Propofol Limits Microglial Activation after Experimental Brain Trauma through Inhibition of Nicotinamide Adenine Dinucleotide Phosphate Oxidase

Tao Luo, M.D., Ph.D.,* Junfang Wu, B.M., Ph.D.,† Shruti V. Kabadi, Ph.D.,‡ Boris Sabirzhanov, Ph.D.,§ Kelsey Guanciale, B.S.,∥ Marie Hanscom, B.S.,∥ Juliane Faden, B.A.,∥ Katherine Cardiff, B.S.,∥ Charles Jeremy Bengson, B.S.,∥ Alan I. Faden, M.D.∥

ABSTRACT

Background: Microglial activation is implicated in delayed tissue damage after traumatic brain injury (TBI). Activation of microglia causes up-regulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, with the release of reactive oxygen species and cytotoxicity. Propofol appears to have antiinflammatory actions. The authors evaluated the neuroprotective effects of propofol after TBI and examined in vivo and in vitro whether such actions reflected modulation of NADPH oxidase.

Methods: Adult male rats were subjected to moderate lateral fluid percussion TBI. Effect of propofol on brain microglial activation and functional recovery was assessed up to 28 days postinjury. By using primary microglial and BV2 cell cultures, the authors examined propofol modulation of lipopolysaccharide and interferon-γ–induced microglial reactivity and neurotoxicity.

Results: Propofol improved cognitive recovery after TBI in novel object recognition test (48 ± 6% for propofol [n = 15] vs. 30 ± 4% for isoflurane [n = 14]; P = 0.005). The functional improvement with propofol was associated with limited microglial activation and decreased cortical lesion volume and neuronal loss. Propofol also attenuated lipopolysaccharide- and interferon-γ–induced microglial activation in vitro, with reduced expression of inducible nitric oxide synthase, nitric oxide, tumor necrosis factor-α, interleukin-1β, reactive oxygen species, and NADPH oxidase. Microglial-induced neurotoxicity in vitro was also markedly reduced by propofol. The protective effect of propofol was attenuated when the NADPH oxidase subunit p22phox was knocked down by small interfering RNA. Moreover, propofol reduced the expression of p22phox and gp91phox, two key components of NADPH oxidase, after TBI.

Conclusion: The neuroprotective effects of propofol after TBI appear to be mediated, in part, through the inhibition of NADPH oxidase.
MICROGLIA are the resident macrophage-like cells in the central nervous system and play an important role in the brain's innate immunity and inflammatory responses. Although microglia have essential protective roles, activated microglia can contribute to neuronal cell death through the production of cytotoxic factors, such as nitric oxide, tumor necrosis factor-α (TNF-α), interleukin-1β, and reactive oxygen species (ROS).

Microglial and astroglial activation is prominent after traumatic brain injury (TBI) and contribute to secondary pathophysiological changes. Both clinical and animal studies indicate that TBI causes chronic microglial activation beginning days after injury and continuing for months to years. These activated microglia contribute to chronic neurodegeneration and related behavioral abnormalities after injury.

Recent studies suggested that the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase plays a critical role in the modulation of microglial phenotype and subsequent inflammatory responses. The NADPH oxidase complex is composed of two membrane-bound subunits (p22phox and p91phox), as well as four cytoplasmic subunits (p40phox, p47phox, p67phox, and the small G-protein Rac). Classically activated microglia generate ROS through NADPH oxidase, which likely contributes to delayed tissue damage after TBI. Genetic or pharmacological modulation of NADPH oxidase can attenuate the detrimental consequences of microglial activation.

The intravenous general anesthetic propofol is frequently used in the management of clinical head injury, including for surgical intervention, mechanical ventilation, or diagnostic imaging. Propofol has been shown to modulate various aspects of the host's inflammatory response. In animal models of endotoxemia, propofol reduced mortality rate and organ injury; promoted expression of Annexin A1, a glucocorticoid-dependent antiinflammatory protein; and inhibited activation of p38 mitogen–activated protein kinases and release of inflammatory factors (interleukin-1β, interleukin-6, and TNF-α). These protective effects may therefore reflect its antiinflammatory capacity and antioxidant activity.

In vitro, propofol can protect BV2 microglia cells against lipopolysaccharide-induced inflammation through down-regulation of toll-like receptor 4 expression and inactivation of glycogen synthase kinase-3β. Propofol has also been shown to reduce endotoxic inflammation in vivo and in vitro by inhibiting the interconnected ROS/Akt/IκB kinase β/ nuclear factor-kB signaling pathways. More recently, Ye et al. reported that propofol strongly reduced the responses of BV2 microglia cells to lipopolysaccharide.

In the current study, we examined the antiinflammatory effects of propofol after lateral fluid percussion TBI in rats. In parallel in vitro studies, lipopolysaccharide and interferon-γ models of microglial activation were used in primary microglial cultures and the BV2 murine microglial cell line to investigate the impact of propofol on microglial reactivity and neurotoxicity. We show that propofol administration improves long-term cognitive outcomes after TBI and attenuates microglial-associated inflammation both in vitro and in vivo, at least in part, through inhibition of NADPH oxide.

