Chronic intermittent propofol attenuates surgery-induced neuroinflammation, apoptosis, and cognitive impairment in aged mice

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Abstract

Background: Postoperative neurocognitive disorder (poNCD), previously also referred to as postoperative cognitive dysfunction (POCD) following surgery and anesthesia, is a significant public health problem in patients over 60 years. Propofol is the most commonly used intravenous anesthetic, acting primarily via $GABA_A$ receptors. However, it has also been shown to have apparent neuroprotective effects in aged mice and mouse models of Alzheimer's disease. Therefore, we postulated that chronic intermittent propofol would attenuate the development of postoperative cognitive deficits in aged mice.

Methods: Abdominal surgery was performed under isoflurane anesthesia in 21-24 months-old mice. Animals received either chronic intermittent propofol (CIP, 75 mg/kg i.p.) or vehicle (Intralipid^R) every 5th day throughout the experiment, starting 17 days before surgery. The levels of α5-$GABA_A$ receptors on cell surface membranes, as well as behavioral or biochemical manifestations of poNCD were studied.

Results: CIP led to a sustained redistribution of the $GABA_A$ receptor α5 subunit to the cell surface membranes. Laparotomy impaired learning and memory functions, as determined using a behavioral test battery that included Y maze alternation, novel object recognition, water maze learning and reversal learning, and cued and contextual fear conditioning, compared to no surgery controls. Strikingly, CIP strongly attenuated the surgery-induced memory impairment. Western blots on hippocampal tissues showed increased expression of the pro-apoptotic markers cleaved caspase-3, cleaved caspase-9, and Bax, indicating that abdominal surgery promoted apoptosis. Moreover, surgery increased expression of Iba-1, a marker of microglial activation. Chronic intermittent propofol prevented this proapoptotic and microglial activation.

Conclusions: Our results suggest that propofol – via mechanisms not fully understood, potentially via the sustained increased availability of α5-$GABA_A$ receptors on cell surface membranes – improves cognitive function by attenuating surgery-induced neuroinflammation and caspase activation and/or independently of this by rebalancing neuronal inhibition in aged mice. These findings support a therapeutic potential of perioperative propofol or of other compounds leading to a sustained redistribution of α5-$GABA_A$ receptors to the cell surface membranes in the perioperative period for reducing the risk of surgery-induced cognitive decline in aged individuals.

1.Introduction

Cognitive decline after surgery, which can persist for months, has been referred to as postoperative cognitive dysfunction (POCD)(Berger et al., 2018; Moller et al., 1998), or more recently as postoperative neurocognitive disorder (poNCD) (Evered et al., 2018), occurs mainly in elderly patients. In patients >60 years of age, approximately 26% of patients display signs of cognitive dysfunction one week after surgery, and 10% of patients still show signs three months after surgery, compared to 3% in controls at both time points (Moller et al., 1998; Berger et al., 2018). There is no evidence in the clinical literature that general anesthetics themselves cause poNCD, as comparisons of regional versus general anesthesia have not found differences in poNCD rates; thus, it is likely that other factors related to surgery are important(Mason et al., 2010). Indeed, there is evidence that poNCD in mice is due to surgery itself, which induces an inflammatory response(C. Zhang et al., 2016), but not the general anesthetic(Walters et al., 2016). In addition, Xu et al. (2014) reported that 18 month- but not 9-month-old mice display cognitive impairments after a laparotomy under local anesthesia, accompanied by changes in β-amyloid that are attenuated by a γ-secretase inhibitor(Xu et al., 2014).

Propofol (2, 6-diisopropyl phenol) is a potent intravenous hypnotic agent widely used for induction and maintenance of anesthesia during surgeries. Apart from its general anesthetic effects, it has been reported to possess anti-oxidative effects (Irwin et al., 2020), anti-inflammatory potential (Samir et al., 2015) and neuroprotective properties (H. Zhang et al., 2019; Y. Zhang et al., 2014). Furthermore, Shao et al. (2014) have shown that chronic intermittent treatment with propofol in aged rodents can improve the cognitive function and attenuate the caspase- 3 activation (Shao et al., 2014). In contrast, multiple exposures of propofol induced persistent neuronal apoptosis, neuronal deficit, synaptic loss, and long-term cognitive impairment in neonatal rodents (Chen et al., 2016). The precise mechanisms involved in propofol's dual effects (neuroprotection and neurotoxicity) of the brain remain unclear.

$GABA_A$ receptors are recognized as major targets for intravenous anesthetics such as propofol and etomidate. The α5-$GABA_A$ receptors play an important role in controlling memory under physiological conditions(Collinson et al., 2002; Crestani et al., 2002). Multiple rigorously controlled studies have shown that the sustained amnestic actions of general anesthetics such as etomidate and isoflurane are mediated by α5-$GABA_A$ receptors(Cheng, 2006; Zurek et al., 2014).

Furthermore, positive modulation of α5-$GABA_A$ receptors in aged rats has been shown to improve cognitive functions, whereas in young adult rats this is not the case (Koh et al., 2013). In addition, propofol has been reported to protect against irreversible neurodegenerative reaction induced by the NMDA antagonist, MK-801(Jevtovic-Todorovic et al., 2001). The pathophysiological mechanisms underlying the various actions of propofol and the finding that chronic intermittent administration of propofol increases cognitive performance in aged mice and in a transgenic mouse model of Alzheimer's Disease (AD Tg) are unknown.

Zurek et al. (2014) reported that etomidate and isoflurane lead to a redistribution of α5-$GABA_A$ receptors to the cell surface membranes which is associated with increased tonic inhibition in CA1 pyramidal neurons and impairments in the novel object recognition task, which can last for 7 days(Zurek et al., 2014). These experiments were done in 3- to 5-month-old mice. We were interested in the question whether propofol also results in a sustained redistribution of α5-$GABA_A$ receptors and whether such a sustained redistribution might prevent or reverse postoperative neurocognitive dysfunction in aged mice.

The objective of the current studies was to assess whether perioperative chronic intermittent propofol could mitigate surgery-induced cognitive impairments in aged mice. We hypothesized that perioperative chronic intermittent propofol administration leads to a redistribution of α5-$GABA_A$ receptors to the cell surface membranes at the time of the surgery, which would prevent the development of or reverse surgery-induced neuroinflammation and apoptosis and would reduce the liability of subsequent postoperative cognitive dysfunction.

2. Materials and methods

Animals

All experiments were conducted under the oversight and with the approval of the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois Urbana-Champaign. C57BL/6J mice (Jackson Laboratory stock# 000664) were purchased at 1-2 months of age and maintained under controlled laboratory conditions; temperature: 22 ± 2°C; humidity: 55 ± 5%; 12 hours/12 hours light/dark cycle. Food and water were available ad libitum. All procedures were consistent with the guideline of National Institute of Health, and all of the efforts were made to minimize the number of animals in the studies.

Preliminary dose finding study

Cell-surface biotinylation

Hippocampal tissue from 3-4 months old C57BL/6 J was used for these experiments. Coronal hippocampal slices were prepared 24 h or 48 h or 72 h or five days, or seven days after treatment with propofol (PropoFlo^{TM}28, Zoetis, USA) (100 mg/kg, i.p) or chronic intermittent propofol (CIP, 75 mg/kg, i.p., every 5th day for 21 days) or vehicle (Intralipid^{R}) and placed in oxygenated aCSF (95% O_2, 5% CO_2) for 30 min to recover. Slices were then placed on ice and incubated twice with 0.75 mg/ml NHS-SS-biotin in PBS (Thermo Scientific, Rockford, Illinois) for 30 min while bubbling with 95% O_2 and 5% CO_2. Excess biotin was quenched with a quenching solution (Pierce cell surface isolation kit, Thermo Scientific) and removed by washing slices three times with ice-cold aCSF. Slices were then lysed with a mild detergent, and the labeled proteins were then isolated with Thermo Scientific™ NeutrAvidin™ Agarose. The bound proteins are released by incubating with an SDS-PAGE sample buffer containing 50mM DTT. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto polyvinylidene fluoride (PVDF) membranes, and incubated at 4◦C overnight with primary antibodies for the α5-$GABA_A$ receptor (1:2, NeuroMab clone N415/24), Na+/K+ ATPase (1:200, Santa Cruz Biotechnology, Catalog # SC-514661) and β-actin (1:2000, Cell Signaling Technology, Catalog # 4970). The membranes were washed with TBST three times and then incubated at room temperature for 60 min with secondary antibody goat anti-rabbit IgG (1:3000, Cell signaling technology, Catalog # 7074) or goat anti-mouse IgG (1:3000, Cell signaling technology, Catalog # 7076). Then the membranes were washed with TBST four times. The SuperSignal West Pico

Chemiluminescent Substrate kit from Thermo Fischer Scientific (Rockford, IL) was used to detect antigens utilizing the manufacturer's instructions.

Experimental protocol and administration of Propofol

To study the effect of chronic intermittent propofol (CIP) on surgery-induced memory impairment in 21-24 months old wild type C57BL/6J mice, a dose of 75 mg/kg, i.p. every 5th day, was selected based on an initial dose-finding study. Vehicle groups, which received the same volume of Intralipid^{R} (i.p.) every 5th day throughout the experiment. After propofol treatment the mice were placed in a heated (37◦C) and humidified chamber flushed continuously with oxygen (100%), and continuously observed for breathing, heart rate, and skin color.

Animals were randomly divided into four groups. Each animal received one of the following treatments.

Group 1 : No surgery + Vehicle- (Intralipid^{R}), every 5th day throughout the experiment.

