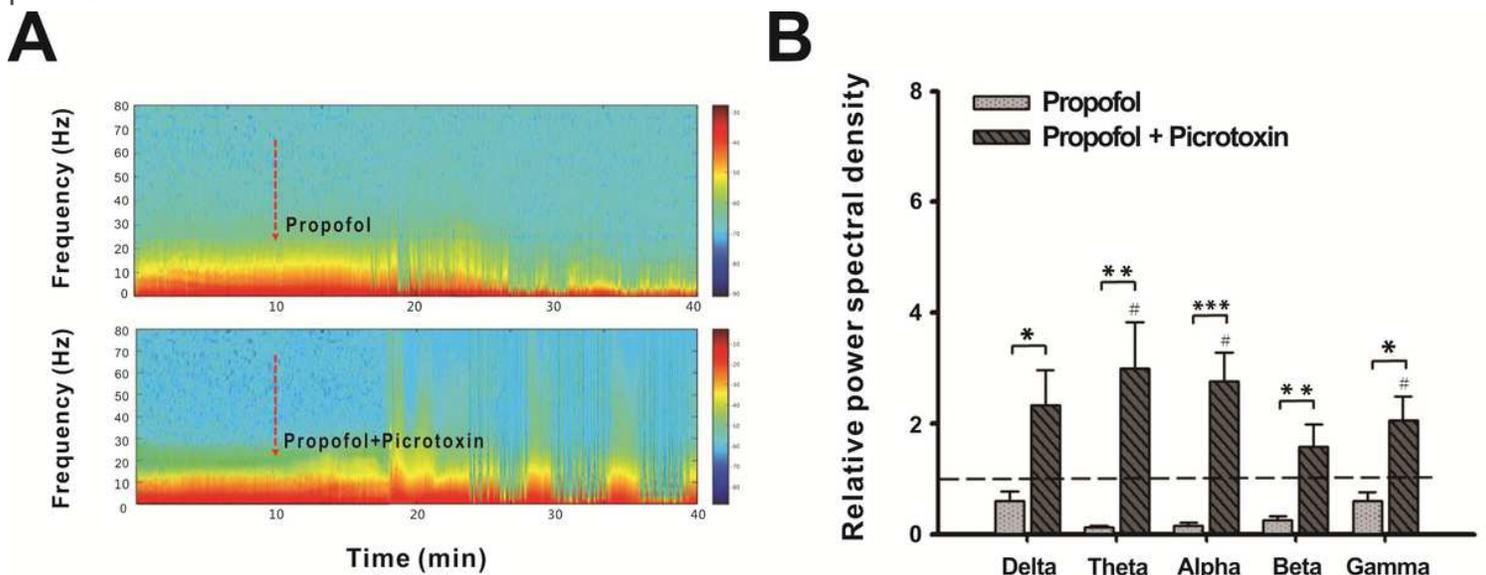


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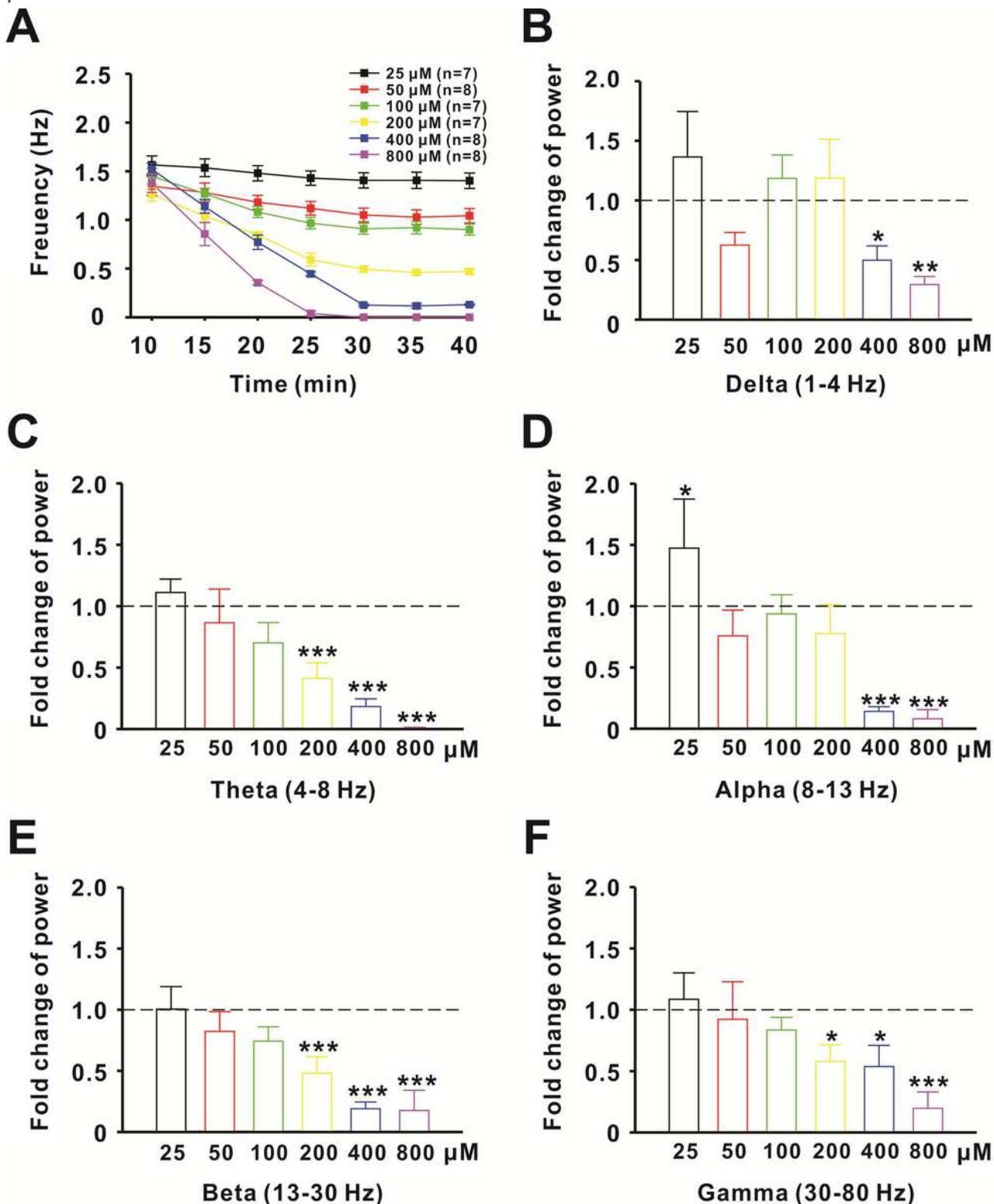


Figure 5

Effects of different concentrations of ketamine on isolated hippocampal action potential and local field potential. (A) Effect of different concentrations of ketamine on the frequency of release of isolated hippocampal action potential. (B~F) Effect of concentrations of ketamine on power spectrum of

Delta~Gamma bands. All data were normalized and the base value before ketamine treatment was set to 1 and the data were compared before ketamine treatment. *P<0.05, **P<0.01, ***P<0.001, two-way ANOVA.