Materials and Methods

Anesthesia Procedures and Surgical Preparations

All procedures were performed under protocols approved by the University of Maryland School of Medicine Animal Care and Use Committee. Adult male Sprague–Dawley rats weighing 300–340 g (Harlan Laboratories, Indianapolis, IN) were randomly assigned to four groups: isoflurane-TBI, isoflurane-sham, propofol-TBI, or propofol-sham. The animals were anesthetized in an induction chamber saturated with 4% isoflurane (Forene; Abbott Laboratories, Abbott Park, IL) in a supply gas mixture of 70% compressed air and 30% oxygen, intubated, and mechanically ventilated (Harvard Apparatus, Holliston, MA) in oxygen and air (fraction of inspired oxygen = 0.33). A temperature probe was placed into the rectum for monitoring and maintenance of temperature at 37°C on a heating pad. Catheters were inserted into the dorsal tail vein for drug administration and the ventral tail artery for arterial blood pressure monitoring, respectively. Upon completion of the endotracheal intubation, propofol (PropoFlo; Abbott Laboratories) was continuously infused via the tail vein (1.3 mg kg−1 min−1 after a bolus injection of 2 mg/kg) in the propofol group, whereas the rats in the isoflurane group were exposed to 2.5% (2 minimum alveolar concentration in rats). Animals with isoflurane received saline 0.9% (0.13 ml kg−1 min−1) to match the volume of infusion in the propofol group. Propofol infusion at 600–650 μg kg−1 min−1 is approximately 1 minimum alveolar concentration equivalent to isoflurane. The timeline of the in vivo experimental design is shown in figure 1.

Rat Lateral Fluid Percussion Trauma Model

Our custom-designed refined lateral fluid percussion trauma model has been previously described in detail. In brief, a 5-mm craniotomy was created between the lambda and bregma sutures over the left parietal cortex where a femaleleur-loc was cemented in place. An isotonic saline-filled fluid

Anesthesiology 2013; 119:1370-88

Luo et al.
percussion device with a 5-mm tube was attached by means of a male leur-loc fitting. A brief 2.0–2.2-atm pressure pulse was given when a pendulum struck a piston at the opposite end of the device. This procedure results in consistent, moderate brain injury of parietal cortex as previously delineated.22 Sham animals received a similarly located craniotomy but no percussion trauma.

Morris Water Maze Tests
Spatial learning and memory was assessed using the acquisition paradigm of the standard Morris water maze (MWM) test on postinjury days (PIDs) 14, 15, 16, and 17 as previously detailed by us.23,24 The MWM protocol included hidden platform training (acquisition) and standard probe test. A white circular pool was divided into four quadrants using the computer-based AnyMaze video tracking system (Stoelting Co., Wood Dale, IL). The maze was surrounded by various distinct extra-maze cues on the walls of the room. A transparent platform was submerged 5 cm below the surface of the opaque water. Spatial learning and memory performance was assessed by determining the latency (in seconds) to locate the submerged hidden platform with a 90-s limit per trial for 4 consecutive days (PID, 14–17). Reference memory was assessed by a probe trial carried out on PID 18, as the time spent (in seconds) with a 60 s limit in the quadrant where the platform had been hidden during the acquisition phase.

Novel Object Recognition Test
Novel object recognition was conducted on PID 21 to evaluate retention or intact memory and exploratory behavior as previously detailed by us.24–27 The apparatus consists of an open field (40 × 80 cm²) with two adjacent located imaginary circular zones. The zones are equally spaced from the sides in the center of the square and designated as “old object” and “novel object” zones, using the AnyMaze video tracking system (Stoelting Co., Wood Dale, IL). Two 5-min trials were performed. The first (training) trial was performed with two old objects in both zones and the second (testing) trial with one old object and one novel object present in the respective zones of the open field. There was an intertrial interval of 1 h, during which the animals were returned to their home cages. The time spent with each object was recorded manually, and the cognitive outcomes were determined as the “discrimination index” for the second trial, which was calculated using the following formula: % discrimination index = Time spent in novel object zone × 100/(time spent in old object zone + time spent in novel object zone).25

Tissue Processing and Immunohistochemistry
Animals were anesthetized with sodium pentobarbital (100 mg/kg, intraperitoneal injection) on PID 28 and transcardially perfused with 200 ml of 0.9% saline followed by 300 ml of 4% paraformaldehyde. The brain was removed and postfixed in 4% paraformaldehyde overnight and cryoprotected in 30% sucrose. Coronal sections were cut and serially collected throughout the injured brain. Standard fluorescent immunohistochemistry was performed on 20 μm sections. The following primary antibodies were used: rabbit anti-Iba-1 (1:1,000; Wako Chemicals, Richmond, VA), and mouse anti-gp91phox (1:200; BD Transduction Laboratories, Franklin Lakes, NJ). Counterstaining was performed with 4’, 6-diamidino-2-phenylindole (1 μg/ml; Sigma-Aldrich, St. Louis, MO). Fluorescence microscopy was performed using a Leica (TCS SP5 II) confocal microscope system (Leica Microsystems Inc., Buffalo Grove, IL).