Group 2 : No Surgery + Propofol- (75 mg/kg, i.p)- every 5th day throughout the experiment

Group 3 : Surgery + Vehicle- (Intralipid^{R}), every 5th day throughout the experiment

Group 4 : Surgery + Propofol- (75 mg/kg, i.p), every 5th day throughout the experiment

Laparotomy

We performed a laparotomy in aged (21-24 months old) mice under 10 min isoflurane (2%) anesthesia. A 2.5 cm incision was made in the middle of the abdomen to open the abdominal cavity, and the abdominal cavity and skin were sutured and closed. All animals received 0.1 mg/kg buprenorphine s.c. immediately before the surgery for pain control. Mice were kept warm on a heating pad as they recovered. Mouse chow moistened with water was placed in the cage once they recovered to encourage eating and provide hydration following surgery. The cages were kept on heating pads as necessary the day following surgery.

Open field test

Open field tests were used to evaluate the locomotor and exploratory activities of mice in a novel environment. An open field box (40 cm × 40 cm × 30 cm) was made from plexiglass. Animals were placed randomly in the center of the open field box, and their activity was observed for 10 minutes using the EthoVision XT video tracking system, which was mounted above the chamber.

The open field chamber was wiped with 70 % ethanol before use and subsequent tests to remove any scent clues left by the previous subject mouse. The total distance traveled (cm) and time spent in inner zone of the open field chamber was observed.

Spontaneous alternation Y-maze test

Spontaneous alternation is a measure of spatial working memory. Each animal was placed in the center of a symmetrical Y-maze and was allowed to explore freely during an 8-min session. The sequence and total number of arms entered were recorded with a video camera that was mounted above the apparatus. Arm entry was defined as entry of the whole body into an arm. Alternation was defined as successive entries (ABC, ACB, BCA, BAC, CBA, CAB) into the three arms in overlapping triple sets. The % of alternation was calculated as the number of triads containing entries into all three arms divided by the maximum possible number of alternations (the total number of arms entered − 1) × 100. To diminish odor cues, the Y maze was cleaned with 70% ethanol between trials and allowed to dry.

Novel object recognition test

Object recognition was assessed in a 40 cm × 40 cm × 30 cm opaque chamber in a dimly lit room. Mice are habituated to the chamber for two days, 10 min per day. During the training phase, on day three, the mouse was allowed to explore two copies of the same object for 10 min. The mouse was then returned to its home cage for a retention period of 1 h. The mouse was reintroduced to the training context and presented with one familiar sample object and one novel object for 10 min. Movement and interaction with the objects were recorded with the EthoVision XT video tracking system, which was mounted above the chamber, and exploratory behavior was measured. Exploratory behavior was defined as sniffing, licking, or touching the object while facing the object. Interaction time was recorded using the multiple body point module. Memory was assessed by measuring the recognition index (i.e., the ratio of time spent exploring the novel object to the time spent exploring both objects). After each mouse had performed the test, the chamber and objects were swabbed thoroughly with alcohol to avoid any interference with tests involving subsequent mice.

Trace -and Contextual Fear conditioning

On the training day of the experiment, the mice were placed in a conditioning box (Med-Associates, Inc., St. Albans, VT) with grid floors. They were allowed to explore the fear conditioning chamber for 180 seconds before presenting a 2-Hz pulsating tone (20 sec,70 dB, 5000 Hz) - shock (2 sec, 0.7 mA) sequence separated by an empty 20 sec trace interval. The pairing of stimuli was repeated five times at 1-min intervals. Sixty seconds after the fifth shock, the mice were removed from the chamber. The first contextual test was performed at ~ 24 hours after the end of the pairing. Each mouse was allowed to stay in the same training chamber for the contextual test for a total of 3 min, with no tones or shocks delivered, and freezing behavior was recorded. At the end of the contextual test, the mice were returned to their home cage. After ~ 24 hours, mice were placed in a novel environment for a cued fear memory test for a total of 9 min. The new environment consisted of a colored Plexiglas sheet that covered the steel rods of the floor, black striped plastic on the chamber's walls, and the introduction into the testing chamber of a novel odor (1% acetic acid as olfactory cue). After 3 min without any stimulus, mice were exposed to the auditory cue for the remaining minutes (6 min), and freezing behavior was scored. Cognitive function in the tone test was also assessed by measuring the freezing time. The same cohorts of mice also tested in the context and tone testing at four weeks post-surgery without additional pairing.

Morris Water Maze and Reversal Learning

The spatial learning and memory function of mice were assessed using the Morris water maze. The test lasted for 14 days: Learning phase Session 1-8 (day1-8); Learning phase probe trials (Day 9); Reversal learning phase Session 1-4 (day 10-13) & Reversal learning probe trials (day 14). Mice are tested in a pool (120 cm in diameter) filled with water (23-25°C) made opaque with the addition of a white nontoxic dye (Premium Grade Tempera, Blick, Galesburg, IL) containing a platform (10 cm in diameter) that is submerged by 1 cm under water surface. Geometric shapes are affixed to the walls to serve as extra-maze cues. Mice are given 3 trials every day released from a different quadrant each time in random order, with the platform location constant. A trial ends either 2 sec after the mouse climbs on the platform, or 60 sec after the start of the trial, with the experimenter guiding the mouse to the platform. If a mouse could not find the platform within 60 sec it was gently guided to the platform and the escape latency was recorded as 60 sec. After the

mouse reached the platform, it was allowed to stay there for 10 sec before the next test was performed. From day 10 to day 13, the reversal learning phase was established by moving the platform from the original location to the nearest quadrant to increase the effects of interference. During probe trial (day 9) and reversal learning probe trial (day 14), the platform was removed, and the mice were left in the pool for 60 seconds. The track of mice was recorded using a camera positioned above the centre of the pool and connected the EthoVision XT software video tracking system.

Biochemical and molecular parameters

Biochemical and molecular parameters estimations were performed in the treatment group. For biochemical assays, animals were deeply anesthetized with ketamine (139 mg/kg i.p.) and xylazine (21 mg/kg i.p.) and then trans-cardially perfused with ice-cold phosphate-buffered saline (PBS). The brain was removed quickly, kept on an ice-cold plate, and then dissected to harvest the hippocampus.

Western blots

Hippocampal tissue was homogenized in RIPA buffer containing protease inhibitors and phosphatase inhibitors. The homogenate was centrifuged at 13,000 rpm for 30 min at 4°C, and supernatant was collected for measurement of protein concentrations with a BCA assay kit. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto polyvinylidene fluoride (PVDF) membranes and incubated at 4°C overnight with primary antibodies for Cleaved Caspase-3 (Asp175) (5A1E) [1:1000, Cell Signaling Technology, catalog # 9664], Cleaved Caspase-9 (Asp353) [1:1000, Cell Signaling Technology, catalog #9509], Bax [1:1000, Cell Signaling Technology catalog #4796], Iba-1 [1:500, Invitrogen, catalog # MA5-36257] and β-actin [1:2,000, Cell Signaling Technology, catalog # 4970]. The membranes were washed with TBST three times and then incubated at room temperature for 60 min with secondary antibody goat anti-rabbit IgG (1:3000, Cell Signaling Technology, catalog # 7074) or goat anti-mouse IgG (1:3,000, Cell Signaling Technology, catalog # 7076). Then the membranes were washed with TBST four times. The SuperSignal West Pico Chemiluminescent Substrate kit from Thermo Fischer Scientific (Rockford, IL) was used to detect antigens utilizing the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed (GraphPad Prism) using 1-way ANOVA followed by Tukey's test for multiple comparisons. Results are represented as mean±S.E.M. and P values, are specified in each figure legend. P values less than 0.05 were considered significant.

3. Results

Propofol induces a sustained redistribution of α5-GABA$_A$ receptors to cell-surface membranes in the hippocampus.

To determine whether propofol affects the amount of GABA$_A$ receptors in the cell surface membranes, we performed biotinylation experiments followed by Western blots. We determined both surface α5 protein expression and total α5 protein expression after propofol treatment at different time points: 24h, 48h, 72h, five days, and seven days. We found increased cell-surface expression of α5-GABA$_A$ receptor in hippocampus after 24h (*p <0.05), 48h (*p<0.05), 72h (*p<0.05), and five days (*p< 0.05), but not at seven days (p>0.05) after treatment with propofol; expression of the α5 subunit in total membrane was unchanged (p>0.05) (Fig 1). Further, we studied the effect of chronic intermittent propofol (CIP), specifically 75 mg/kg, i.p., every 5th day for 21 days on α5-GABA$_A$ receptor expression in surface and total membrane. We found that CIP treatment significantly up-regulated the levels of cell-surface expression of α5 subunits (*p<0.05), with no change of the amount of the a5 subunit in total membranes (p>0.05) (Fig. 2). These results suggest that there is a redistribution of α5-GABA$_A$ receptors to the cell surface membranes and that apparently no tolerance develops with repeated injection.

Examination of the effects of CIP treatment on surgery-induced behavioral and biochemical changes

In order to assess the effects of CIP treatment in the postoperative phase, we used a protocol outline in Figure 3. Mice received 75 mg/kg propofol i.p. every 5 days throughout the experiment starting with day 1. Laparotomy was performed on day 17, followed by open field test on day 19, Y maze test on day 20, novel object recognition (NOR) test on day 21-22, fear conditioning on days 23-25 (day 23: training; day 24: contextual test; day 25: tone test), Morris water maze on days 26-39, fear conditioning testing on days 40 (contextual test) and 41 (tone test). Mice were euthanized for molecular analysis on day 42 (Fig. 3).