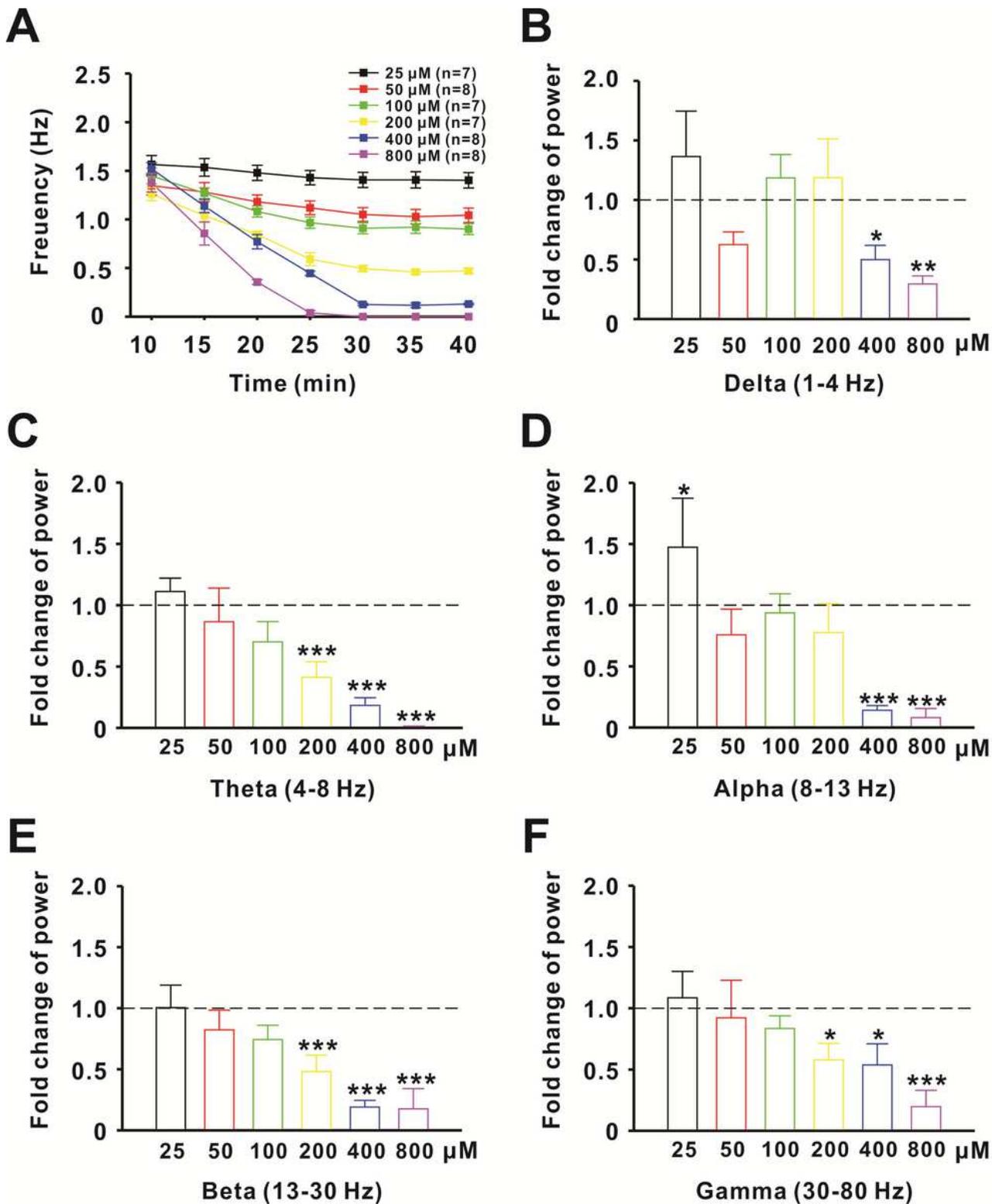


Figure 5

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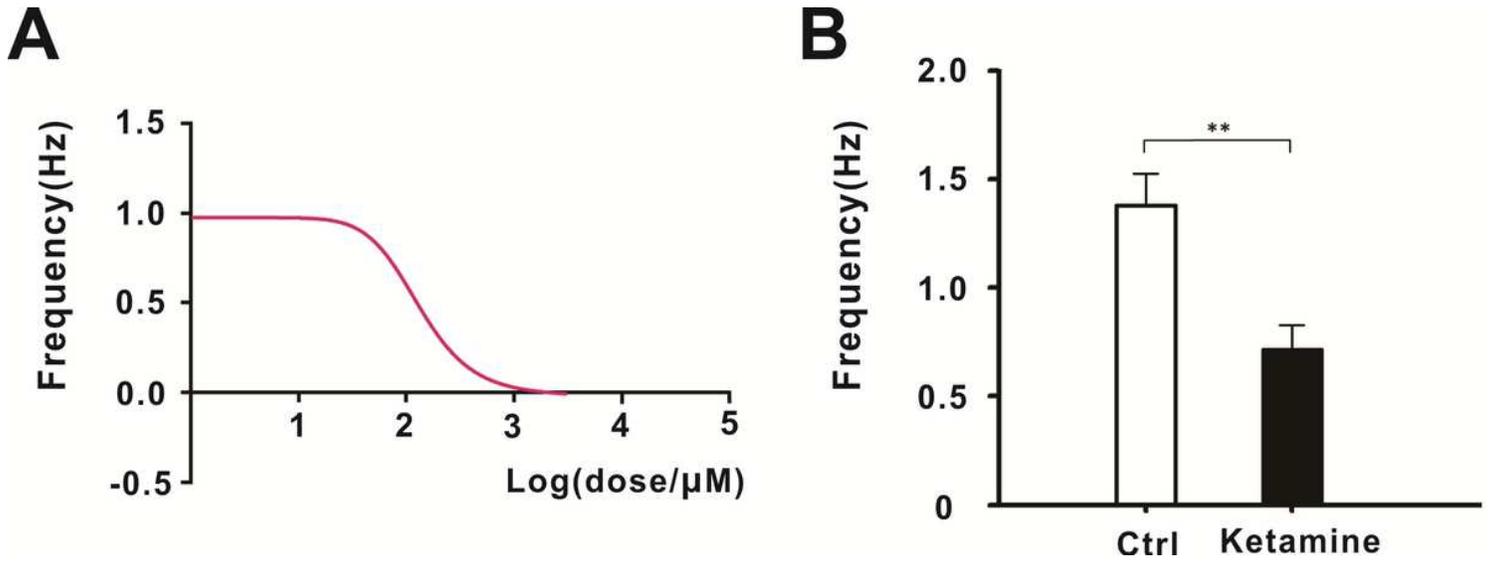


Figure 6

The half-effect concentration of ketamine on the spontaneous discharge of hippocampal CA1 neurons (A) The dose-response curves of ketamine were fitted according to the effects of different concentrations of ketamine on the frequency of action potential, and the half-effect concentration of ketamine on the spontaneous discharge of hippocampal CA1 neurons was 136.46 μ M. (B) The effect of half-effect concentration of ketamine on the frequency of isolated hippocampal action potentials. *P<0.05, **P<0.01, paired t test.

