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RESEARCH ARTICLE

Subhypnotic doses of propofol impair spatial memory retrieval in rats

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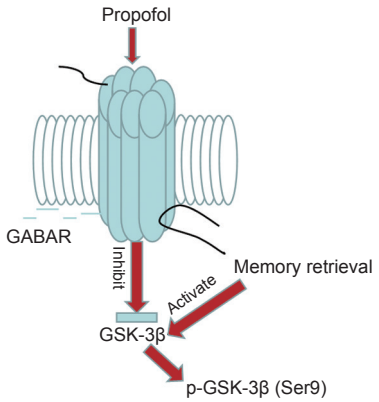
Graphical Abstract

Propofol affects memory retrieval through attenuated glycogen synthase kinase-3β (GSK-3β) signaling in the hippocampus



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Abstract

Abundant evidence indicates that propofol profoundly affects memory processes, although its specific effects on memory retrieval have not been clarified. A recent study has indicated that hippocampal glycogen synthase kinase-3β (GSK-3β) activity affects memory. Constitutively active GSK-3β is required for memory retrieval, and propofol has been shown to inhibit GSK-3β. Thus, the present study examined whether propofol affects memory retrieval, and, if so, whether that effect is mediated through altered GSK-3β activity. Adult Sprague-Dawley rats were trained on a Morris water maze task (eight acquisition trials in one session) and subjected under the influence of a subhypnotic dose of propofol to a 24-hour probe trial memory retrieval test. The results showed that rats receiving pretest propofol (25 mg/kg) spent significantly less time in the target quadrant but showed no change in locomotor activity compared with those in the control group. Memory retrieval was accompanied by reduced phosphorylation of the serine-9 residue of GSK-3β in the hippocampus, whereas phosphorylation of the tyrosine-216 residue was unaffected. However, propofol blocked this retrieval-associated serine-9 phosphorylation. These findings suggest that subhypnotic propofol administration impairs memory retrieval and that the amnestic effects of propofol may be mediated by attenuated GSK-3β signaling in the hippocampus.

Key Words: *nerve regeneration; glycogen synthase kinase-3β; propofol; memory retrieval; Morris water maze; neural regeneration*

Introduction

Propofol, an alkylphenol derivative, is a commonly used intravenous anesthetic and sedative. Many pharmacological effects of propofol have been elucidated, including sedative (Franks and Lieb, 1994; Jurd et al., 2003), antinociceptive (Guindon et al., 2007; Takechi et al., 2013) and organ-protecting (Andrews et al., 2012; Cui et al., 2013; Sahin et al., 2015) effects. Studies have also demonstrated effects of propofol on learning and memory, although there is some controversy about the nature of these effects. For example, subhypnotic doses of propofol have been shown to produce anterograde amnesia of inhibitory

avoidance (Ren et al., 2008) and also to accelerate extinction of conditioned taste aversion (Ishitobi et al., 2003). By contrast, Hauer et al. (2011) reported that anesthetic doses of propofol enhance memory consolidation *via* the endocannabinoid system.

Memory function is complex but is classically divided into three distinct phases: encoding, consolidation, and retrieval (Abel and Lattal, 2001). Most previous studies examining the effects of propofol on memory were conducted with administration of the drug shortly before or immediately after learning, thereby addressing effects on memory encoding and consolidation (Pain et al., 2002; Zhang et al., 2013). However, the effects of propofol on memory retrieval and retrieval-related mechanisms have not been addressed.

Glycogen synthase kinase-3β (GSK-3β) is a serine/threonine protein kinase. Phosphorylation of the serine-9 residue of GSK-3β inhibits the function of the enzyme (Sutherland et al., 1993; Jung et al., 2015), whereas phosphorylation of its tyrosine-216 residue increases its activity (Hughes et al., 1993). Recent studies have suggested that the phosphorylating activity of GSK-3β plays a functional role in memory processes (Liu et al., 2003; Hooper et al., 2007). Constitutively active GSK-3β is required for memory retrieval, and memory retrieval can be inhibited by intrahippocampal administration of the GSK-3β inhibitor SB216763 (Hong et al., 2012). The retrieval phase is required for development of long-term depression (Zhang et al., 2008). Propofol not only inhibits GSK-3β (Whittington et al., 2011) but also facilitates the development of long-term depression (Wei et al., 2002). These findings suggest that propofol may affect memory retrieval through modulation of GSK-3β activity.