Open field test

We assessed the effects of the CIP treatment and/or laparotomy surgery on open-field behavior. CIP treatment and/or surgery did not significantly change the total distance traveled by mice in the

open field test as compared to the control condition ($p>0.05$). The CIP treatment ($p>0.05$) and/or surgery ($p>0.05$), did not significantly alter the time spent in the center of the open field as compared to the control condition in the mice (Fig. 4). Collectively, our data suggest that the CIP treatment and/or surgery did not disturb the open field behavior (e.g., total distance moved, and time spent in the center).

Y maze and novel object recognition (NOR) tests

In the Y maze test, surgery reduced in the percentage of correct alternations ($p<0.05$) (Fig.5A). In the novel object recognition test, surgery reduced the recognition index in the NOR test compared with no-surgery control ($p<0.01$) (Fig.5B). In contrast, CIP treatment significantly increased the percentage of correct alternations in the Y-maze test ($p<0.05$) (Fig.5A) and the recognition index in the NOR test ($p<0.05$) (Fig.5B) compared to controls. Strikingly, in animals with surgery CIP treatment reversed the surgery-induced reduction of the percentage correct alternations in the Y maze ($p<0.05$) (Fig.5A) as well as the surgery-induced reduction of the recognition index in the NOR test ($p<0.001$) (Fig.5B). We observed no significant differences between males and females in the Y maze and NOR test (data not shown). The results indicate that CIP treatment can reverse the surgery-induced impairments of working memory in the Y maze test and in the NOR test, and that in these tests CIP treatment alone (i.e., in the absence of surgery) is sufficient to improve performance.

Morris water maze (MWM)

There were significant differences in escape latency ($p<0.05$, Figs. 6A and 7A) and path length ($p<0.05$, Figs. 6B and 7B) in the mice that have undergone surgery compared to control mice without surgery compared to mice with surgery in both learning phases (Day 1-8) (Figs. 6A and B) and reversal learning phase (Day 9-13) (Figs. 7A and 7B). Surgery led to an increase in the escape latency and path length in both learning and reversal learning phases. CIP treatment decreased the escape latency ($p<0.05$, Figs. 6A and 7A) and path length ($p<0.05$, Figs. 6B and 7B) in the surgery group (Surgery + Propofol) as compared to the vehicle-treated group (Surgery + Vehicle) in both learning phase (Day 1-8, Figs. 6A and 6B) and reversal learning phase (Day 9-

13, Figs. 7A and 7B), thus reversing the effects induced by surgery. The treatment with CIP did not significantly alter the escape latency ($p>0.05$, Fig.6A & 7A) and path length ($p>0.05$, Fig.6B and 7B) in no-surgery control mice. There were no significant differences in swimming speed between the surgery, no-surgery controls, and Propofol-treated groups (data not shown).

Probe trials were conducted to assess spatial reference memory after both learning phase (learning phase probe trials) (Day 9), and reversal learning phase (reversal learning probe trials) (day 14). Cumulative duration (Time in target zone) and frequency to enter the target platform area in learning phase probe trials and reversal learning probe trials were recorded. In the learning phase probe trials, surgery reduced both time to the target zone ($p<0.05$) (Fig. 6C) and frequency to enter the target zone ($p<0.05$) (Fig. 6D) compared with no-surgery controls. Likewise, in the reversal learning phase probe trials surgery also reduced time in target zone ($p<0.05$) (Fig. 7C) and frequency to enter the target zone ($p<0.05$) (Fig. 7D) compared with the no-surgery control. The results suggest that surgery impaired reference memory. CIP treatment of mice in the surgery group significantly increased time in the target quadrant ($p<0.05$, Fig 6C) and frequency of entering the target platform area ($p<0.05$, Fig. 6D) in the learning phase probe trial but not in the reversal learning probe trials ($p>0.05$, Fig 7C & $p>0.05$, Fig 7D). Our findings indicate that surgery impairs learning and reversal learning, and that this impairment can be reversed at least in the learning phase.

Trace and contextual fear conditioning.

Fear conditioning is among the most commonly used behavioral tests to detect cognitive impairment induced by anesthesia and anesthesia plus surgery (Xu et al., 2014; W. Zhang et al., 2020). We found that surgery reduces freezing time in both tone test ($p<0.05$, Fig 8A) and context test ($p<0.05$, Fig 8B) both 1-2 days after training (1 week after surgery) and 17-18 days after training. CIP improves the cognitive function in the tone test ($p<0.05$, Fig 8A) and contextual test ($p<0.05$, Fig 8B) 1-2 days after training, whereas 17-18 days after training the improvement is only seen in the contextual test ($p<0.01$, Fig. 8B) but not in the tone test ($p>0.05$, Fig. 8A). These results indicate that CIP treatment can reverse surgery-induced cognitive impairments.

Expression of microglial activation and proapoptotic markers.

Postoperative neurocognitive dysfunction (poNCD) (Evered et al., 2018) is associated with the inflammatory activation of hippocampal microglia (Feng et al., 2017) and apoptosis(Yin et al., 2020). To examine the potential role of microglia activation and apoptosis in surgery-induced cognitive dysfunction, we studied the expression levels of the microglial activation marker Iba-1, and of the proapoptotic markers cleaved caspase-9, cleaved caspase-3, and Bax in the hippocampus using Western blots (Fig. 9). Surgery induced an increase in the expression of Iba-1 ($p<0.05$, Fig.9B), cleaved caspase-9 ($p<0.05$, Fig. 9C), cleaved caspase-3 ($p<0.05$, Fig.9D) and Bax ($p<0.05$, Fig. 9E) protein expression compared to no-surgery controls. This surgery-induced increase in expression was attenuated by CIP treatment for Iba-1 ($p<0.05$,Fig. 9B), cleaved caspase-9 ($p<0.05$, Fig. 9C), cleaved caspase-3 ($p<0.05$, Fig. 9D), and Bax ($p<0.05$,Fig. 9E). There were no significant differences between the Control (No surgery control) and the Propofol per se (No surgery + Propofol) groups ($p>0.05$, Fig. 9B-E).

4.Discussion

The goal of this study was to investigate whether chronic intermittent treatment with propofol is able to attenuate cognitive impairments that occur postoperatively in mice and, if this is the case, whether the mechanism may involve $GABA_A$ receptors, the target of the anesthetic and amnestic actions of intravenous anesthetics. It has been reported that chronic intermittent propofol (CIP) improves spatial memory in aged mice (20-22 months) (Shao et al. 2014). In our study, abdominal surgery caused an increase in the microglial activation marker, Iba-1, and in the proapoptotic markers, cleaved caspase-9, cleaved caspase-3, and Bax in the hippocampus, and impaired spatial learning and memory ability, while locomotor activity was unaffected. We found that CIP treatment results in a sustained redistribution of α5-$GABA_A$ receptors and suppression of microglial activation and apoptosis in the hippocampus of aged mice (21-24 months of age). These changes may underly at least in part the protective role of CIP treatment against surgery-induced memory-impairments.

Zurek et al. (2014) reported that etomidate and isoflurane lead to a redistribution of α5-$GABA_A$ receptors to the cell surface membranes, which is associated with increased tonic inhibition in CA1 pyramidal neurons and impairments in the novel object recognition task, which can last for seven days (Zurek et al., 2014). In the present study, we were interested in whether propofol also results in a sustained redistribution of α5-$GABA_A$ receptors and whether such a sustained redistribution might prevent or reverse postoperative neurocognitive dysfunction in aged mice. We found that a single treatment with the injectable anesthetic propofol increased cell-surface expression of α5-$GABA_A$ receptors in the hippocampus after 24 h (*$p < 0.05$), 48 h (*$p<0.05$), 72 h (*$p<0.05$), five days (*$p< 0.05$) and seven days ($p>0.05$). CIP treatment over 21 days also significantly up-regulated the levels of cell-surface expression of α5 subunits (*$p<0.05$), indicating that there is no development of tolerance to this effect. However, the total expression of α5 subunits was unchanged at all time points ($p>0.05$), which indicates that propofol most likely affects the distribution of α5-$GABA_A$ receptors in the neurons by an unknown mechanism, possibly by inhibiting endocytosis.

Therefore, in the current study we assessed whether CIP treatment (75 mg/kg propofol i.p. every 5^{th} day throughout the experiment) administered perioperatively could prevent or reverse surgery-induce cognitive impairments as well as microglia and caspase activation. We found that

abdominal surgery caused significant cognitive impairment in aged mice. At the same time, CIP treatment attenuates surgery-induced memory impairment in the Y-maze, NOR, Trace and contextual fear conditioning, and MWM tests. These findings are in accordance with previous observations by Shao et al. (2014), who demonstrated that the effects of CIP administration (50 mg/kg/week i.p.) improved cognitive function in the water maze and attenuated Aβ-induced mitochondrial dysfunction and brain caspase-3 and caspase-9 activation in both aged wild type and AD-Tg mice. The propofol-induced reduction of Aβ-induced caspase-3 activation and the reduction of Aβ-induced opening of the mitochondrial permeability transition pore (mPTP) were attenuated by flumazenil, which is an antagonist at the benzodiazepine binding site of $GABA_A$ receptors(Hunkeler et al., 1981). This effect was difficult to interpret until it was reported that there are shared structural mechanisms of general anesthetics and benzodiazepines, providing a potential mechanism for anesthetic reversal by flumazenil (Kim et al., 2020). Limon et al. (2012) reported that patients with Alzheimer's disease (AD) had decreased numbers of $GABA_A$ receptors (including a decreased level of α5 subunit mRNA), an age dependent reduction of GABA currents, a faster rate of desensitization and less sensitivity to GABA(Limon et al., 2012), suggesting deficits in $GABA_A$-mediated neurotransmission in AD. In the current study, CIP treatment results in increased surface expression of α5-$GABA_A$ receptors, which may underly the improvement of cognitive function in aged animals. Recently, it has been shown that propofol, administered 30 min after fear conditioning, led to reduced contextual freezing 2, 4, and 6 weeks after training, and restored shock-induced spatial memory deficits in the MWM, which is somewhat reminiscent of our findings; while the authors showed that propofol blocks shock-induced reductions of LTP and LTD, the mechanisms behind these observations are not entirely clear(Niu et al., 2022).

