# Propofol effectively inhibits lithium-pilocarpine-induced status epilepticus in rats *via* downregulation of N-methyl-D-aspartate receptor 2B subunit expression

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## Abstract

Status epilepticus was induced *via* intraperitoneal injection of lithium-pilocarpine. The inhibitory effects of propofol on status epilepticus in rats were judged based on observation of behavior, electroencephalography and 24-hour survival rate. Propofol (12.5−100 mg/kg) improved status epilepticus in a dose-dependent manner, and significantly reduced the number of deaths within 24 hours of lithium-pilocarpine injection. Western blot results showed that, 24 hours after induction of status epilepticus, the levels of N-methyl-D-aspartate receptor 2A and 2B subunits were significantly increased in rat cerebral cortex and hippocampus. Propofol at 50 mg/kg significantly suppressed the increase in N-methyl-D-aspartate receptor 2B subunit levels, but not the increase in N-methyl-D-aspartate receptor 2A subunit levels. The results suggest that propofol can effectively inhibit status epilepticus induced by lithium-pilocarpine. This effect may be associated with downregulation of N-methyl-D-aspartate receptor 2B subunit expression after seizures.
**Key Words:** propofol; status epilepticus; N-methyl-D-aspartate receptor 2A, 2B subunit; cerebral cortex; hippocampus; electroencephalogram
**Abbreviations:** NMDA, N-methyl-D-aspartate; Li-pilo, lithium-pilocarpine; NR2A, N-methyl-D-aspartate receptor subunit 2A

## INTRODUCTION

Status epilepticus is defined as a period of continuous seizure activity[1]. Different anesthetics are used to treat refractory status epilepticus in the clinic, but no definite recommendations or guidelines have been established scientifically. It was reported that propofol in combination with phenobarbital may be efficacious for the management of refractory status epilepticus in dogs[2]. Can propofol be used as an anti-epileptic drug to treat patients with status epilepticus? Activation of N-methyl-D-aspartate (NMDA) receptors plays an important role in status epilepticus and brain damage in the lithium-pilocarpine (Li-pilo) model[3]. N-methyl-D-aspartate receptor subunit 2A (NR2A) and 2B (NR2B) are abundant in the adult cortex and hippocampus[4-5]. Activation of NR2A and NR2B receptors is involved in seizure-induced neuronal cell death[6]. There is increasing evidence indicating the enhanced expression of NR2B-containing receptors in both human epilepsy and

animal models[7-10].
Propofol is a short-acting intravenous anesthetic that can enhance GABAergic neurotransmission mediated by the $GABA_A$ receptor complex, as shown in biochemical, electrophysiologic, and molecular biological studies[11-15]. In addition, it was reported on the basis of neurochemical and electrophysiologic studies[16-18] that propofol inhibits glutamate-mediated excitatory neurotransmission and NMDA receptors. However, seizures or seizure-like phenomena, which are mostly convulsive, have been observed during the induction, maintenance and withdrawal phases of propofol administration. To date, it has been unclear whether propofol can be used to treat status epilepticus patients, and it is unknown if propofol can inhibit NR2A and NR2B receptor subunits. In this study, we selected the first-line anticonvulsant diazepam and the non-selective NMDA receptor antagonist MK801 as positive controls, and the anticholinergic drug scopolamine as a negative control, and investigated the effects of propofol on status

epilepticus induced by Li-pilo in adult male rats. In particular, we observed the effects of propofol on NR2A/NR2B expression in the cortex and hippocampus of rats with status epilepticus induced by Li-pilo, and explored whether the inhibition of status epilepticus by propofol in rats is associated with NR2A or NR2B subunits.

## RESULTS

### Quantitative analysis of experimental animals

One-hundred Sprague-Dawley rats were equally and randomly divided into 10 groups, including blank, status epilepticus, propofol at different doses (12.5, 25, 50, 75, 100 mg/kg), diazepam (positive control), MK801 (positive control) and scopolamine (negative control) groups. All rats underwent behavioral and electroencephalographic analysis. Rats from the blank group were fed normally, but the rats in all other groups (status epilepticus models) were given Li-pilo. From each of the blank, status epilepticus, propofol at 50 mg/kg, MK801 and scopolamine groups, we randomly selected six rats for western blot analysis. None of the rats were affected by infection. All rats ($n = 100$) were used for the behavioral observation and survival analysis, and six surviving rats were randomly selected from each group (a total of 36 rats) and used for the final analysis.