In the present study, we examined the hypothesis that propofol affects memory retrieval through an effect on GSK-3β activity. We trained rats on a continuous multiple-trial Morris water maze (MWM) task and then subjected them to a 24-hour probe trial retrieval test while they were under the influence of a subhypnotic dose of propofol. We investigated the involvement of GSK-3β in memory retrieval by assessing levels of serine-9- and tyrosine-216-phosphorylated GSK-3β, abbreviated herein as p-GSK-3β(Ser9) and p-GSK-3β(Tyr216), respectively, following MWM retrieval testing, with or without pretest propofol administration, relative to those in untested rats.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (10 weeks of age) were purchased from the Center of Experimental Animals, Xuzhou Medical College, China, a specific-pathogen-free level laboratory authorized by the Jiangsu provincial government of China (production license for experimental animals, SCXK2010-0003). On their arrival, the rats were housed four per cage at a constant temperature of 24 ± 1°C, under a 12-hour light-dark cycle, with unlimited access to food and water for 1 week before behavioral training. All experiments were approved by the Animal Ethics Committee of Xuzhou Medical College, and the experimental procedures were in compliance with the National Institutes of Health's *Guide for Care and Use of Laboratory Animals* (Publication No. 80-23, revised 1996). For the water maze experiment, the rats were divided into three groups: one control group and two propofol dose groups (10 mg/kg, and 25 mg/kg; n = 10 per group). The rats were subjected to training and pharmacological interventions and then sacrificed after retrieval to obtain tissue for the western blot assays. For determining the change in GSK-3β phosphorylation in molecular biology experiments, rats were decapitated before, immediately after, 5 minutes after, or 10 minutes after completing the MWM retrieval test. Untrained rats were included in this experiment (group N) for comparison of GSK-3β phosphorylation with trained rats 24 hours after training. After the effective behavioral dosages and times at which protein levels changed were determined, these rats were divided into two groups: non-retrieval and retrieval. The rats in the non-retrieval group were treated with saline and propofol 24 hours after training, but did not undergo the retrieval test. The rats in the retrieval group also received saline and propofol and were compared with those in the non-retrieval group.

Drug administration

Rats in the propofol groups received a single intraperitoneal injection of propofol (10 and 25 mg/kg Diprivan at 10 mg/mL, Corden Pharma S.p.A., Sermoneta, Italy) 5 minutes before the probe test. The doses of propofol were chosen based on previous studies that demonstrated that these doses produced amnesia of an aversive event (Alkire et al., 2001; Pain et al., 2002). The control group received a single intraperitoneal injection of saline (2.5 mL/kg). Following their injections, the rats were returned to their home cages, where they remained until behavioral testing.

MWM testing

A continuous multiple-trial MWM paradigm was used for evaluating spatial learning and memory as described elsewhere (Moosavi et al., 2007, 2012). Briefly, a black circular water tank (diameter, 150 cm; height, 50 cm) was filled with water at a temperature of 24 ± 0.5°C to a depth of 32 cm. A round black platform (diameter, 17 cm) located in the middle of one quadrant and was submerged 1 cm beneath the surface of the water. The motion and path of the rats swimming in the pool were tracked and analyzed automatically using WaterMaze software. The MWM system was obtained from Coulbourn Instruments (Allentown, PA, USA).