Lesion Volume and Neuronal Number Assessment
Sections were stained with cresyl violet (FD NeuroTechnologies, Baltimore, MD), dehydrated, and mounted for analysis (n = 6–8 per group). Lesion volume was quantified based on the Cavalieri method of unbiased stereology using Stereolo- gizer 2000 program software (Systems Planning and Analysis, Alexandria, VA). The lesion volume was quantified by outlining the missing tissue on the injured hemisphere using the Cavalieri estimator with a grid spacing of 0.1 mm. From 96 total 60-μm sections, every fourth section was analyzed beginning from a random start point.

The total number of surviving neurons was quantified in the cortex, thalamus, CornuAmmonis 1–3, and dentate gyrus subregions of the hippocampus using the optical fractionator method of unbiased stereology. The optical dissector had a size of 50 μm by 50 μm in the x- and y-axes, respectively, with a height of 10 μm and guard zone of 4 μm from the top of the section. A grid spacing of 400 μm in the x-axis and 400 μm in the y-axis was used, resulting in an area fraction of one-sixty-fourth. The estimated number of surviving neurons in each field was divided by the volume of the region of interest to obtain the neuronal cellular density, expressed as counts/mm³.

Stereological Assessment of Cortical Microglia
Brain sections were obtained as described above and immunostained for microglia marker ionized calcium-binding adapter molecule 1 (Iba-1). Stereoinvestigator software (MBF Biosciences, Williston, VT) was used to count the number of cortical microglia in each of the three microglial morphologic phenotypes (namely ramified, hypertro- phic, and bushy) using the optical fractionator method of unbiased stereology.4,28 The sampled region was the ipsilateral cortex between −1.22 mm and −2.54 mm from the bregma and dorsal to a depth of 2.0 mm from the surface. Every fourth 60-mm section was analyzed beginning from a random start point. Sections were analyzed using a Leica DM4000B microscope (Leica: Leica Microsystems Inc.). The optical dissector had a size of 50 × 50 mm² in the x- and y-axes with a height of 10 mm and guard zone of 4 mm from the top of the section. Dissectors were positioned every 150 mm in the x- and y-axes. Microglial phenotypic classification was based on the length and thickness of the
projections, the number of branches, and the size of the cell body as described previously. The volume of the region of interest was measured using the Cavalieri estimator method with a grid spacing of 150 μm for the cortex. The estimated number of microglia in each phenotypic class was divided by the volume of the region of interest to obtain cellular density expressed in counts/mm³.

Western Blot

At 7 days after injury, a 5-mm area surrounding the lesion epicenter on the ipsilateral cortex was rapidly dissected and stored at −80°C until processing. The samples were lysed in Radio-Immunoprecipitation Assay buffer and centrifuged at 15,000 rpm for 10 min at 4°C. The supernatant was removed, and protein concentration was determined using the Pierce bicinchoninic acid Protein Assay kit (Thermo Scientific, West Palm Beach, FL) with a bovine serum albumin standard. Each sample contains proteins from one animal. Equal amounts of protein were electrophoretically separated on 4–12% NuPAGE Novex Bis-Tris gradient gels (Invitrogen, Grand Island, NY) and transferred to nitrocellulose membranes (Invitrogen). After blocking in 5% nonfat milk for 1 h at room temperature, membranes were incubated with respective antibodies overnight at 4°C followed by horseradish peroxidase–conjugated secondary antibodies (GE Healthcare, Pittsburgh, PA) for 1.5 h at room temperature. The immunoreactivity was detected using SuperSignal West Dura Extended Duration Substrate (Thermo Scientific) and quantified by band densitometry of scanned films using the Gel-Pro Analyzer program (Media Cybernetics, Inc., Rockville, MD). Some blots were further stripped in a stripping buffer (Thermo Scientific) for 45 min at 55°C. The loading and blotting of equal amounts of protein were verified by reprobing the membrane with antiglyceraldehyde 3-phosphate dehydrogenase (Chemicon, Billerica, MA).

Microglial Cell Cultures

Primary microglia were cultured from the cerebral cortices of 1- to 3-day-old rat pups as described. In brief, the cerebral cortices were dissected, chopped, triturated, and plated on tissue culture flasks that had been coated with poly-D-lysine (50 ng/ml; Sigma-Aldrich). The cells were grown in Dulbecco’s Modified Eagle’s Medium/F12 (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), 1% penicillin/streptomycin at 37°C with 5% CO₂. When the cells had grown to confluence, the flask were shaken at 100 rpm for 1 h at 37°C to isolate microglia. The immortalized murine BV2 microglial cells were grown and maintained in Dulbecco’s Modified Eagle’s Medium supplemented with 10% fetal bovine serum at 37°C in a 5% CO₂ incubator.

Drug Treatments

Propofol (12.5–200 μM; Sigma-Aldrich) reconstituted in 0.02% dimethylsulfoxide (final concentration) was applied to microglia for 1 h before lipopolysaccharide (10–50 ng/ml; Sigma-Aldrich) or recombinant mouse interferon-γ (0.5 ng/ml; R&D Systems, Minneapolis, MN) stimulation. The γ-aminobutyric acid (GABA) A receptor antagonist picrotoxin or bicuculline (500 μM; Sigma-Aldrich) was administered 30 min before propofol administration. All drugs were prepared and stored according to the manufacturer’s instructions.