### Li-pilo induced typical status epilepticus behavioral alterations in rats

To observe the behavioral alterations in status epilepticus rats, we injected pilocarpine (30 mg/kg, i.p.) in lithium chloride-pretreated rats. Injection of Li-pilo produced a sequence of behavioral alterations including initial akinesia, body tremor and/or incomplete limbic gustatory automatisms. The gustatory automatisms were considered to be a type of behavior occurring before convulsions, characterized by myoclonic twitching; these were restricted to the head and face and were accompanied by salivation. These changes were followed by episodes of motor limbic seizures, ranging from stage 1, the start phase of seizures, to stage 5, the status epilepticus attack stage.

### Propofol improved the behavioral alterations and elevated survival rate in status epilepticus rats during the first 24 hours of status epilepticus

Propofol was given 30 minutes after seizure onset, and blocked the ongoing seizures in a dose-dependent manner, moreover, the survival rate of status epilepticus rats was significantly improved 24 hours after Li-pilo treatment ($P < 0.01$; Table 1). Diazepam and MK801 also effectively inhibited onset of status epilepticus ($P < 0.05$), but the anticholinergic agent scopolamine was ineffective after 30 minutes when the status epilepticus occurred (Table 1).
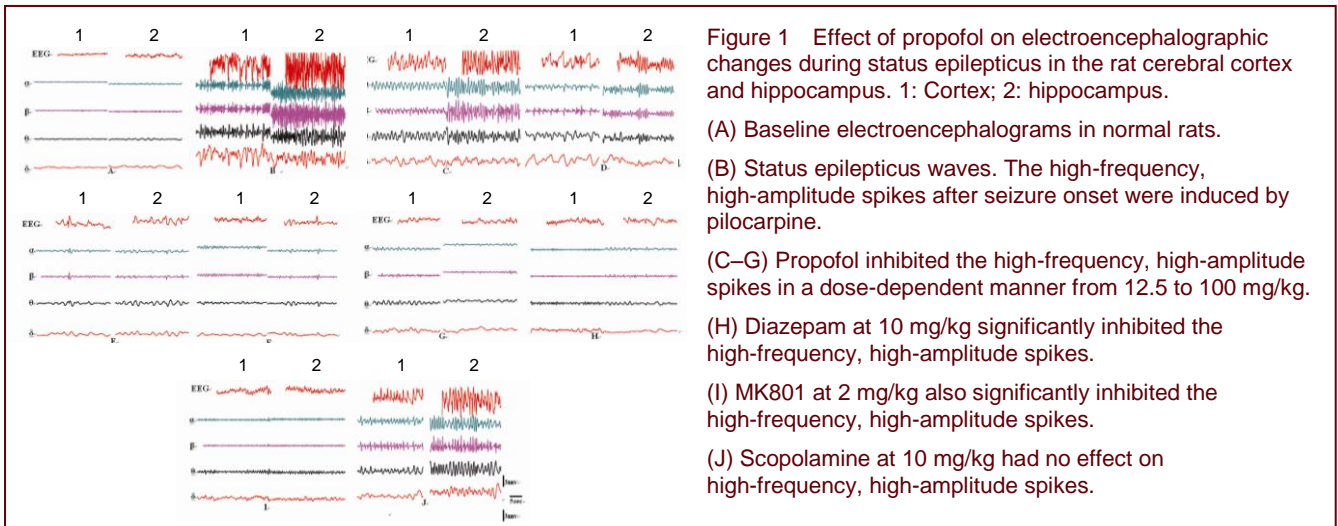
### Effects of propofol effect on the change in electroencephalograms in rats with status epilepticus induced by Li-pilo