Similar to previous studies (Moosavi et al., 2007, 2012), the training session consisted of eight acquisition trials, with four different starting locations that were distributed in a balanced manner around the maze. The rats were required to find the hidden platform using spatial cues within 90 seconds. After finding the platform, the rat was allowed to stay on it for 20 seconds and was then placed in its home cage for 30 seconds until the start of the next acquisition trial. If a rat did not find the platform within 90 seconds, it was guided onto the platform and allowed to remain on it for 20 seconds. After training, the rats were placed back in their

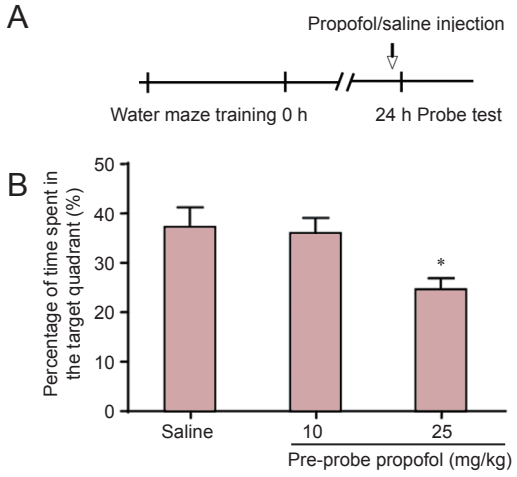


Figure 1 Propofol administered 5 minutes before Morris water maze probe test dose-dependently impairs memory retrieval.
(A) Schematic of the experimental procedure. (B) Percentage of time spent in the target quadrant during the retention test. Data are expressed as the mean ± SEM (n = 10 per group) and were analyzed by Tukey's test after one-way analysis of variance. *P < 0.05, *vs.* saline group. h: Hours.

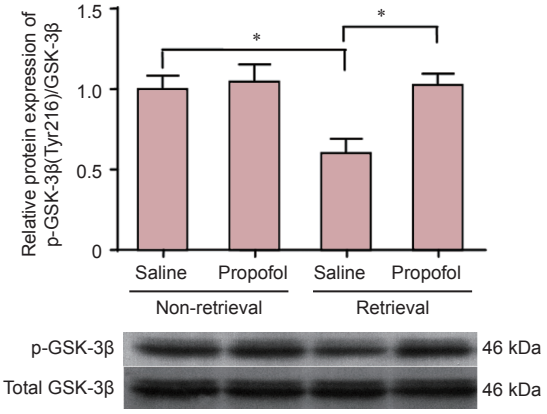
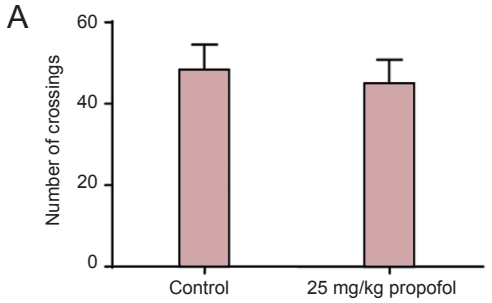
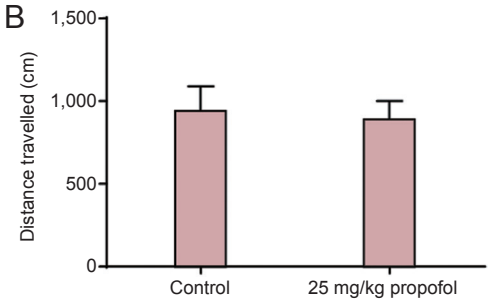


Figure 4 Propofol (25 mg/kg) administered 5 minutes before the test blocks retrieval-induced changes in hippocampal p-GSK-3β(Ser9) levels.
Rats in the non-retrieval group were treated with saline and propofol 24 hours after training but did not perform the retrieval test. Rats in the retrieval group received saline and propofol and were compared with rats in the non-retrieval group. p-GSK-3β protein expression was normalized to total GSK-3β (grayscale value ratio). Data are expressed as the mean ± SEM (n = 5 per group) and were analyzed by Tukey's test after one-way analysis of variance. *P < 0.05. GSK-3β: Glycogen synthase kinase-3β; p-GSK-3β(Ser9): serine-9–phosphorylated GSK-3β.





(A) Number of crossings and (B) distance traveled do not differ between saline-injected and propofol-treated rats during the 5-minute open-field test observation (P = 0.70, P = 0.79, respectively). Data are expressed as the mean ± SEM (n = 8/group) and were analyzed by independent-sample t tests.

Figure 2 Propofol (25 mg/kg) administered 5 minutes before an open-field activity test does not alter spontaneous locomotor behavior.