NADPH Oxidase Activity Assay

NADPH oxidase activities were measured by a spectrophotometric assay of cytochrome c reduction. In brief, BV2 cells were pretreated with propofol followed by coincubation with lipopolysaccharide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich) or recombinant mouse interferon-γ (0.5 ng/ml; R&D Systems, Minneapolis, MN) stimulation. The γ-aminobutyric acid (GABA) A receptor antagonist picrotoxin or bicuculline (500 μM; Sigma-Aldrich) was administered 30 min before propofol administration. All drugs were prepared and stored according to the manufacturer’s instructions.
with lipopolysaccharide for 4 h. The membrane proteins were extracted from treated cells and the membrane fractions (50 μg) diluted in Dulbecco’s Modified Eagle’s Medium without phenol red were distributed in 96-well culture plates. Cytochrome c (100 mM; Sigma-Aldrich) and NAPDH (100 mM; Sigma-Aldrich) were added in the presence or absence of superoxide dismutase (200 units/ml; Sigma-Aldrich) and incubated at 37°C for 30 min. Cytochrome c reduction was measured by reading absorbance at 550 nm on the microplate reader. The relative NAPDH oxidase activity was expressed as a percentage of the untreated control.

**Neurotoxicity Assay**

Rat primary cortical neuronal cultures were derived from E18 rat cortices as previously described. At 24 h after stimulation, the medium from the primary cultured microglia was removed and the cells were grown in fresh media for 48 h. The conditioned media harvested from the stimulated microglia was added to primary cortical neurons for an additional 24 h. Cell death was measured by lactate dehydrogenase release assay (CytoTox96TM nonradioactive cytotoxicity assay; Promega, Madison, WI) according to the manufacturer’s instructions. Data are presented as a percentage of control-treated values.

**Transient Transfection with Small Interfering RNA**

Transfection of cells with small interfering RNA (siRNA) against p22-phox, gp91-phox, and control-siRNA (Santa Cruz Biotechnology, Inc., Dallas, TX) was performed using the Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer’s instructions. In brief, subconfluent BV2 cells grown in antibiotic-free medium were transfected with 1.2 pmol of siRNA per well for 96-well plate and with 50 pmol for 60-mm dish, respectively. After 6 h of transfection, cells were washed and pretreated with propofol for 1 h, stimulated with lipopolysaccharide, and cultured for an additional 24 h for nitric oxide and TNF-α assay. Gene knockdown was verified by Western blotting.

**Statistical Analysis**

Data obtained from independent measurements are presented as the mean ± SEM. For the acquisition trials of the MWM test, repeated-measures one-way ANOVAs between-subjects were conducted, followed by multiple pairwise comparisons between groups using the Student–Newman–Keuls post hoc test. One-way ANOVA analysis followed by Student–Newman–Keuls post hoc test or two-tailed unpaired Student t test was performed for the other behavioral tests, Western blotting, microglial phenotype, and in vitro assays. Statistical analysis was performed using SigmaPlot Program, Version 12 (Systat Software, San Jose, CA) or GraphPad Prism software, version 4.00 for windows (GraphPad Software, Inc., San Diego, CA). Differences were considered significant at a P value of less than 0.05.

**Results**

**Animal Physiology**

There were no significant differences in body weight and duration of surgery among the four groups. The hemodynamic parameters from all groups of animals including intraoperative heart rate and mean arterial blood pressure remained ±20% of the baseline values in each study group, respectively; there were no significant differences between groups (table 1). The average values of rectal temperature and SpO2 were normal in all groups for the duration of the surgery.

**Propofol Anesthesia Improves Cognitive Recovery and Reduces Lesion Size after TBI**

To evaluate the neuroprotective potential of propofol anesthesia against TBI-induced cognitive functional impairments, rats were administrated propofol by intravenous injection, and 2.5% isoflurane anesthesia served as a control. As there were no significant differences across all tests between propofol- and isoflurane-anesthetized sham animals, the data shown for the sham group are combined in order to minimize the use of animals. Spatial learning and memory was tested using the acquisition phase of MWM test. Fluid percussion resulted in learning impairments on days 15, 16, and 17 postinjury (fig. 2A). The factors of “PIDs (F(3,152) = 33.6; P < 0.001) and groups (F(2,152) = 7.943; P < 0.001)” were found to be statistically significant after performing repeated-measures one-way ANOVA between-subjects. However, the interaction

<p>| Table 1. Intraoperative Physiological Parameters in Animals Anesthetized with Isoflurane or Propofol |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>MAP</th>
<th>Start Surgery</th>
<th>Craniotomy</th>
<th>Before LFP</th>
<th>After LFP</th>
<th>End of Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane</td>
<td>88 ± 2</td>
<td>91 ± 1</td>
<td>93 ± 2</td>
<td>93 ± 1</td>
<td>91 ± 1</td>
</tr>
<tr>
<td>Propofol</td>
<td>86 ± 2</td>
<td>94 ± 2</td>
<td>95 ± 2</td>
<td>95 ± 1</td>
<td>92 ± 1</td>
</tr>
<tr>
<td>HR</td>
<td>Isoflurane</td>
<td>312 ± 8</td>
<td>308 ± 6</td>
<td>306 ± 6</td>
<td>301 ± 7</td>
</tr>
<tr>
<td>Propofol</td>
<td>306 ± 8</td>
<td>315 ± 7</td>
<td>308 ± 9</td>
<td>302 ± 8</td>
<td>294 ± 10</td>
</tr>
</tbody>
</table>

Shown as mean ± SEM (N = 13 for isoflurane and n = 14 for propofol). Analysis was performed by repeated-measures two-way ANOVA between-subjects, followed by Student–Newman–Keuls post hoc test, P > 0.05.