Electroencephalography was used to observe the effect of propofol on electroencephalographic findings in rats with status epilepticus induced by Li-pilo. Compared with the blank group, electroencephalograms of rats in the status epilepticus group showed continuous high-frequency, high-amplitude spikes in the cortex and hippocampus about 10–20 minutes after pilocarpine injection (Figures 1A and B). Propofol at 50 to 100 mg/kg markedly inhibited the high-frequency, high-amplitude spikes in a dose-dependent manner, and the α, β, θ and δ waves were all inhibited (Figures 1E, F and G). However, propofol at 12.5 and 25 mg/kg had no effect on the electroencephalographic changes induced by status epilepticus (Figures 1C and D). Although the propofol 50, 75 and 100 mg/kg groups still occasionally showed sharp waves, no epileptic seizures occurred. Compared with the status epilepticus group, the frequency and amplitude of sharp waves were significantly decreased. Although MK801 and diazepam were effective at preventing the status epilepticus induced by Li-pilo, their effectiveness was no greater than that of propofol (Figures 1G, H and I). The scopolamine had no effect on the electroencephalographic changes after 30 minutes when the status epilepticus occurred (Figure 1J).

**Table 1  Effects of propofol on lithium-pilocarpine-induced status epilepticus (SE) in rats**

| Group | Latency (minute) | Induced SE number | Controlled SE numbers | | | | Numbers of surviving rats 24 hours after SE onset |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | | 10 minutes | 1 hour | 3 hours | 5 hours | |
| SE | 10.2±2.1 | 10 | 0 | 0 | 0 | 0 | 6 |
| Blank | – | – | – | – | – | – | 10 |
| MK801 | 10.5±2.3 | 10 | 10 | 10 | 10 | 10 | 8[a] |
| DZP | 10.3±2.0 | 10 | 10 | 10 | 10 | 10 | 8[a] |
| Scop | 10.7±2.3 | 10 | 0 | 0 | 0 | 0 | 6 |
| Propofol (mg/kg) | | | | | | | |
| 12.5 | 10.9±2.4 | 10 | 0 | 0 | 1 | 1 | 6 |
| 25 | 10.4±1.9 | 10 | 0 | 0 | 2 | 2 | 7 |
| 50 | 10.2±2.3 | 10 | 10[b] | 10[b] | 10[b] | 10[b] | 9[b] |
| 75 | 10.7±2.0 | 10 | 10[b] | 10[b] | 10[b] | 10[b] | 10[b] |
| 100 | 10.3±2.1 | 10 | 10[b] | 10[b] | 10[b] | 10[b] | 10[b] |