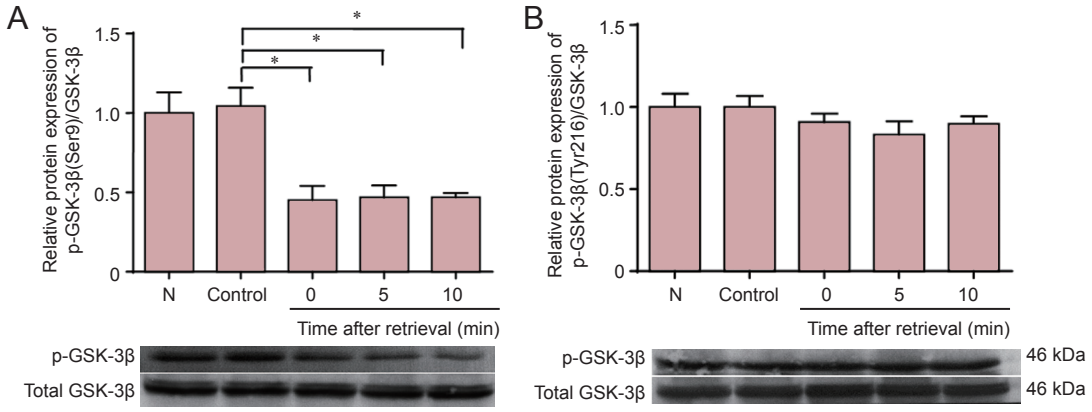


Figure 3 Morris water maze retrieval testing decreases hippocampal p-GSK-3β(Ser9) (A) but not p-GSK-3β(Tyr216) (B) levels.
p-GSK-3β protein expression was normalized to total GSK-3β (grayscale value ratio). Data are expressed as the mean ± SEM (n = 5 per group) and were analyzed by Tukey's test after one-way analysis of variance. *P < 0.05. N: Untrained rats; Control: rats that received Morris water maze test only; GSK-3β: glycogen synthase kinase-3β; p-GSK-3β(Ser9): serine-9–phosphorylated GSK-3β; p-GSK-3β(Tyr216): tyrosine-216–phosphorylated GSK-3β; min: minute.

home cages until retention testing 24 hours later. A 60-second probe trial was performed 5 minutes after a propofol or saline injection. During the probe test, the platform was not present, and the time spent in the target quadrant was recorded.

Spontaneous locomotor behavior

A 5-minute open-field test was performed 5 minutes after the propofol injection to investigate the effect of the drug on spontaneous locomotor behavior. Rats were placed in the center of an open-field box (50 cm × 50 cm × 39 cm), which consisted of four black plywood walls and a brown floor divided by virtual lines into nine equally sized squares (Institute of Biomedical Engineering, Tianjin, China). The number of line crossings and the distance travelled were automatically recorded by a computer during the 5-minute test.

Western blot assay

Rat hippocampi were rapidly isolated and placed in liquid nitrogen. Tissue samples were homogenized with radioimmunoprecipitation assay lysis buffer in the presence of phenylmethylsulfonyl fluoride (both from Beyotime, Jiangsu Province, China). Protein concentrations were determined by the Bradford method, with bovine serum albumin as a standard. Protein aliquots (80 μg) were loaded onto a 10% sodium dodecyl sulfate-polyacrylamide gel for electrophoresis, and after separation, the protein bands were transferred onto polyvinylidene fluoride membranes. The membranes were blocked with 5% nonfat milk for 2 hours at room temperature and incubated overnight at 4°C with primary antibodies. The following rabbit polyclonal primary antibodies were applied at a dilution of 1:1,000: anti-GSK-3β, anti-p-GSK-3β(Ser9), and anti-p-GSK-3β(Tyr216) (all from Cell Signaling Technology, Beverly, MA, USA). After the membranes were washed with Tris-buffered saline supplemented with Tween 20, they were incubated for 2 hours with alkaline phosphatase-labeled goat anti-rabbit secondary antibody (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 hours at room temperature. The immunoreactive complexes were visualized after reaction with nitroblue tetrazolium using a nitroblue tetrazolium/5–bromo-4-chloro-3-phosphate assay kit (Sigma, St. Louis, MO, USA). Scans of the membranes were first processed with Adobe Photoshop software (Adobe, San Jose, CA, USA) to acquire the grayscale values of the bands. The bands were then subjected to densitometric analysis using GraphPad Prism 5 software (Graph Pad Software Inc., San Diego, CA, USA).

Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). GraphPad Prism 5 was used to perform statistical analyses. Data from the western blot assays and water maze tests were analyzed by one-way analysis of variance followed by Tukey's tests. Independent-sample *t* tests were used to compare the locomotor activity between groups. In all cases, $P < 0.05$ was considered to be statistically significant.

Results

Propofol impaired spatial memory retrieval

As shown in **Figure 1**, rats given 25 mg/kg propofol 5 minutes prior to the MWM probe trial showed impaired memory retrieval, as demonstrated by a lower percentage of time spent in the target quadrant compared with that for the control group ($P < 0.05$). The percentage of time spent in the target quadrant by the rats in the 10 mg/kg propofol group did not differ significantly from that of the control group.

Propofol did not affect spontaneous locomotor behavior

Because 25 mg/kg propofol effectively impaired memory retrieval memory, we selected this dose for use in the open-field test and molecular biology analysis. As shown in **Figure 2**, there was no significant difference in basal locomotor activity between the propofol (25 mg/kg) and control groups. These results demonstrate that a dose of propofol that impairs memory retrieval does not affect spontaneous locomotion.

Propofol-impaired memory retrieval was associated with inhibition of decreased hippocampal p-GSK-3β(Ser9) levels

The ratio of p-GSK-3β(Ser9) to total GSK-3β in the hippocampus was significantly decreased for at least 10 minutes after MWM retrieval testing ($P < 0.05$ *vs.* non-tested controls; **Figure 3A**). By contrast, hippocampal p-GSK-3β(Tyr216) levels were unaffected by the test (**Figure 3B**). The retrieval test-associated decrease in p-GSK-3β(Ser9) was blocked in rats that were injected with 25 mg/kg propofol 5 minutes before the MWM test ($P < 0.05$ *vs.* saline control group; **Figure 4**).

Discussion

In the present study, pretest administration of propofol (25 mg/kg) significantly impaired spatial memory retrieval in the MWM task. A similar nonsignificant trend was observed at a dose of 10 mg/kg. The retrieval-impairing propofol dose did not affect spontaneous locomotor behavior in an open-field activity task. Performing the MWM retrieval test decreased rat hippocampal p-GSK-3β(Ser9) levels, and this decrease did not occur in rats given the memory retrieval-impairing dose of propofol before the test.

We investigated whether GSK-3β may mediate the propofol effects on memory retrieval based on previous findings that indicated that propofol has specific effects on memory processes beyond its sedative properties (Leslie et al., 1995; Veselis et al., 1997, 2001) and that GSK-3β is required for memory retrieval (Hong et al., 2012). Although the effects of propofol on memory encoding and consolidation have been characterized extensively, its effects on memory retrieval have been ambiguous. For example, 9 mg/kg propofol reportedly induces anterograde amnesia of aversive and nonaversive experiences (Pain et al., 2002), and 25 mg/kg propofol impairs spatial memory consolidation (Zhang et al., 2013). The present work is the first to demonstrate that a subhypnotic dose of propofol affects behavior in a long-term memory retrieval test.

We used a single multi-trial training session in the MWM task to obtain a single consolidation period without multiple reconsolidation events to focus specifically on the effects of propofol on memory retrieval. Because MWM performance can be affected by basal locomotor activity, which could potentially be influenced by anesthetics, including propofol (Kembro et al., 2012), we investigated the behavior of rats exposed to 25 mg/kg propofol 5 minutes before being placed in the open-field apparatus. Our finding that propofol at this dose did not reduce basal locomotor activity within a time interval concordant with the MWM probe test indicates that the differences in swim behavior observed in the MWM probe test are unlikely to be attributable to a nonspecific drug effect on motor behavior. Additionally, the same propofol dose immediately after training was insufficient to affect consolidation (data not shown). It was previously reported that propofol needs to be administered within 30 minutes of training to affect memory consolidation (Hauer et al., 2011). Thus, given the 24-hour delayed post-training used here, a rapid effect on consolidation, even late consolidation, is highly unlikely to have occurred here.