Numerous studies have shown that microglia are required for postoperative hippocampal inflammation and cognitive decline in mice, and that microglial activation is a key factor in the pathophysiology of poNCD (Feng et al., 2017; Subramaniyan and Terrando, 2019). Systemic inflammation caused by surgery could induce neuroinflammation, mainly through destroying the permeability of the blood-brain barrier (Bowman et al., 2018), hence, promoting the activation of microglia. Activated microglia subsequently release more inflammatory cytokines and trigger apoptosis (Dai et al., 2015; Rajasekar et al., 2017) . In the present study, we have detected an increase in activated microglia marker Iba-1 after abdominal surgery in aged mice with an increase in pro-apoptotic markers, cleaved caspase-9, cleaved caspase-3, and Bax in the hippocampus. The

results indicate that significant inflammation and apoptotic activation occur in the hippocampus after laparotomy surgery. On the other hand, we found that the CIP treatment attenuated the expression of activated microglia and pro-apoptotic markers in mice exhibiting memory impairment following surgery in aged mice. These findings align with a report where CIP administration attenuated Aβ-induced caspase activation and Aβ-induced mPTP opening in a flumazenil-senstitive manner and thus likely via $GABA_A$ receptors(Shao et al., 2014). The precise mechanism for this is unclear but could involve upstream radical scavenging (Kobayashi et al., 2008) or a direct cellular effect on cytokine responsiveness or secretion, or both. For example, propofol completely ablated lipopolysaccharide-stimulated cytokine secretion in cultured microglial cells, compared with volatile anesthetics(Ye et al., 2013). Perioperative depletion of microglia attenuates surgery-induced cognitive decline in mice (Feng et al., 2017). Furthermore, in humans undergoing anesthesia with propofol reduced gene expression of pro-inflammatory cytokines was found in macrophages (Kotani et al., 1999). Taken together, these findings suggest a potential association between GABA neurotransmission and caspase activation, neuroinflammation, and cognitive function. Future studies may use a different anesthetic agent, e.g., etomidate to test this hypothesis further. These findings may promote more research, leading to new concepts of poNCD neuropathogenesis and new intervention (s) of poNCD.

Our studies have several limitations. First, we did not determine the dose- or time-dependent effects of propofol on surgery-induced memory impairment in aged mice, in part due to logistical issues such as aged mice being an order of magnitude more expensive. Since in the literature, propofol showed dual effects, i.e., neuroprotection(Mardini et al., 2017; Shao et al., 2014) and neurotoxicity(Chen et al., 2016; Yan et al., 2017) in the brain, it is possible that propofol treatment with different doses or administered at different times may have different effects. Nevertheless, our current studies have demonstrated that propofol induces redistribution of α5-$GABA_A$ receptors in the hippocampus and proposed a new concept to further determine the effect of CIP on poNCD.

Second, we did not directly assess the role and the cellular basis of α5-containing α5-$GABA_A$ receptors in the memory-enhancing effect of CIP in surgery-induced memory impairment in aged mice. Future studies could evaluate whether the cognition-enhancing effect is mediated by α5-$GABA_A$ receptors in hippocampal principal or other neurons. However, the main objective of the current studies was to determine whether chronic intermittent propofol results in a reduction in

poNCD in aged mice. Future studies will systematically investigate the underlying mechanism by which CIP improves memory function.

In conclusion, our studies revealed that chronic intermittent treatment with the anesthetic propofol improved cognitive function postoperatively, led to a sustained redistribution of α5-GABA$_A$ receptors to the cell surface membranes, attenuation of a surgery-induced increase of pro-apoptotic proteins (caspase-9, cleaved caspase-3, and Bax) and attenuation of microglial cell activation in the hippocampus in aged mice with surgery-induced memory-impairment. Our findings suggest that chronic intermittent propofol treatment might prevent or attenuate surgery-induced cognitive dysfunction and thus could potentially reduce the liability for undesired long-term neurocognitive outcomes.

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Declaration of Interests

The authors declare no competing interests.

5. References

Berger, M., Schenning, K. J., Brown, C. H., Deiner, S. G., Whittington, R. A., Eckenhoff, R. G., Angst, M. S., Avramescu, S., Bekker, A., Brzezinski, M., Crosby, G., Culley, D. J., Eckenhoff, M., Eriksson, L. I., Evered, L., Ibinson, J., Kline, R. P., Kofke, A., Ma, D., … Zuo, Z. (2018). Best Practices for Postoperative Brain Health. *Anesthesia & Analgesia*, *127*(6), 1406–1413. https://doi.org/10.1213/ANE.0000000000003841

Bowman, G. L., Dayon, L., Kirkland, R., Wojcik, J., Peyratout, G., Severin, I. C., Henry, H., Oikonomidi, A., Migliavacca, E., Bacher, M., & Popp, J. (2018). Blood-brain barrier breakdown, neuroinflammation, and cognitive decline in older adults. *Alzheimer's & Dementia*, *14*(12), 1640–1650. https://doi.org/10.1016/j.jalz.2018.06.2857

Chen, B., Deng, X., Wang, B., & Liu, H. (2016). Persistent neuronal apoptosis and synaptic loss induced by multiple but not single exposure of propofol contribute to long-term cognitive dysfunction in neonatal rats. *The Journal of Toxicological Sciences*, *41*(5), 627–636. https://doi.org/10.2131/jts.41.627

Cheng, V. Y. (2006). 5GABAA Receptors Mediate the Amnestic But Not Sedative-Hypnotic Effects of the General Anesthetic Etomidate. *Journal of Neuroscience*, *26*(14), 3713–3720. https://doi.org/10.1523/JNEUROSCI.5024-05.2006

Collinson, N., Kuenzi, F. M., Jarolimek, W., Maubach, K. A., Cothliff, R., Sur, C., Smith, A., Otu, F. M., Howell, O., Atack, J. R., McKernan, R. M., Seabrook, G. R., Dawson, G. R., Whiting, P. J., & Rosahl, T. W. (2002). Enhanced Learning and Memory and Altered GABAergic Synaptic Transmission in Mice Lacking the α5 Subunit of the GABA $_A$ Receptor. *The Journal of Neuroscience*, *22*(13), 5572–5580. https://doi.org/10.1523/JNEUROSCI.22-13-05572.2002

Crestani, F., Keist, R., Fritschy, J.-M., Benke, D., Vogt, K., Prut, L., Blüthmann, H., Möhler, H., & Rudolph, U. (2002). Trace fear conditioning involves hippocampal α $_5$ GABA $_A$ receptors. *Proceedings of the National Academy of Sciences*, *99*(13), 8980–8985. https://doi.org/10.1073/pnas.142288699

Dai, X., Li, N., Yu, L., Chen, Z., Hua, R., Qin, X., & Zhang, Y.-M. (2015). Activation of BV2 microglia by lipopolysaccharide triggers an inflammatory reaction in PC12 cell apoptosis through a toll-like receptor 4-dependent pathway. *Cell Stress and Chaperones*, *20*(2), 321–331. https://doi.org/10.1007/s12192-014-0552-1

Evered, L., Silbert, B., Knopman, D. S., Scott, D. A., DeKosky, S. T., Rasmussen, L. S., Oh, E. S., Crosby, G., Berger, M., & Eckenhoff, R. G. (2018). Recommendations for the Nomenclature of Cognitive Change Associated with Anaesthesia and Surgery—2018. *Anesthesiology*, *129*(5), 872–879. https://doi.org/10.1097/ALN.0000000000002334

Feng, X., Valdearcos, M., Uchida, Y., Lutrin, D., Maze, M., & Koliwad, S. K. (2017). Microglia mediate postoperative hippocampal inflammation and cognitive decline in mice. *JCI Insight*, *2*(7). https://doi.org/10.1172/jci.insight.91229

Hunkeler, W., Möhler, H., Pieri, L., Polc, P., Bonetti, E. P., Cumin, R., Schaffner, R., & Haefely, W. (1981). Selective antagonists of benzodiazepines. *Nature*, *290*(5806), 514–516. https://doi.org/10.1038/290514a0

Irwin, M. G., Chung, C. K. E., Ip, K. Y., & Wiles, M. D. (2020). Influence of propofol-based total intravenous anaesthesia on peri-operative outcome measures: a narrative review. *Anaesthesia*, *75*(S1). https://doi.org/10.1111/anae.14905

Jevtovic-Todorovic, V., Wozniak, D. F., Powell, S., & Olney, J. W. (2001). Propofol and sodium thiopental protect against MK-801-induced neuronal necrosis in the posterior cingulate/retrosplenial cortex. *Brain Research*, *913*(2), 185–189. https://doi.org/10.1016/S0006-8993(01)02800-1

Kim, J. J., Gharpure, A., Teng, J., Zhuang, Y., Howard, R. J., Zhu, S., Noviello, C. M., Walsh, R. M., Lindahl, E., & Hibbs, R. E. (2020). Shared structural mechanisms of general anaesthetics and benzodiazepines. *Nature*, *585*(7824), 303–308. https://doi.org/10.1038/s41586-020-2654-5

Kobayashi, K., Yoshino, F., Takahashi, S.-S., Todoki, K., Maehata, Y., Komatsu, T., Yoshida, K.-I., & Lee, M.-C.-I. (2008). Direct Assessments of the Antioxidant Effects of Propofol Medium Chain Triglyceride/Long Chain Triglyceride on the Brain of Stroke-prone Spontaneously Hypertensive Rats Using Electron Spin Resonance Spectroscopy. *Anesthesiology*, *109*(3), 426–435. https://doi.org/10.1097/ALN.0b013e318182a903

Koh, M. T., Rosenzweig-Lipson, S., & Gallagher, M. (2013). Selective GABAA α5 positive allosteric modulators improve cognitive function in aged rats with memory impairment. *Neuropharmacology*, *64*, 145–152. https://doi.org/10.1016/j.neuropharm.2012.06.023

Kotani, N., Hashimoto, H., Sessler, D. I., Yasuda, T., Ebina, T., Muraoka, M., & Matsuki, A. (1999). Expression of genes for proinflammatory cytokines in alveolar macrophages during propofol and isoflurane anesthesia. *Anesthesia and Analgesia*, *89*(5), 1250–1256.