HR = heart rate; LFP = lateral fluid percussion; MAP = mean arterial pressure.
of “PIDs X groups” \((F(6,152) = 1.107; P = 0.361)\) was not statistically significant. The mean escape latency on the last day of training was 35.5 ± 5.1 s for the sham-injured \((n = 12)\) group, 53.1 ± 7.1 s for the TBI-propofol \((n = 15)\), and 61.7 ± 5.9 s for TBI-isoflurane \((n = 14)\) group, respectively.

Reference memory was assessed using the MWM probe trial on day 18 after injury. The average time spent in the target quadrant was 26.8 ± 2.0 s for sham, 21.2 ± 1.7 s for TBI-propofol rats, and 18.1 ± 1.3 s with TBI-isoflurane, respectively. TBI caused significant cognitive impairments in both propofol and isoflurane groups when compared with sham-injured rats (fig. 2B; \(P = 0.003\) isoflurane vs. sham and \(P = 0.024\) propofol vs. sham). No statistically significant differences were observed between the two injured groups.

Retention or intact memory after TBI was assessed by the novel object recognition test on PID21. As shown in figure 2C, during the training phase, animals from both sham injury and TBI showed an equal preference to the identical objects. During the test phase, sham-injured rats showed the predicted preference for the novel object. In contrast, TBI rats had reduced preference to the novel object, and the discrimination index was 65 ± 3% for sham \((n = 12)\), 30 ± 4% for isoflurane \((n = 14)\), and 48 ± 6% for propofol \((n = 15)\), respectively (fig. 2C; \(P < 0.001\) for TBI-isoflurane and \(P = 0.012\) TBI-propofol vs. sham, one-way ANOVA analysis followed by Student–Newman–Keuls post hoc test). Propofol anesthesia caused significant increase in the discrimination index (fig. 2C; \(P = 0.005\), TBI-propofol vs. TBI-isoflurane, one-way ANOVA analysis followed by
Student–Newman–Keuls post hoc test), indicating improvement in retention memory performance.

TBI-induced lesion volume was measured by unbiased stereological techniques (fig. 2D). Histological assessment showed that propofol anesthesia (6.14 ± 1.36 mm³, n = 8) resulted in a significant reduction in lesion size after TBI as compared with isoflurane (11.99 ± 1.42 mm³; n = 6; \( P = 0.0125 \) vs. propofol, two-tailed unpaired Student t test).

**Propofol Reduces Neuronal Cell Loss in the Cortex, but Not in the Hippocampus and Thalamus after TBI**

To evaluate the differences in neuropathology between the injured groups treated with propofol and isoflurane, we further performed a quantitative assessment of neuronal cell loss in various brain regions: cortex (A), hippocampal subregions (CA1 (C), CA2/3 (D), and DG (E)), thalamus (B), TBI resulted in significant neuronal cell loss in the cortex, thalamus, and hippocampus. Propofol significantly improved neuronal survival in the cortex (310,081 ± 26,616 vs. 191,315 ± 16,679 counts/mm³ for propofol-treated [n = 5] and isoflurane-treated [n = 7] samples, respectively; \( P < 0.05 \) vs. TBI-isoflurane). However, there were no statistically significant differences between the injured treatment groups with respect to neuronal survival in the thalamus or hippocampus. Analysis by one-way ANOVA followed by post hoc adjustments using Student–Newman–Keuls test. Mean ± SEM. * \( P < 0.05 \), ** \( P < 0.01 \), *** \( P < 0.001 \) versus sham; # \( P < 0.05 \) versus isoflurane. CA = CornuAmmonis; DG = dentate gyrus.

**Propofol Suppresses Microglial Activation after TBI**

To examine whether the observed behavioral improvement may be related to reduced inflammation, Western blotting for inflammatory markers Iba-1 and ED1 and stereological assessment for microglial phenotypes were performed in ipsilateral-injured rat cerebral cortex after TBI. At 7 days after TBI, Western blot analysis of ED1 protein expression, a marker for activated phagocytic microglia, indicated a 6.4-fold increase in injured cortex extracts from TBI-isoflurane group compared with sham tissue (fig. 4, A and B). In the propofol-treated samples, ED1 expression was increased to a 1.7-fold of sham, but significantly \( (P < 0.001; \) TBI-propofol vs. TBI-isoflurane; \( n = 4; \) one-way ANOVA analysis followed by Student–Newman–Keuls post hoc test) reduced compared with TBI-isoflurane group. TBI also significantly increased expression of Iba-1 as compared with sham (fig. 4, A and C); these changes were attenuated by propofol treatment.