Phosphorylation of GSK-3β is known to play a role in the neuroprotective and cardioprotective effects of propofol (Kamada et al., 2008; Gui et al., 2012). Additionally, GSK-3β has been shown to be involved in multiple memory processes, including consolidation, retrieval, and reconsolidation (Kimura et al., 2008; Maguschak and Ressler, 2008; Hong et al., 2012). In the present study, GSK-3β(Ser9) phosphorylation decreased markedly immediately after spatial memory retrieval, and this decrease was maintained for at least 10 minutes without modification of GSK-3β(Tyr216). In a prior inhibitory avoidance study, GSK-3β(Ser9) phosphorylation effects were observed 10 minutes after recall (Hong et al., 2012). This timeline difference may be due to the different behavioral approaches used. Importantly, our finding that retrieval-induced reduction of GSK-3β(Ser9) phosphorylation was abrogated by 25 mg/kg propofol suggests that inhibition of GSK-3β activity may contribute to the propofol-mediated impairment of memory retrieval.

This study has some limitations. First, because specific pharmacological GSK-3β activators are lacking, we were unable to obtain direct evidence showing whether enhancement of GSK-3β activity in the hippocampus could prevent propofol-mediated impairment of memory retrieval. Transgenic overexpression of GSK-3β in rat hippocampus would not provide clarity on this issue, because the rats would overexpress GSK-3β during learning and consolidation as well (Hooper et al., 2007; Maguschak and Ressler, 2008). New approaches may enable us to address this issue in the future. A second study limitation is that the structure and function of GSK-3α are similar to those of GSK-3β, and GSK-3α is also expressed at high levels in the brain (Meijer et al., 2004; Asuni et al., 2006). To date, much greater attention has been paid to GSK-3β signaling than to GSK-3α signaling. Therefore, latent effects of propofol on GSK-3α should be considered. Finally, propofol could affect memory function at different levels, such as through effects on enzyme activity (Fibuch and Wang, 2007), neuroapoptosis (Yu et al., 2013), neurotransmitters (Semba et al., 2005), ion channels (Wang et al., 2006), and even cytoskeletal proteins (Ren et al., 2008). The downstream changes consequent to propofol-induced GSK-3β(Ser9) phosphorylation are unclear. Nevertheless, GSK-3β inhibition by serine-9 phosphorylation increases cAMP-responsive element-binding protein (CREB) activation (Grimes and Jope, 2001), and chronic increases in hippocampal CREB activity interfere with the retrieval of spatial information (Viosca et al., 2009). Therefore, we speculate that propofol impairment of retrieval may involve activation of CREB downstream of GSK-3β inhibition.

In summary, memory retrieval in the MWM task was impaired by 25 mg/kg propofol administered 5 minutes before retrieval testing, and this effect was associated with a suppression of GSK-3β activity, which may underlie this effect, at least in part. The present results in rats suggest that propofol may potentially disrupt memory recall in humans following minor ambulatory surgery performed under propofol sedation.

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References

Abel T, Lattal KM (2001) Molecular mechanisms of memory acquisition, consolidation and retrieval. Curr Opin Neurobiol 11:180-187.
Alkire MT, Vazdarjanova A, Dickinson-Anson H, White NS, Cahill L (2001) Lesions of the basolateral amygdala complex block propofol-induced amnesia for inhibitory avoidance learning in rats. Anesthesiology 95:708-715.
Andrews DT, Royse C, Royse AG (2012) The mitochondrial permeability transition pore and its role in anaesthesia-triggered cellular protection during ischaemia-reperfusion injury. Anaesth Intensive Care 40:46-70.
Asuni AA, Hooper C, Reynolds CH, Lovestone S, Anderton BH, Killick R (2006) GSK3α exhibits β-catenin and tau directed kinase activities that are modulated by Wnt. Eur J Neurosci 24:3387-3392.
Cui DR, Wang L, Jiang W, Qi AH, Zhou QH, Zhang XL (2013) Propofol prevents cerebral ischemia-triggered autophagy activation and cell death in the rat hippocampus through the NF-κB/p53 signaling pathway. Neuroscience 246:117-132.
Fibuch EE, Wang JQ (2007) Inhibition of the MAPK/ERK cascade: a potential transcription-dependent mechanism for the amnesic effect of anesthetic propofol. Neurosci Bull 23:119-124.
Franks NP, Lieb WR (1994) Molecular and cellular mechanisms of general anaesthesia. Nature 367:607-614.
Grimes CA, Jope RS (2001) CREB DNA binding activity is inhibited by glycogen synthase kinase-3β and facilitated by lithium. J Neurochem 78:1219-1232.
Gui B, Su M, Chen J, Jin L, Wan R, Qian Y (2012) Neuroprotective effects of pretreatment with propofol in LPS-induced BV-2 microglia cells: role of TLR4 and GSK-3β. Inflammation 35:1632-1640.
Guindon J, LoVerme J, Piomelli D, Beaulieu P (2007) The antinociceptive effects of local injections of propofol in rats are mediated in part by cannabinoid CB1 and CB2 receptors. Anesth Analg 104:1563-1569.