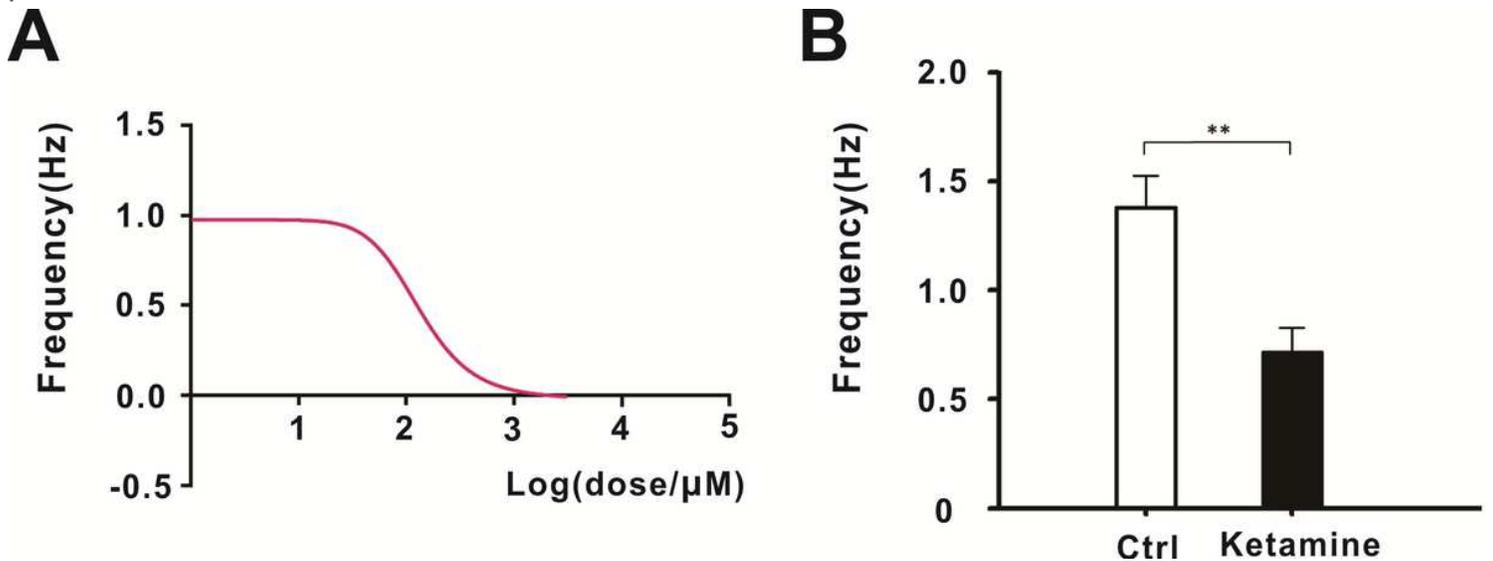


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The half-effect concentration of ketamine on the spontaneous discharge of hippocampal CA1 neurons (A) The dose-response curves of ketamine were fitted according to the effects of different concentrations of ketamine on the frequency of action potential, and the half-effect concentration of ketamine on the spontaneous discharge of hippocampal CA1 neurons was 136.46 μM . (B) The effect of half-effect concentration of ketamine on the frequency of isolated hippocampal action potentials. * $P < 0.05$, ** $P < 0.01$, paired t test.