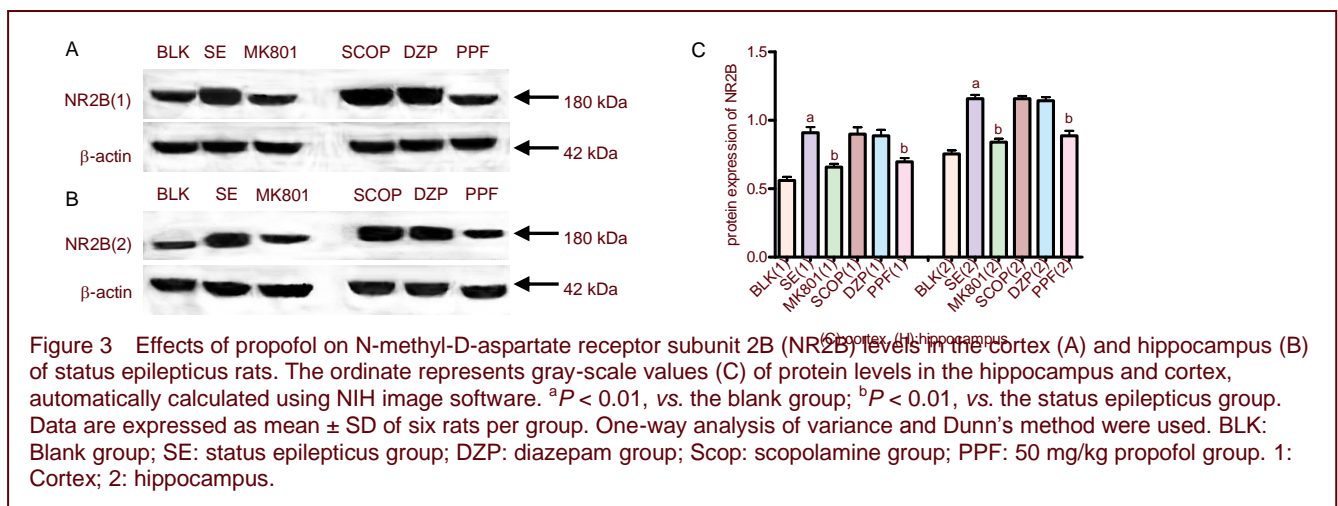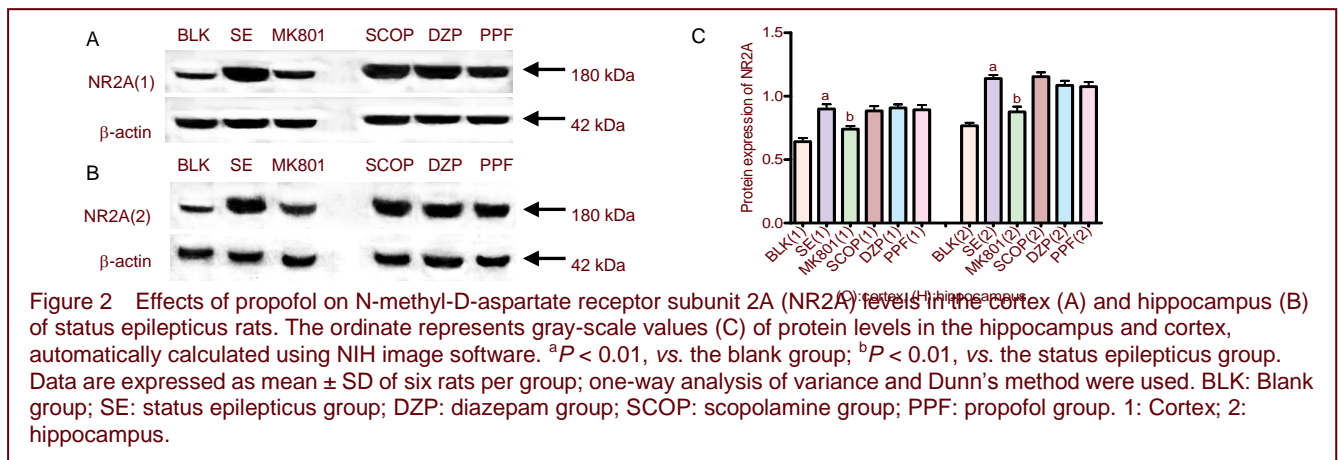[a]$P < 0.05$, [b]$P < 0.01$, *vs*. the SE group. Measurement data are expressed as mean ± SD of 10 rats per group. Chi-square test and one-way analysis of variance were used. DZP: Diazepam; Scop: scopolamine.

Figure 1  Effect of propofol on electroencephalographic changes during status epilepticus in the rat cerebral cortex and hippocampus. 1: Cortex; 2: hippocampus.

(A) Baseline electroencephalograms in normal rats.

(B) Status epilepticus waves. The high-frequency, high-amplitude spikes after seizure onset were induced by pilocarpine.

(C–G) Propofol inhibited the high-frequency, high-amplitude spikes in a dose-dependent manner from 12.5 to 100 mg/kg.

(H) Diazepam at 10 mg/kg significantly inhibited the high-frequency, high-amplitude spikes.

(I) MK801 at 2 mg/kg also significantly inhibited the high-frequency, high-amplitude spikes.

(J) Scopolamine at 10 mg/kg had no effect on high-frequency, high-amplitude spikes.

## Effects of propofol on NMDA receptor subunit 2A and 2B expression in the cortex and hippocampus of status epilepticus rats

Western blot analysis of proteins was performed to detect changes in the levels of NR2A and NR2B subunits in the cortex and hippocampus of rats at 24 hours after onset of status epilepticus. Significant increases in NR2A and NR2B protein levels (compared with the blank group) were observed in the rat cortex and hippocampus 24 hours after onset of status epilepticus by Li-pilo. Propofol, at a dose of 50 mg/kg, significantly reduced the level of NR2B subunit in the cortex and hippocampus compared with the level in the status epilepticus group ($P < 0.01$). However, compared with the status epilepticus group, the level of NR2A subunit was unchanged both in the cortex and the hippocampus of rats in the 50 mg/kg propofol group. MK801 treatment caused remarkable down-regulation of NR2A and NR2B subunits in the cortex and hippocampus, whereas diazepam and scopolamine did not affect the levels of these subunits (Figures 2, 3).