Limon, A., Reyes-Ruiz, J. M., & Miledi, R. (2012). Loss of functional $GABA_A$ receptors in the Alzheimer diseased brain. *Proceedings of the National Academy of Sciences*, *109*(25), 10071–10076. https://doi.org/10.1073/pnas.1204606109

Mardini, F., Tang, J. X., Li, J. C., Arroliga, M. J., Eckenhoff, R. G., & Eckenhoff, M. F. (2017). Effects of propofol and surgery on neuropathology and cognition in the 3xTgAD Alzheimer transgenic mouse model. *British Journal of Anaesthesia*, *119*(3), 472–480. https://doi.org/10.1093/bja/aew397

Mason, S. E., Noel-Storr, A., & Ritchie, C. W. (2010). The Impact of General and Regional Anesthesia on the Incidence of Post-Operative Cognitive Dysfunction and Post-Operative Delirium: A Systematic Review with Meta-Analysis. *Journal of Alzheimer's Disease*, *22*(s3), S67–S79. https://doi.org/10.3233/JAD-2010-101086

Moller, J., Cluitmans, P., Rasmussen, L., Houx, P., Rasmussen, H., Canet, J., Rabbitt, P., Jolles, J., Larsen, K., Hanning, C., Langeron, O., Johnson, T., Lauven, P., Kristensen, P., Biedler, A., van Beem, H., Fraidakis, O., Silverstein, J., Beneken, J., & Gravenstein, J. (1998). Long-term postoperative cognitive dysfunction in the elderly: ISPOCD1 study. *The Lancet*, *351*(9106), 857–861. https://doi.org/10.1016/S0140-6736(97)07382-0

Niu, W., Duan, Y., Kang, Y., Cao, X., & Xue, Q. (2022). Propofol improves learning and memory in post-traumatic stress disorder (PTSD) mice via recovering hippocampus synaptic plasticity. *Life Sciences*, *293*, 120349. https://doi.org/10.1016/j.lfs.2022.120349

Rajasekar, N., Nath, C., Hanif, K., & Shukla, R. (2017). Intranasal Insulin Administration Ameliorates Streptozotocin (ICV)-Induced Insulin Receptor Dysfunction, Neuroinflammation, Amyloidogenesis, and Memory Impairment in Rats. *Molecular Neurobiology*, *54*(8), 6507–6522. https://doi.org/10.1007/s12035-016-0169-8

Samir, A., Gandreti, N., Madhere, M., Khan, A., Brown, M., & Loomba, V. (2015). Anti-inflammatory effects of propofol during cardiopulmonary bypass: A pilot study. *Annals of Cardiac Anaesthesia*, *18*(4), 495. https://doi.org/10.4103/0971-9784.166451

Shao, H., Zhang, Y., Dong, Y., Yu, B., Xia, W., & Xie, Z. (2014). Chronic Treatment with Anesthetic Propofol Improves Cognitive Function and Attenuates Caspase Activation in Both Aged and Alzheimer's Disease Transgenic Mice. *Journal of Alzheimer's Disease*, *41*(2), 499–513. https://doi.org/10.3233/JAD-132792

Subramaniyan, S., & Terrando, N. (2019). Neuroinflammation and Perioperative Neurocognitive Disorders. *Anesthesia & Analgesia*, *128*(4), 781–788. https://doi.org/10.1213/ANE.0000000000004053

Walters, J. L., Chelonis, J. J., Fogle, C. M., Orser, B. A., & Paule, M. G. (2016). Single and repeated exposures to the volatile anesthetic isoflurane do not impair operant performance in aged rats. *NeuroToxicology*, *56*, 159–169. https://doi.org/10.1016/j.neuro.2016.07.012

Xu, Z., Dong, Y., Wang, H., Culley, D. J., Marcantonio, E. R., Crosby, G., Tanzi, R. E., Zhang, Y., & Xie, Z. (2014). *Age-dependent postoperative cognitive impairment and Alzheimer-related neuropathology in mice OPEN*. https://doi.org/10.1038/srep03766

Yan, Y., Qiao, S., Kikuchi, C., Zaja, I., Logan, S., Jiang, C., Arzua, T., & Bai, X. (2017). Propofol Induces Apoptosis of Neurons but Not Astrocytes, Oligodendrocytes, or Neural Stem Cells in the Neonatal Mouse Hippocampus. *Brain Sciences*, *7*(12), 130. https://doi.org/10.3390/brainsci7100130

Ye, X., Lian, Q., Eckenhoff, M. F., Eckenhoff, R. G., & Pan, J. Z. (2013). Differential General Anesthetic Effects on Microglial Cytokine Expression. *PLoS ONE*, *8*(1), e52887. https://doi.org/10.1371/journal.pone.0052887

Yin, L., Gao, S., & Li, C. (n.d.). *Exogenous hydrogen sulfide alleviates surgery-induced neuroinflammatory cognitive impairment in adult mice by inhibiting NO signaling*. https://doi.org/10.1186/s12871-019-0927-z

Zhang, C., Li, C., Xu, Z., Zhao, S., Li, P., Cao, J., & Mi, W. (2016). The effect of surgical and psychological stress on learning and memory function in aged C57BL/6 mice. *Neuroscience*, *320*, 210–220. https://doi.org/10.1016/j.neuroscience.2016.02.015

Zhang, H., Tu, X., Chen, Q., & Shi, S. (2019). Propofol Reduces Inflammatory Brain Injury after Subarachnoid Hemorrhage: Involvement of PI3K/Akt Pathway. *Journal of Stroke and*

Cerebrovascular Diseases, *28*(12), 104375. https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.104375

Zhang, W., Xiong, B., Zhang, L., Huang, X., Zhou, W., Zou, Q., Manyande, A., Wang, J., Tian, X., & Tian, Y. (2020). Disruption of the GABAergic system contributes to the development of perioperative neurocognitive disorders after anesthesia and surgery in aged mice. *CNS Neuroscience & Therapeutics*, *26*(9), 913–924. https://doi.org/10.1111/cns.13388

Zhang, Y., Shao, H., Dong, Y., Swain, C. A., Yu, B., Xia, W., & Xie, Z. (2014). Chronic treatment with anesthetic propofol attenuates β-amyloid protein levels in brain tissues of aged mice. *Translational Neurodegeneration*, *3*(1), 8. https://doi.org/10.1186/2047-9158-3-8

Zurek, A. A., Yu, J., Wang, D.-S., Haffey, S. C., Bridgwater, E. M., Penna, A., Lecker, I., Lei, G., Chang, T., Salter, E. W. R., & Orser, B. A. (2014). Sustained increase in α5GABAA receptor function impairs memory after anesthesia. *Journal of Clinical Investigation*, *124*(12), 5437–5441. https://doi.org/10.1172/JCI76669