Stereological assessment of microglial phenotypes in the cerebral cortex was performed at 28 days post-TBI. Brain injury results in a switch from a resting ramified microglia to more activated forms displaying hypertrophic or bushy
Fig. 4. Propofol attenuates microglia activation after traumatic brain injury (TBI). (A–C) Western blot analysis showed a significant increase in microglia markers ED1 and Iba-1 at 7 days after fluid percussion injury. Propofol-treated (n = 4) TBI tissue had significantly reduced ED1 and Iba-1 expression when compared with isoflurane-treated (n = 4) TBI tissue. (D) Representative Iba-1 immunohistochemical images displaying ramified, hypertrophic, and bushy microglial phenotypes based on cellular morphological features. (E) Unbiased stereological quantification of microglial cell number and activation phenotype in each group 28 days postinjury. Propofol-treated TBI tissue had significantly reduced numbers of bushy microglia when compared with isoflurane-treated TBI tissue, N = 4 rats per group. *P < 0.05, **P < 0.01, ***P < 0.001 versus sham; #P < 0.05, ##P < 0.01, ###P < 0.001 versus isoflurane. Statistical analyses were performed by one-way ANOVA followed by Student–Newman–Keuls post hoc test. GAPDH = glyceraldehyde-3-phosphate dehydrogenase; Iba-1 = ionized calcium-binding adapter molecule 1.
Propofol reduces microglial activation after TBI

Luo et al.

Propofol attenuates the production of lipopolysaccharide (LPS)-stimulated proinflammatory mediators and microglia-mediated neurotoxicity in vitro. (A–E) Propofol pretreatment dose dependently attenuated LPS-induced production of (A) nitric oxide (NO), (B) reactive oxygen species (ROS), (C) tumor necrosis factor-α (TNF-α), (D) interleukin (IL)-1β, and (E) cell proliferation in primary rat microglial cells. N = 6 independent measurements. (F) Conditioned medium from LPS-stimulated microglia induced increased release of lactate dehydrogenase (LDH) in embryonic cortical neuronal. Pretreatment of microglia with propofol before LPS stimulation and addition of conditioned media to neurons resulted in reduced cell death. N = 6 independent measurements. (G–I) LPS stimulation also increased Iba-1 and inducible NO synthase (iNOS) expression in primary cortical microglia, and propofol pretreatment significantly reduced Iba-1 and iNOS expression after 24 h. N = 4 independent measurements. *P < 0.05, **P < 0.01, ***P < 0.001 versus LPS group. Statistical analyses were performed by one-way ANOVA with Student–Newman–Keuls post hoc test. GAPDH = glyceraldehyde-3-phosphate dehydrogenase; Iba-1 = ionized calcium-binding adapter molecule 1.

morphologies. Representative images of the ramified (small cell body with elongated and thin projections), hypertrophic (large cell body with shorter and thicker projections), and bushy (multiple short processes that form thick bundles around enlarged cell bodies) are presented (fig. 4D). No significant differences were observed in the number of ramified
and hypertrophic microglia across the groups. Activated microglial bushy phenotypes were significantly increased in both TBI-propofol and TBI-isoflurane animals compared with sham group. Notably, propofol significantly reduced the number of bushy microglial phenotypes compared with TBI-isoflurane group (fig. 4E; \( P < 0.001 \); propofol vs. isoflurane; \( n = 4 \); one-way ANOVA analysis followed by Student–Newman–Keuls post hoc test).

**Propofol Reduces Lipopolysaccharide-stimulated Primary Microglia Activation and Neurotoxicity**

Lipopolysaccharide- or interferon-\( \gamma \)-induced microglia activation in vitro has been extensively used as complementary tools to investigate the mechanisms underlying microglial activation in central nervous system injury. To determine whether propofol exerted a direct effect on microglia-mediated neuroinflammation, rat primary microglial cells were cultured in 96-well plates, and propofol was added alone or in combination with lipopolysaccharide. Pretreatment with propofol (50–200 \( \mu M \)) for 1 h before additional 24 h of lipopolysaccharide (50 ng/ml) stimulation reduced the expression of several independent markers of microglial activation, including ROS, nitric oxide, cell proliferation, TNF-\( \alpha \), and interleukin-1\( \beta \) (fig. 5, A–E). These effects of propofol were dose dependent, with maximal actions at 200 \( \mu M \). The cell viability remained above 100% of control value with incubation of propofol alone, and propofol by itself had no effect on nitric oxide, ROS, TNF-\( \alpha \), and interleukin-1\( \beta \) release.

Addition of activated microglia to neuronal cultures is known to induce neuronal cell death. In order to determine whether propofol affects microglia-induced neurotoxicity, conditioned medium prepared from lipopolysaccharide-treated microglia with or without propofol was applied to cultured cerebral cortical neurons and the neuronal cell death was assessed by determining lactate dehydrogenase release. As shown in figure 5F, when conditioned medium from lipopolysaccharide-stimulated microglia was added to cultured neurons, lactate dehydrogenase release was significantly increased after 24 h. However, pretreatment of microglia with propofol before lipopolysaccharide stimulation and addition of conditioned medium to neurons significantly reduced neuronal cell death (fig. 5F; \( P = 0.002 \) vs. lipopolysaccharide; \( n = 6 \); one-way ANOVA analysis followed by Student–Newman–Keuls post hoc test). Neither lipopolysaccharide nor propofol had any direct effect on neuronal viability (data not shown).