Hauer D, Ratano P, Morena M, Scaccianoce S, Briegel I, Palmery M, Cuomo V, Roozendaal B, Schelling G, Campolongo P (2011) Propofol enhances memory formation via an interaction with the endocannabinoid system. Anesthesiology 114:1380-1388.

Hong JG, Kim DH, Lee CH, Park SJ, Kim JM, Cai M, Jang DS, Ryu JH (2012) GSK-3β activity in the hippocampus is required for memory retrieval. Neurobiol Learn Mem 98:122-129.

Hooper C, Markevich V, Plattner F, Killick R, Schofield E, Engel T, Hernandez F, Anderton B, Rosenblum K, Bliss T, Cooke SF, Avila J, Lucas JJ, Giese KP, Stephenson J, Lovestone S (2007) Glycogen synthase kinase-3 inhibition is integral to long-term potentiation. Eur J Neurosci 25:81-86.

Hughes K, Nikolakaki E, Plyte SE, Totty NF, Woodgett JR (1993) Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. EMBO J 12:803-808.

Ishitobi S, Miyamoto T, Oi K, Toda K (2003) Subhypnotic doses of propofol accelerate extinction of conditioned taste aversion. Behav Brain Res 141:223-228.

Jung EM, Moffat JJ, Kim WY (2015) Regenerative potential of targeting glycogen synthase kinase-3 signaling in neural tissues. Neural Regen Res 10:1912-1913.

Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, Zaugg M, Vogt KE, Ledermann B, Antkowiak B, Rudolph U (2003) General anesthetic actions in vivo strongly attenuated by a point mutation in the GABAA receptor β3 subunit. FASEB J 17:250-252.

Kamada N, Kanaya N, Hirata N, Kimura S, Namiki A (2008) Cardioprotective effects of propofol in isolated ischemia-reperfused guinea pig hearts: role of KATP channels and GSK-3β. Can J Anaesth 55:595-605.

Kembro JM, Guzman DA, Perillo MA, Marin RH (2012) Temporal pattern of locomotor activity recuperation after administration of propofol in Japanese quail (Coturnix coturnix japonica). Res Vet Sci 93:156-162.

Kimura T, Yamashita S, Nakao S, Park JM, Murayama M, Mizoroki T, Yoshiike Y, Sahara N, Takashima A (2008) GSK-3beta is required for memory reconsolidation in adult brain. PLoS One 3:e3540.

Leslie K, Sessler DI, Schroeder M, Walters K (1995) Propofol blood concentration and the Bispectral Index predict suppression of learning during propofol/epidural anesthesia in volunteers. Anesth Analg 81:1269-1274.

Liu SJ, Zhang AH, Li HL, Wang Q, Deng HM, Netzer WJ, Xu H, Wang JZ (2003) Overactivation of glycogen synthase kinase-3 by inhibition of phosphoinositol-3 kinase and protein kinase C leads to hyperphosphorylation of tau and impairment of spatial memory. J Neurochem 87:1333-1344.

Maguschak KA, Ressler KJ (2008) Beta-catenin is required for memory consolidation. Nat Neurosci 11:1319-1326.