Figure-1

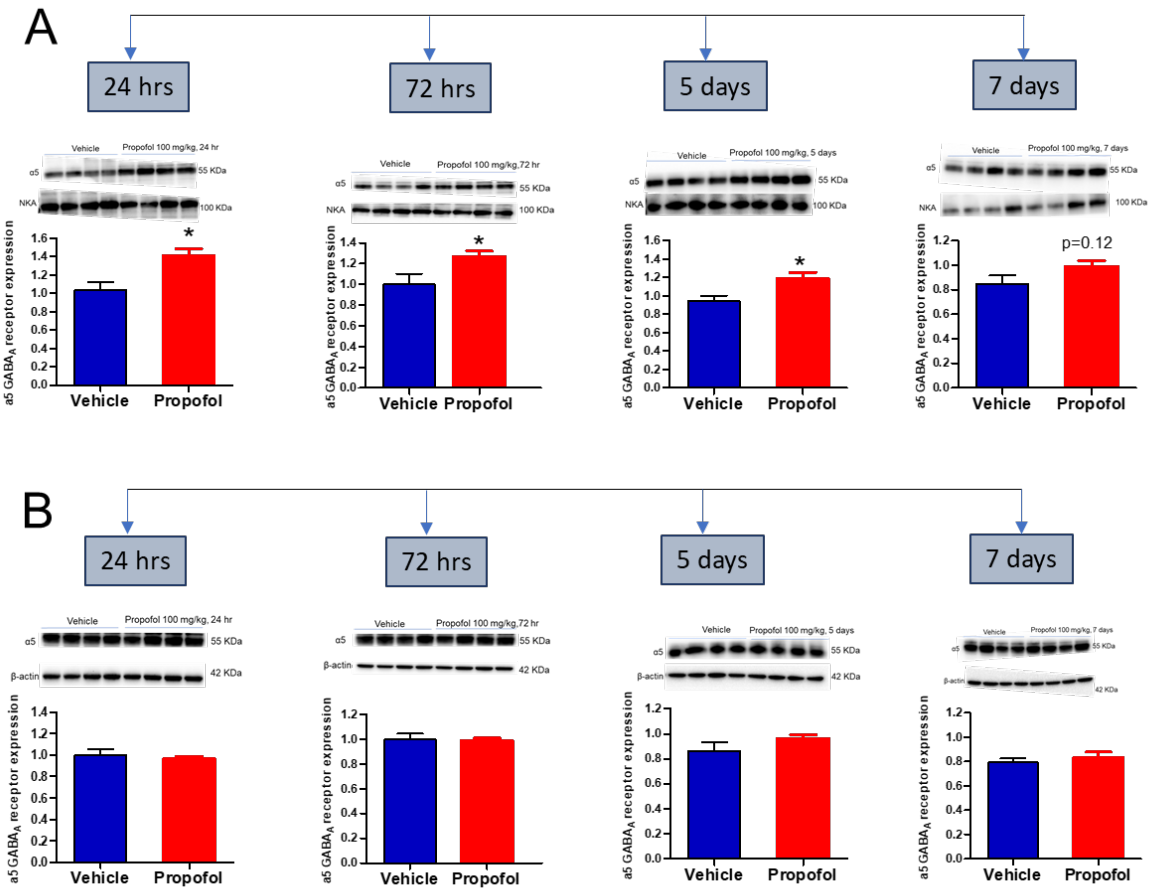

Fig. 1: Time course of cell surface expression of α5-GABA$_A$ receptors in hippocampus after a single dose of propofol. Biotinylation was performed followed by Western blots. The α5-GABA$_A$ receptor expression in cell surface membranes was normalized with Na+/K+ ATPase (NKA) (A), and the α5-GABA$_A$ receptors expression on total membranes was normalized with β-actin (B). Bar graph images showing mean ± S.E.M. of the relative density of protein (n=4). (*) Significant difference (*$p < 0.05$) in comparison to the of vehicle control. MW is shown in kDa.

Figure-2

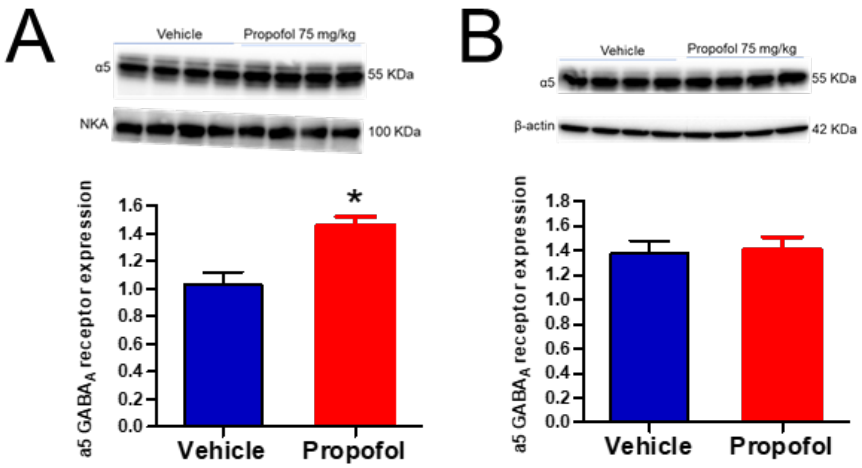

Fig. 2: Cell-surface expression of α5GABA$_A$ receptors in hippocampus after chronic intermittent propofol (CIP) treatment. Animals received either chronic intermittent propofol or vehicle (Intralipid^R) every 5th day for 21 days. The cell surface membrane α5-GABA$_A$ receptor expression was normalized with Na+/K+ ATPase (NKA) **(A),** α5-GABA$_A$ receptors expression with β-actin **(B).** Bar graph images showing mean ± S.E.M. of the relative density of protein (n=4). (*) Significant difference (*$p < 0.05$) in comparison to the of vehicle control. MW is shown in kDa.

Figure-3

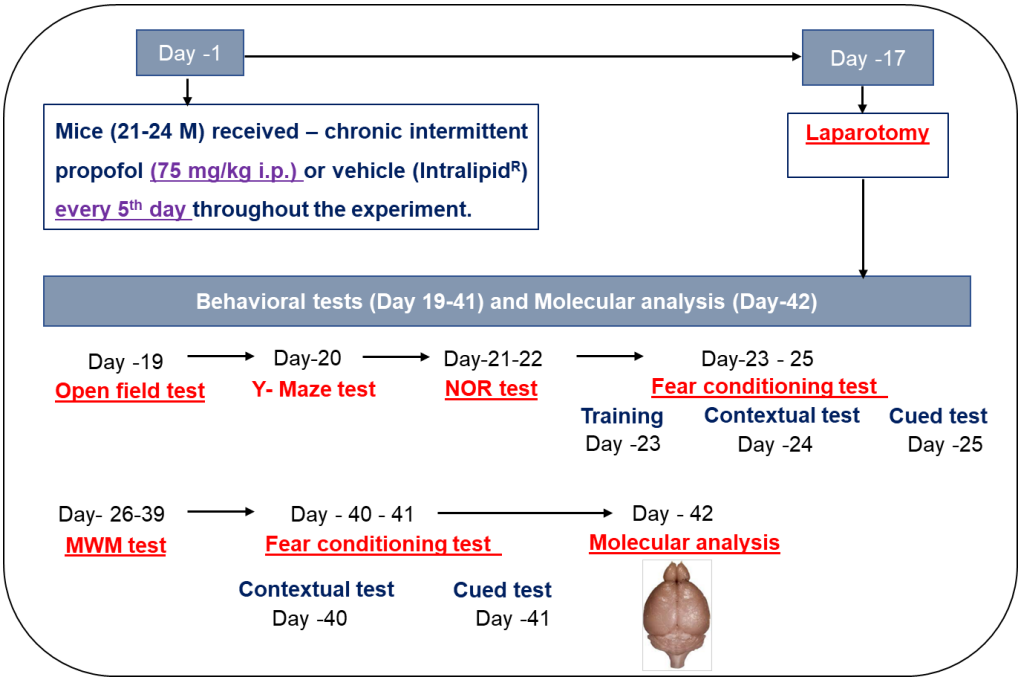

Fig. 3: Schematic overview of the experimental protocol for studying the effects of perioperative chronic intermittent propofol (CIP). NOR - Novel object recognition; MWM - Morris water maze.

Figure-4

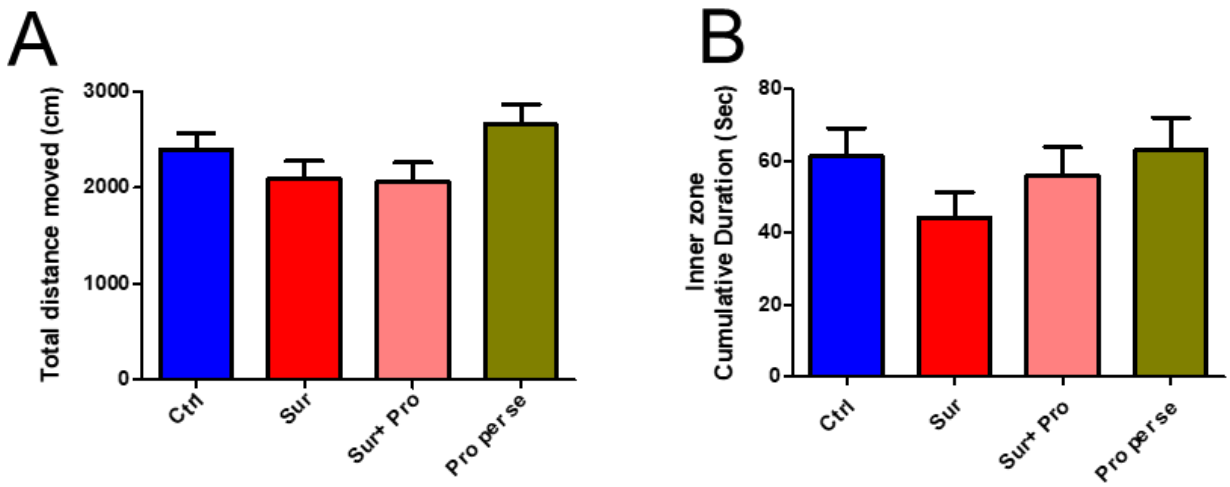

Fig 4: Effects of surgery and CIP in the open field test. A) Total distance moved (cm) and B) cumulative duration in the inner zone (sec) in the open field test. Results are expressed as mean ± SEM .Ctrl – No surgery control; Sur- Surgery; Sur+Pro – Surgery + Propofol; Pro per se – No surgery + Propofol.