Western blot analysis of Iba-1 and inducible nitric oxide in primary microglia also showed that pretreatment with propofol resulted in a dose-dependent reduction of Iba-1 and inducible nitric oxide expression (fig. 5, G–I). Thus, propofol limits the effects of lipopolysaccharide on microglia activation.

**Propofol Attenuates Lipopolysaccharide- and Interferon-\( \gamma \)-induced Microglial Activation in BV2 Cells**

BV2 cells are derived from raf/myc-immortalized murine neonatal microglia and are the most frequently used substitute for primary microglia; their response pattern parallels that of primary microglia. The antiinflammatory effects of propofol were therefore further tested in BV2 cells. Propofol, at clinically relevant concentrations, showed a significant suppression of nitric oxide, ROS, and TNF-\( \alpha \) production induced by lipopolysaccharide 10 ng/ml (fig. 6, A–C). Furthermore, when BV2 microglia were stimulated with 0.5 ng/ml interferon-\( \gamma \), pretreatment with propofol reduced in a dose-dependent manner the expression of several independent markers of microglial activation—including nitric oxide, TNF-\( \alpha \), and ROS (fig. 7, A–C).

**Propofol Reduces Microglial Activation via Inhibition of NADPH Oxidase Activity, but Not the GABA\(_{A}\) Receptor**

Activation of NADPH oxidase is a major mechanism for intracellular ROS production and subsequent inflammatory responses.\(^7,8\) To determine whether changes in NADPH oxidase activity might be related to the observed changes in proinflammatory factors by propofol, the enzymatic activity of NADPH oxidase was assessed in lipopolysaccharide-stimulated BV2 microglia, with or without pretreatment with propofol. Lipopolysaccharide caused a 1.8-fold increase in NADPH oxidase activity after 4 h of stimulation compared with the control cells (fig. 8A), which was significantly decreased upon propofol treatment (fig. 8A; \( P = 0.005 \) vs. lipopolysaccharide; \( n = 6 \); one-way ANOVA analysis followed by Student–Newman–Keuls post hoc test).

Activation of the NADPH oxidase requires membrane translocation of the cytosolic proteins to assemble with the membrane-spanning catalytic subunit flavocytochrome b558, which is composed of gp91\( \text{phox} \) and p22\( \text{phox} \), to form the active system. We next determined the expression of the membrane subunits of the NADPH oxidase complex, p22\( \text{phox} \) and gp91\( \text{phox} \), after lipopolysaccharide stimulation in primary microglia. Western blot analysis showed an increase in p22\( \text{phox} \) and gp91\( \text{phox} \) at 24 h after exposure to lipopolysaccharide (fig. 8, B–D). The expression of p22\( \text{phox} \) and gp91\( \text{phox} \) was significantly reduced with propofol treatment (fig. 8, B–D), suggesting that propofol may inhibit production of proinflammatory factors by propofol, the enzymatic activity of NADPH oxidase was assessed in lipopolysaccharide-stimulated BV2 microglia, with or without pretreatment with propofol. Lipopolysaccharide caused a 1.8-fold increase in NADPH oxidase activity after 4 h of stimulation compared with the control cells (fig. 8A), which was significantly decreased upon propofol treatment (fig. 8A; \( P = 0.005 \) vs. lipopolysaccharide; \( n = 6 \); one-way ANOVA analysis followed by Student–Newman–Keuls post hoc test).

To further identify the role of individual NADPH oxidase components involved in propofol antiinflammation, siRNA-mediated silencing of the p22\( \text{phox} \) messenger RNA and gp91\( \text{phox} \) messenger RNA was transfected into growing BV2 cells. The expression of p22\( \text{phox} \) or gp91\( \text{phox} \) protein was reduced by 50 and 48%, respectively, when compared with control siRNA-transfected cell levels (fig. 9, A–C). Then, effect of propofol on lipopolysaccharide-stimulated nitric oxide and TNF-\( \alpha \) production was determined in control-, p22\( \text{phox} \)-, and gp91\( \text{phox} \)-siRNA-transfected cells. In BV2 microglia that expressed the scrambled control-siRNA, propofol at concentrations of 100 and 200 \( \mu M \) resulted in a 21%
Fig. 6. Propofol dose dependently attenuates lipopolysaccharide (LPS)-stimulated release of proinflammatory mediators in BV2 microglial cells. LPS stimulation induced significant increases in the production of (A) nitric oxide (NO), (B) reactive oxygen species (ROS), and (C) tumor necrosis factor-α (TNF-α) in BV2 cells, whereas propofol pretreatment significantly attenuated LPS-stimulated NO, ROS, and TNF-α production. N = 6 independent measurements. ### $P < 0.001$ versus control, ** $P < 0.01$. Statistical analyses were performed by one-way ANOVA with Student–Newman–Keuls post hoc test.