Figure 2  Effects of propofol on N-methyl-D-aspartate receptor subunit 2A (NR2A) levels in the cortex (A) and hippocampus (B) of status epilepticus rats. The ordinate represents gray-scale values (C) of protein levels in the hippocampus and cortex, automatically calculated using NIH image software. [a]$P < 0.01$, *vs.* the blank group; [b]$P < 0.01$, *vs.* the status epilepticus group. Data are expressed as mean ± SD of six rats per group; one-way analysis of variance and Dunn's method were used. BLK: Blank group; SE: status epilepticus group; DZP: diazepam group; SCOP: scopolamine group; PPF: propofol group. 1: Cortex; 2: hippocampus.



Figure 3  Effects of propofol on N-methyl-D-aspartate receptor subunit 2B (NR2B) levels in the cortex (A) and hippocampus (B) of status epilepticus rats. The ordinate represents gray-scale values (C) of protein levels in the hippocampus and cortex, automatically calculated using NIH image software. [a]$P < 0.01$, *vs.* the blank group; [b]$P < 0.01$, *vs.* the status epilepticus group. Data are expressed as mean ± SD of six rats per group. One-way analysis of variance and Dunn's method were used. BLK: Blank group; SE: status epilepticus group; DZP: diazepam group; Scop: scopolamine group; PPF: 50 mg/kg propofol group. 1: Cortex; 2: hippocampus.

## DISCUSSION

The first-line treatment for status epilepticus in the clinic is still intravenous benzodiazepines. Diazepam is a first-choice drug for treatment of early status epilepticus and acute repetitive seizures[19-20]. However, there are many potential safety issues associated with benzodiazepine use. Benzodiazepines may cause amnesia, memory impairment, confusion, withdrawal syndrome, tolerance, cognitive impairment and aggravate depression[20].

Therefore, there is an urgent need to find a medicine with few side effects to treat status epilepticus in the clinic. In the present study, we found that propofol effectively improved the seizures induced by Li-pilo in a dose-dependent manner. Electroencephalographic findings showed that propofol at doses of 50, 75 and 100 mg/kg effectively decreased high-frequency, high-amplitude spikes after onset of status epilepticus. The anticholinergic drug scopolamine had no effect on these changes, which may imply that the mechanism by which status epilepticus is induced by pilocarpine in the secondary phase is not associated with the cholinergic system.

It is unclear whether propofol can improve early status epilepticus and acute repetitive seizures, as there have been no reports of its use in this context in the clinic or laboratory experiments. Our results show for the first time that propofol effectively inhibits the early status epilepticus and acute repetitive seizures induced by Li-pilo. Of course, it is necessary to study this effect in more detail in both laboratory experiments and in the clinic.

It is thought that propofol may induce excitation of the central nervous system, such as generalized paroxysmal fast activity observed on electroencephalograms following the use of propofol for anesthesia, but the patients showing this activity remained hemodynamically stable and surgery was uneventful, with blood sugar, serum electrolytes and arterial blood gas analyses all normal[21-27]. Moreover, there was no evidence of a postictal phase; serum electrolytes and the findings of postoperative CT scanning of the head were normal, and patients had uneventful recovery postoperatively. Moreover, there was no clear reason why propofol could not be used in patients with epilepsy.

Many different studies have already confirmed that overexpression of NR2A or NR2B in the cortex and hippocampus plays an important role in the development of status epilepticus[6-10, 28-29]. Our results showed that the NR2A and NR2B subunits are significantly upregulated in the cortex and hippocampus 24 hours after onset of status epilepticus.