Figure-5

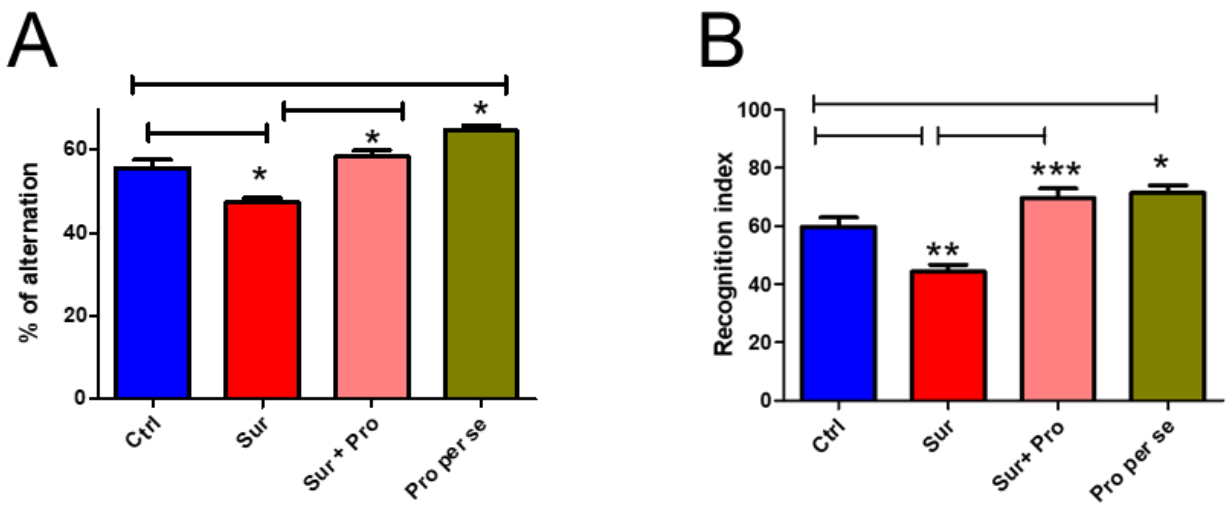

Fig. 5: Effects of surgery and CIP in the Y maze test and the novel object recognition test. A) Spontaneous arm alternation percentage in the Y-maze test and B) Recognition index in the novel object recognition (NOR) test. Results are expressed as mean ± SEM and statistically evaluated by one-way analysis of variance followed by Tukey's Multiple Comparison Test.*$p<0.05$, **$p<0.01$, ***$p<0.001$. Ctrl – No surgery control; Sur- Surgery; Sur+Pro – Surgery + Propofol; Pro per se – No surgery + Propofol.

Figure-6

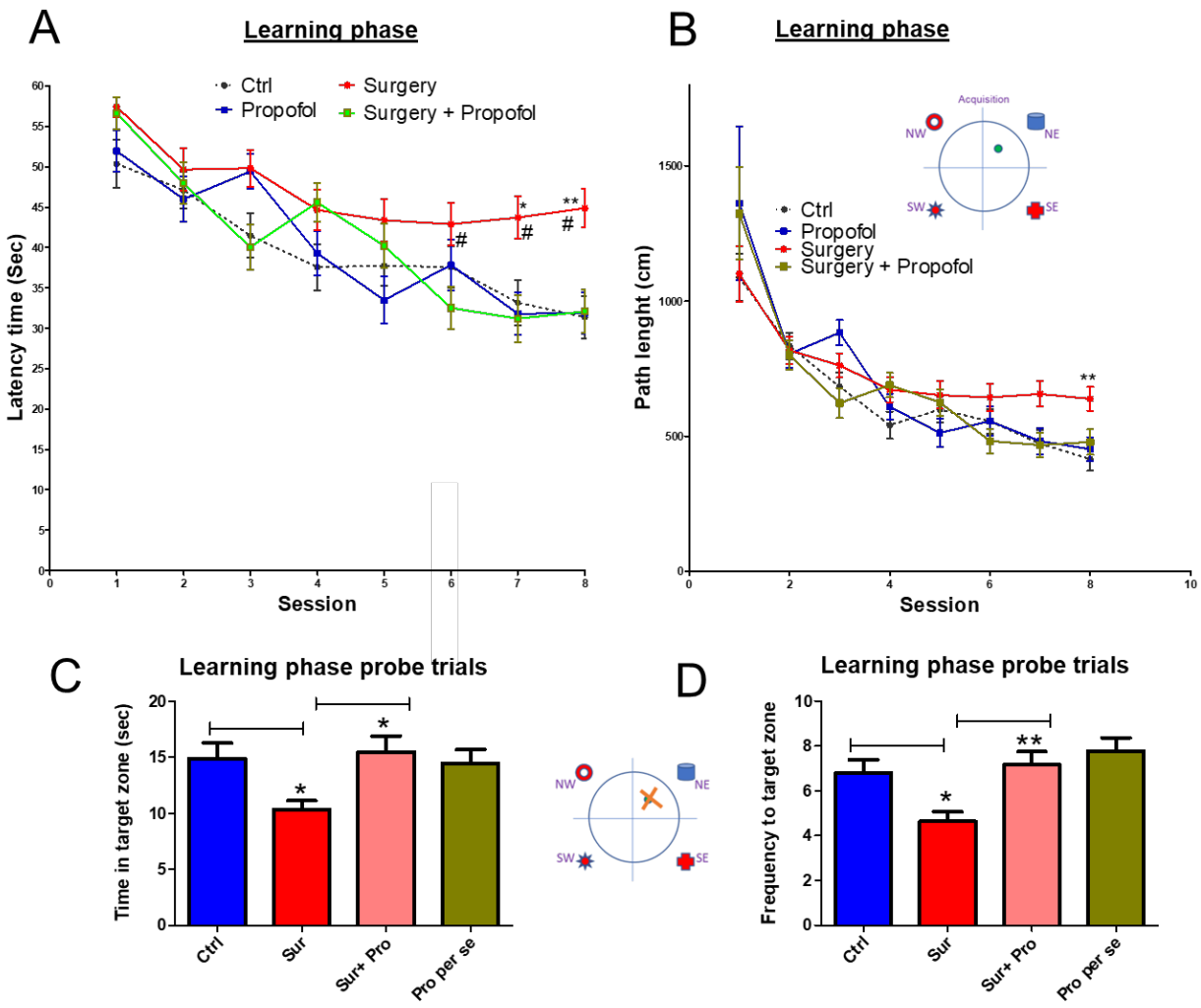

Fig. 6: Effects of surgery and CIP in the learning phase of the Morris water maze task. Morris water maze with learning phase in Sessions 1-8 (day1-8) and learning phase probe trials in session 9 (Day 9). A and B, latency time (sec) and Path length (cm) to the hidden platform in the learning phase. ** $p<0.01$, *$p<0.05$ Surgery vs Ctrl; #$p<0.05$ Surgery vs Surgery + Propofol. C & D, Cumulative duration (Time in target zone) and frequency of entering the target platform area in learning phase probe trials. *$p<0.05$ & **$p<0.01$. Ctrl – No surgery control; Sur- Surgery; Sur+Pro – Surgery + Propofol; Pro per se – No surgery + Propofol.