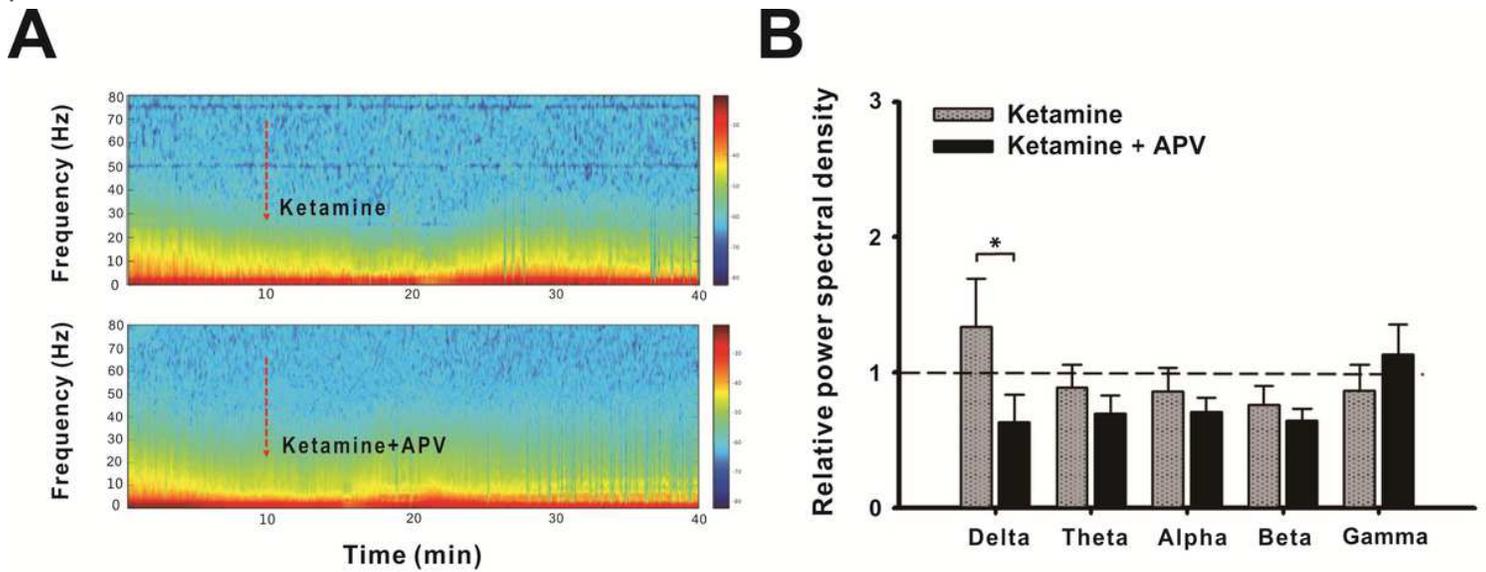


Figure 7

Mechanism of inhibition of ketamine on frequency bands of local field potential power spectrum. Comparison of effects of ketamine perfusion group and ketamine plus APV perfusion group on power spectrum of hippocampus field potential. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, two-way ANOVA.

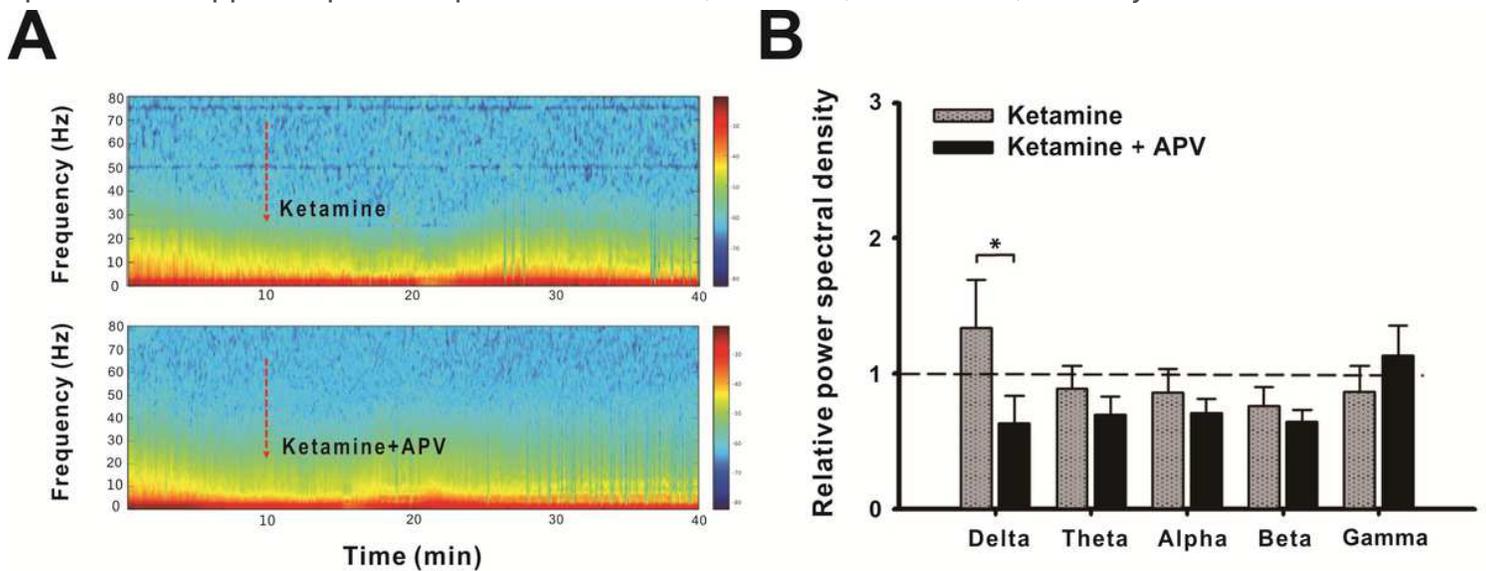


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Mechanism of inhibition of ketamine on frequency bands of local field potential power spectrum. Comparison of effects of ketamine perfusion group and ketamine plus APV perfusion group on power spectrum of hippocampus field potential. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, two-way ANOVA.

Supplementary Files

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