Meijer L, Flajolet M, Greengard P (2004) Pharmacological inhibitors of glycogen synthase kinase 3. Trends Pharmacol Sci 25:471-480.

Moosavi M, Naghdi N, Maghsoudi N, Zahedi Asl S (2007) Insulin protects against stress-induced impairments in water maze performance. Behav Brain Res 176:230-236.

Moosavi M, Yadollahi Khales G, Rastegar K, Zarifkar A (2012) The effect of sub-anesthetic and anesthetic ketamine on water maze memory acquisition, consolidation and retrieval. Eur J Pharmacol 677:107-110.

Pain L, Angst MJ, LeGourrier L, Oberling P (2002) Effect of a nonsedative dose of propofol on memory for aversively loaded information in rats. Anesthesiology 97:447-453.

Ren Y, Zhang FJ, Xue QS, Zhao X, Yu BW (2008) Bilateral inhibition of gamma-aminobutyric acid type A receptor function within the basolateral amygdala blocked propofol-induced amnesia and activity-regulated cytoskeletal protein expression inhibition in the hippocampus. Anesthesiology 109:775-781.

Sahin M, Gullu H, Peker K, Sayar I, Binici O, Yildiz H (2015) Does the intrathecal propofol have a neuroprotective effect on spinal cord ischemia? Neural Regen Res 10:1825-1829.

Semba K, Adachi N, Arai T (2005) Facilitation of serotonergic activity and amnesia in rats caused by intravenous anesthetics. Anesthesiology 102:616-623.

Sutherland C, Leighton IA, Cohen P (1993) Inactivation of glycogen synthase kinase-3 beta by phosphorylation: new kinase connections in insulin and growth-factor signalling. Biochem J 296:15-19.

Takechi K, Carstens MI, Klein AH, Carstens E (2013) The antinociceptive and antihyperalgesic effects of topical propofol on dorsal horn neurons in the rat. Anesth Analg 116:932-938.

Veselis RA, Reinsel RA, Feshchenko VA (2001) Drug-induced amnesia is a separate phenomenon from sedation: electrophysiologic evidence. Anesthesiology 95:896-907.

Veselis RA, Reinsel RA, Feshchenko VA, Wroński M (1997) The comparative amnestic effects of midazolam, propofol, thiopental, and fentanyl at equisedative concentrations. Anesthesiology 87:749-764.

Viosca J, Malleret G, Bourtchouladze R, Benito E, Vronskava S, Kandel ER, Barco A (2009) Chronic enhancement of CREB activity in the hippocampus interferes with the retrieval of spatial information. Learn Mem 16:198-209.

Wang W, Wang H, Gong N, Xu TL (2006) Changes of K^+-Cl^- cotransporter 2 (KCC2) and circuit activity in propofol-induced impairment of long-term potentiation in rat hippocampal slices. Brain Res Bull 70:444-449.

Wei H, Xiong W, Yang S, Zhou Q, Liang C, Zeng BX, Xu L (2002) Propofol facilitates the development of long-term depression (LTD) and impairs the maintenance of long-term potentiation (LTP) in the CA1 region of the hippocampus of anesthetized rats. Neurosci Lett 324:181-184.

Whittington RA, Virág L, Marcouiller F, Papon MA, Khoury NBl, Julien C, Morin F, Emala CW, Planel E (2011) Propofol directly increases tau phosphorylation. PLoS One 6:e16648.

Yu D, Jiang Y, Gao J, Liu B, Chen P (2013) Repeated exposure to propofol potentiates neuroapoptosis and long-term behavioral deficits in neonatal rats. Neurosci Lett 534:41-46.

Zhang H, Zhang SB, Zhang QQ, Liu M, He XY, Zou Z, Sun HJ, You ZD, Shi XY (2013) Rescue of cAMP response element-binding protein signaling reversed spatial memory retention impairments induced by subanesthetic dose of propofol. CNS Neurosci Ther 19:484-493.

Zhang Z, Gong N, Wang W, Xu L, Xu TL (2008) Bell-shaped D-serine actions on hippocampal long-term depression and spatial memory retrieval. Cereb Cortex 18:2391-2401.

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