Figure-7

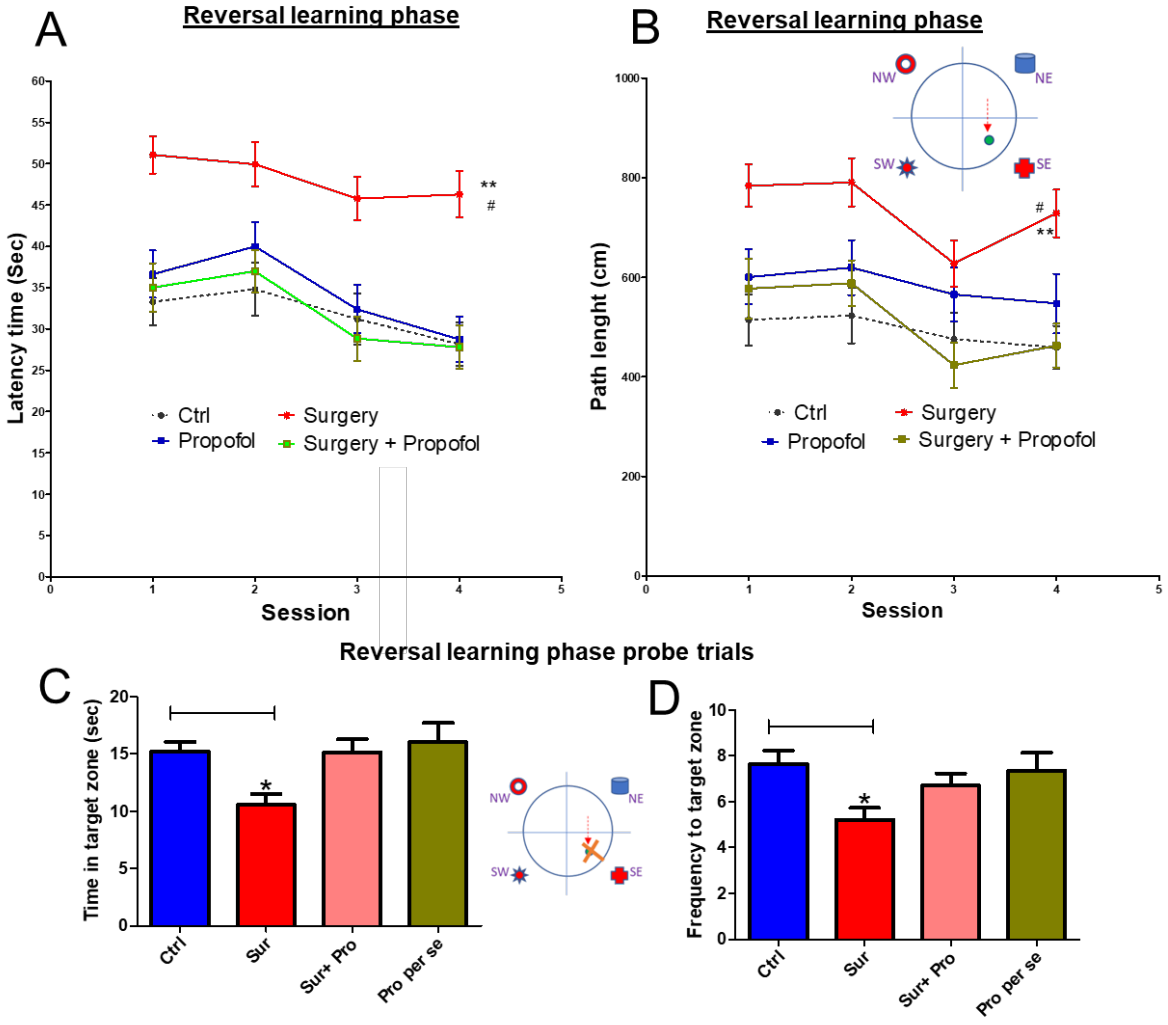

Fig. 7: Effects of surgery and CIP in the reversal learning phase of the Morris water maze task. Morris water maze reversal learning phase with Sessions 1-4 (day 10-13) and reversal learning probe trials (day 14). A and B, latency time (sec) and Path length (cm) to the hidden platform in the reversal learning phase. ** $p<0.01$, *$p<0.05$ Surgery vs Ctrl; #$p<0.05$ Surgery vs Surgery + Propofol. C and D, Cumulative duration (Time in target zone) and frequency to enter the target platform area in the reversal learning phase- probe trials. *$p<0.05$. Ctrl – No surgery control; Sur- Surgery; Sur+Pro – Surgery + Propofol; Pro per se – No surgery + Propofol.

Figure-8

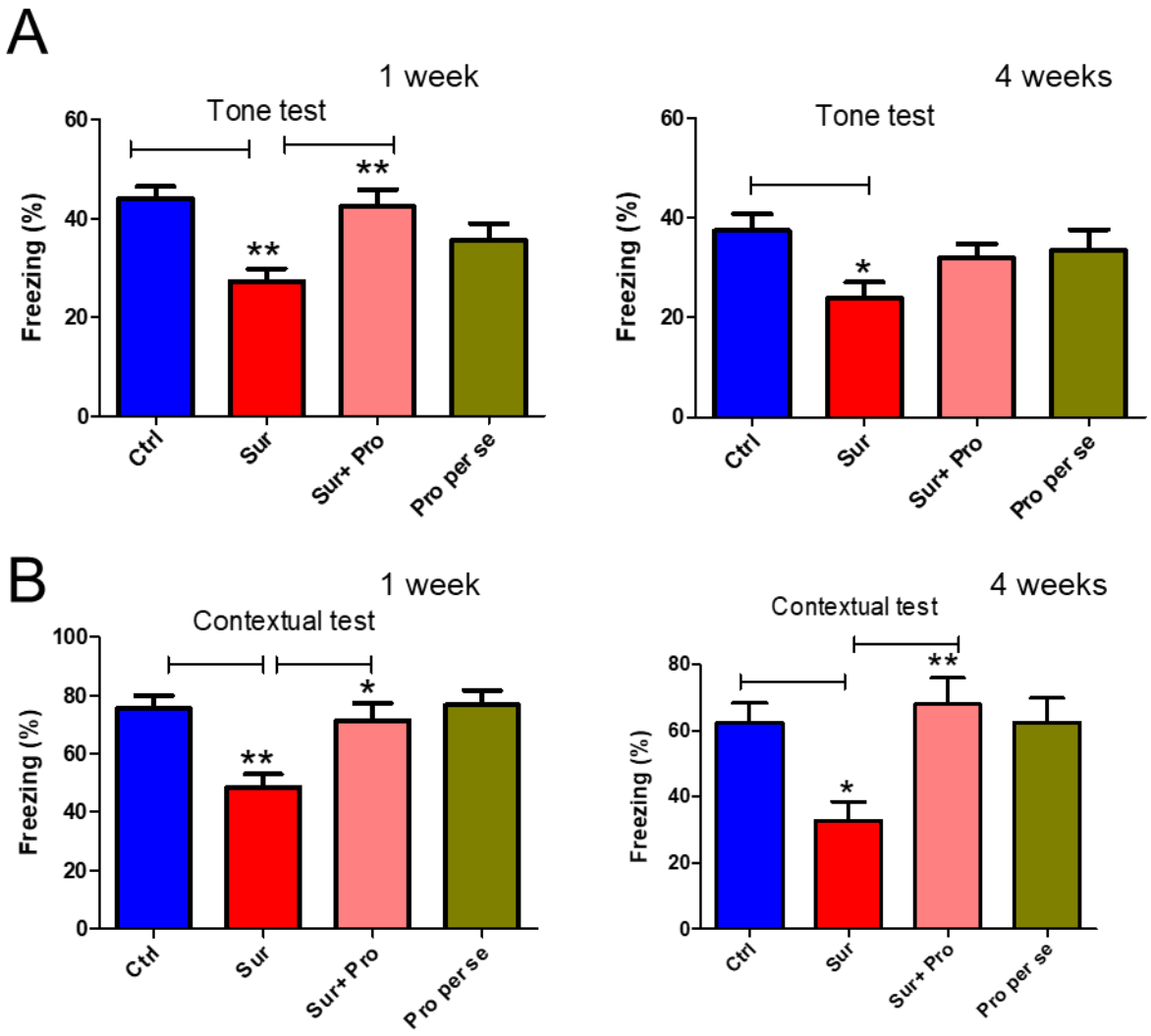

Fig. 8: Effects of surgery and CIP in trace fear conditioning and contextual conditioning. Fear conditioning was performed with recall approximately 1 week and 4 weeks after surgery. (A) Tone test; (B) contextual test. Values are expressed as mean ± SEM . *$p<0.05$ & **$p<0.01$. Ctrl – No surgery control; Sur- Surgery; Sur+Pro – Surgery + Propofol; Pro per se – No surgery + Propofol.

Figure-9

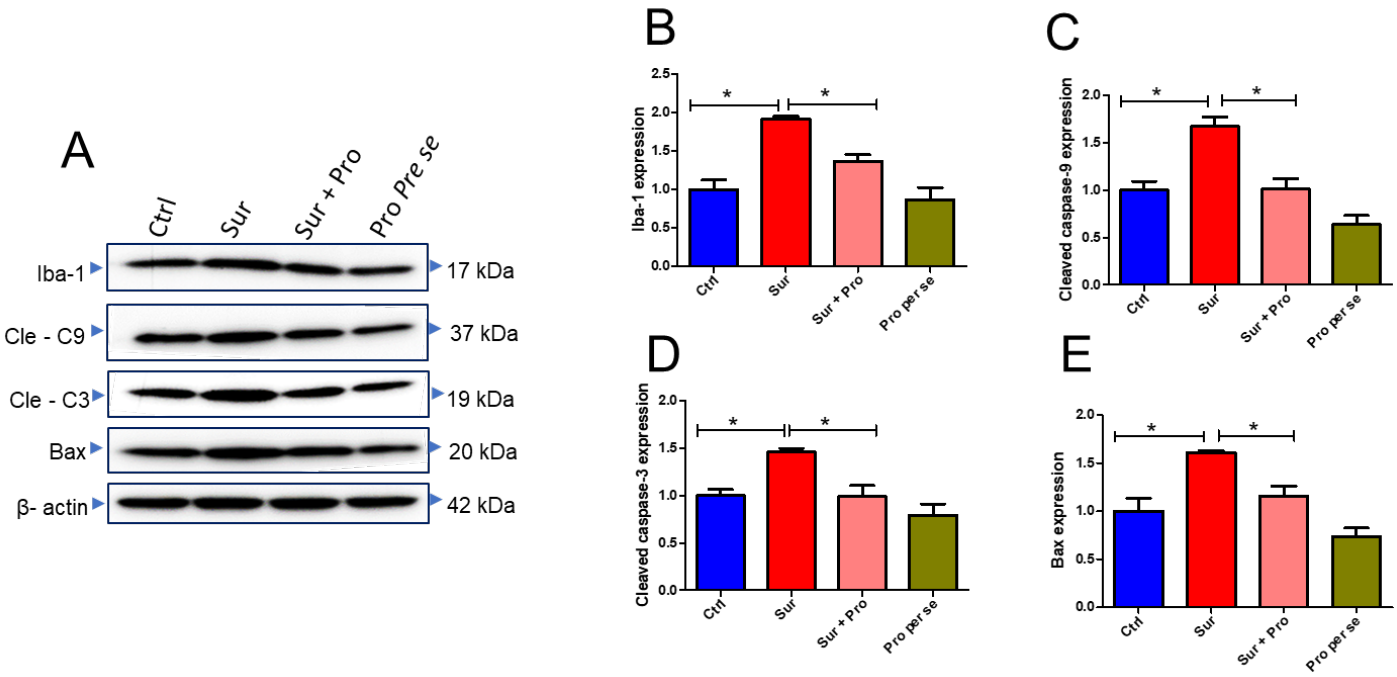

Fig. 9: Effects of surgery and CIP on a microglial activation and apoptotic markers. A) Representative Western blot images of Iba-1, cleaved caspase-9, cleaved caspase-3, Bax and β-actin in different groups of mice. Banding patterns shown are from one out of three experiments. B) Bar graph images B) Iba-1, C) cleaved caspase-9, D) cleaved caspase-3, and E) Bax showing mean ± S.E.M. of relative protein expression. The expression levels were normalized with β-actin. *p<0.05. Cle-C9- cleaved caspase-9; Cle-C3- cleaved caspase-3. Ctrl – No surgery control; Sur- Surgery; Sur+Pro – Surgery + Propofol; Pro per se – No surgery + Propofol.