Kingston *et al* [28] confirmed that propofol inhibits NMDA receptor phosphorylation in neurons. Grasshoff *et al* [29] showed that propofol reduces the NMDA receptor-induced increase in intraneuronal calcium concentration, and this inhibition might contribute to the observed neuroprotective effects of propofol. Concurrent inhibition of transcriptional activity also occurs as a result of inhibiting the responses of ERK1/2 to NMDA[30]. However, whether propofol inhibits NR2A or NR2B subunits and whether the inhibition of status, epilepticus induced by pilocarpine by propofol is related to NR2A or NR2B, remain unclear. The results of our present study indicate that propofol 50 mg/kg, which is an anesthetic dose, significantly decreased the level of NR2B subunit both in the cortex and hippocampus after onset of status epilepticus induced by pilocarpine, whereas the level of NR2A was unchanged. These results suggest that the inhibition of status epilepticus induced by pilocarpine by propofol may be mediated by its inhibition of NR2B.

In conclusion, propofol effectively inhibited status epilepticus induced by Li-pilo, improved EEG changes and increased the survival rates of rats. Propofol can remarkably downregulate the levels of NR2B both in the cortex and hippocampus of status epilepticus rats. It is suggested that the inhibition of status epilepticus induced by Li-pilo in rats, by propofol, may be, at least in part, mediated by its downregulation of the NR2B subunit.

## MATERIALS AND METHODS

### Design
A randomized, controlled animal experiment.

### Time and setting
The experiment was conducted at the Experimental Center of Institute of Pharmacology and Toxicology, Academy of Military Medical Science, China, from October 2009 to March 2011.

### Materials
A total of 100 male Sprague-Dawley rats weighing 250−300 g, aged 3 months, were purchased from the Experimental Animal Center of the Academy of Military Medical Sciences (license No. SCXK (Jing) 2009-0005). Animal disposal was in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of the People's Republic of China[31].

### Methods

#### *Intracranial electrode implantation of cerebral cortices and hippocampus in rats*
Adult Sprague-Dawley rats were housed in controlled standard conditions (12-hour light/dark cycle, lights on at 7:00 a.m.) with free access to chow and water. Temperature was maintained between 20 and 21°C. Rats were anesthetized with chloral hydrate (Sigma, St. Louis, MO, USA) (400 mg/kg, i.p.) and mounted on a small-animal stereotaxic apparatus (Academy of Military Medical Science, Beijing, China). Electrode implantation was performed under sterile conditions and pairs of cuprum wires (250 µm in diameter) with a 0.1-mm vertical tip separation were implanted bilaterally at symmetrical points into the hippocampus, according to the standardized atlas plates of Paxinos and Watson[32]

(anteroposterior, −3.3 mm from the bregma; mediolateral, 2.0 mm from the bregma; dorsoventral, 2.5 mm from the neocortex). Two electrodes were implanted at the following stereotaxic coordinates: anteroposterior, 2.8 mm; mediolateral, 3 mm to record the frontal cortical electroencephalogram. Another stainless screw was placed into the skull over the right cerebellar hemisphere as a ground electrode. The electrodes were anchored to the skull using dental base acrylic resin powder. Electrode placement was verified histologically.

### Establishment of the Li-pilo kindling model, behavioral observations and electrocardiographic recordings from the cortices and hippocampus

Twenty-four hours prior to induction of status epilepticus, the rats were administered i.p. 3 mmol/kg lithium chloride (Sigma) dissolved in saline. On the next day, immediately after pilocarpine (Sigma) administration (30 mg/kg, i.p)[33], the animals ($n = 10$) were placed in a seizure observation cage and the onset and duration of status epilepticus within a period of 5 hours were measured. Simultaneously, electroencephalogram activity was collected through a BIOPIC MP-150 System (BIOPIC inc., Santa Monica, CA, USA) for 5 hours. Pilocarpine dissolved in normal saline was administered i.p. at a dose of 30 mg/kg following 15 minutes of baseline electrocardiogram recording. The onset of status epilepticus was determined based on behavior and clearly identifiable high-frequency, high-amplitude spikes were seen on electroencephalograms. Convulsion intensity was quantified according to the rating scale adapted from Racine[34] and described in Table 2.

Table 2 Signs and symptoms observed in lithium- pilo-carpine-treated rats

| Seizure severity | Behaviors |
| --- | --- |
| Stage 1 | Immobility, staring, irregular breathing, piloerection |
| Stage 2 | Tremor and agitation |
| Stage 3 | Weak limbic type seizures, automatisms: facial and jaw clonus, chewing and forelimb clonus. |
| Stage 4 | Severe limbic type seizures, rearing, loss of postural control and falling |
| Stage 5 | Tonic-clonic convulsive seizures |

After onset of status epilepticus, rats were continuously recorded for 30 minutes. Propofol at doses of 12.5, 25, 50, 75 and 100 mg/kg, scopolamine 10 mg/kg, MK801 2 mg/kg or diazepam 10 mg/kg were injected (i.p.) into animals in different groups. In all groups, electroencephalograms were continuously recorded for 5 hours after the onset of status epilepticus. Changes in wave frequency and amplitude were observed. The changes in electroencephalogram findings and survival rates after 24 hours were criteria for observing the inhibition of status epilepticus in rats by propofol. The blank control was only administered i.p. normal saline. Simultaneously, the latency of status epilepticus, induced status epilepticus number, controlled status epilepticus numbers, and numbers of surviving rats were recorded after 24 hours.

### Western blot analysis for NR2A and NR2B protein expression in cortices and hippocampus

To quantify the protein levels of the NR2A and NR2B subunits, western blot analysis was used. We choose propofol at a dose of 50 mg/kg, an anesthetic dose, to explore the mechanisms by which propofol inhibits status epilepticus induced by Li-pilo in rats. Twenty-four hours after the onset of status epilepticus, living rats from the blank, status epilepticus, propofol, diazepam, MK801 and scopolamine groups ($n = 6$) were anesthetized with pentobarbital (80 mg/kg), and decapitated. The frontal cortices and hippocampi were rapidly removed, weighed and homogenized at 4°C in lysis buffer. Before homogenization, a protease inhibitor cocktail was added to the lysis buffer. The homogenates were sonicated for 30 seconds and centrifuged at 12 000 × $g$ at 4°C. Cortex and hippocampus proteins were resuspended in sodium dodecyl sulfate-bromophenol blue reducing buffer containing 40 mDTT (mixed with an equal volume of 2× sample buffer). The proteins were denatured in a boiling-water bath for 5 minutes. The protein concentration was measured according to the Lowry method[35]. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (5% stacking gel and 8% separating gel) and a high molecular weight marker (40−200 kDa) was also loaded onto gels (80 V, 110 minutes). The separated proteins were electrotransferred to nitrocellulose membranes (180 mA, 2.5 hours). Membranes were blocked with 5% non-fat dry milk in Tris-buffered saline with Tween-20 0.1% for 2 hours to block nonspecific binding of antibodies. After rinsing in Tween-20/Tris-buffered saline, the membranes were incubated overnight at 4°C with rabbit anti-NR2A (1:400) and rabbit anti-NR2B (1:400) (primary polyclonal antibodies, Wuhan, China) diluted in Tris-buffered saline with Tween 0.1% buffer. The next day, the membranes were washed three times (10 minutes each time) in Tris-buffered saline with Tween-20 0.1% buffer, incubated at 37°C for 1 hour with horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit polyclonal antibodies [Pierce, Rockford, IL, USA] diluted 1:2 000 with Tris-buffered saline with Tween-20 0.1% buffer). The membranes were washed twice (10 minutes once) in Tween-20/Tris-buffered saline and once in Tris-buffered saline. Immunostaining was revealed by an enhanced chemiluminescence western blot analysis system (Amersham Pharmacia, Cambridge, England). Films were assessed by densitometry and the intensity of bands was quantified using National Institutes of Health image analysis software (National Institutes of Health, Washington, USA).

### Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was performed using the SPSS 16.0 software package (SPSS, Chicago, IL, USA). Statistical analysis on different treatment groups was carried out using different

methods. For controlled status epilepticus numbers and the survival rates of rats, the Chi-square test was used. For western blot analyses, one-way analysis of variance, and the Dunn's method were used owing to the small n. A value of $P < 0.05$ was considered to represent statistical significance.

**Author contributions:** The present study was designed by Henglin Wang, Weidong Mi and Yongan Wang. Henglin Wang and Haipeng Sun participated in data analysis. All authors contributed to experimental operational procedures.

**Conflicts of interest:** None declared.

**Ethical approval:** This study was approved by the Animal Ethics Committee of Academy of Military Medical Science, Beijing, China.

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