Fig. 7. Propofol dose dependently attenuates interferon-γ (IFN-γ)-stimulated release of proinflammatory mediators in BV2 microglial cells. IFN-γ stimulation induced significant increase in the production of (A) nitric oxide (NO), (B) reactive oxygen species (ROS), and (C) tumor necrosis factor-α (TNF-α) in BV2 cells, whereas propofol pretreatment significantly attenuated IFN-γ-stimulated NO, ROS, and TNF-α production. N = 6 independent measurements. ### $P < 0.001$ versus control, * $P < 0.05$, *** $P < 0.001$ versus IFN-γ group. Statistical analyses were performed by one-way ANOVA followed by Student–Newman–Keuls post hoc test.
Propofol inhibits lipopolysaccharide (LPS)-stimulated nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation in microglial cells. (A) NADPH oxidase activity was evaluated with cytochrome c reduction assay in BV2 microglial cells pretreated with propofol for 1 h and stimulated with LPS for a 4 h incubation period. N = 6 independent measurements. (B–D) The protein expressions of NADPH oxidase p22phox and gp91phox subunits were measured in primary microglial cells pretreated with propofol for 1 h and stimulated with LPS for a 24 h incubation period. Data are presented as percentage of control-treated values. N = 4 independent measurements. ***P < 0.001, ****P < 0.0001 versus control; *P < 0.05, **P < 0.01 versus LPS group. Statistical analyses were performed by one-way ANOVA with Student–Newman–Keuls post hoc test. GAPDH = glyceraldehydes-3-phosphate dehydrogenase.

Fig. 8. Propofol inhibits lipopolysaccharide (LPS)-stimulated nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation in microglial cells. (A) NADPH oxidase activity with LPS stimulation (24 h treatment) (fig. 11, D–F). nor muscimol affected the microglial responses to lipopolysaccharide stimulation (24 h treatment) (fig. 11, A–C). However, neither picrotoxin nor muscimol affected the microglial responses to lipopolysaccharide stimulation (24 h treatment) (fig. 11, A–C).

Propofol Anesthesia Limits TBI-induced Up-regulation of gp91phox and p22phox Expression Associated in Activated Microglia

Finally, we evaluated effect of propofol on NADPH oxidase activation after TBI. Western blot analysis showed that expression of gp91phox and p22phox is significantly increased at 7 days postinjury compared with sham-injured rats. A marked reduction of both gp91phox (P = 0.004 for vs. TBI-isoflurane group; n = 4; one-way ANOVA analysis followed by Student–Newman–Keuls post hoc test) and p22phox (P = 0.038 vs. TBI-isoflurane group; n = 4; one-way ANOVA analysis followed by Student–Newman–Keuls post hoc test) expression was observed in propofol-treated rats compared with TBI-isoflurane animals (fig. 12, A–C).

Immunohistochemistry at 28 days postinjury demonstrated an increase in immunolabeling of gp91phox in contrast to sham tissue (fig. 12D). Moreover, double-labeling immunohistochemistry revealed that large numbers of gp91phox-positive cells in the injured coronal sections were colabeled with Iba-1 (fig. 12D). Notably, there were fewer gp91phox-positive cells in the propofol-treated TBI samples, and Iba-1 expression was also reduced in these cells.
In a rat lateral fluid percussion injury model, we show that propofol treatment decreased microglial activation, lesion volume, cortical neuronal cell loss, and improved cognitive recovery after injury compared with isoflurane-treated animals. Lipopolysaccharide and interferon-γ induce microglial activation in vitro, leading to proliferation, induction of inflammatory factors (nitric oxide, ROS, TNF-α, and interleukin-1β) and Iba-1 and inducible nitric oxide expression. These effects, as well as microglia-induced neurotoxicity, were significantly attenuated by pretreatment with propofol. Moreover, propofol blocked NADPH oxidase enzymatic activity and reduced the expression of its membrane subunits p22phox and gp91phox both in vitro and in vivo. In addition, the inhibitory effects of propofol on microglial activation were significantly reduced when the p22phox subunit of NADPH oxidase complex was knocked down by siRNA. Together, these findings support the conclusion that propofol reduces microglial activation and associated neurotoxicity in part by limiting the activation of NADPH oxidase.

Previous reports addressing the effects of propofol on microglial function have been conflicting. Yu et al. found that propofol inhibits extracellular pressure-stimulated phagocytosis, proliferation, and secretion of TNF-α, interleukin-1β, and total nitrate in a human microglial cell line (Fig. 9). Small interfering RNA (siRNA) knockdown of p22phox reduces the protective effects of propofol in vitro. Subconfluent BV2 microglia cells were transfected with nontargeting siRNAs (control), siRNA targeting p22phox or gp91phox. After the transfection, cells were grown for 6 h and then exposed to propofol and lipopolysaccharide (LPS) treatment for 24 h. Western blot demonstrated a 50% reduction in p22phox and 48% reduction in gp91phox protein expression as compared with scrambled control-siRNA-transfected cells. N = 4 independent measurements. 

### Discussion

In a rat lateral fluid percussion injury model, we show that propofol treatment decreased microglial activation, lesion volume, cortical neuronal cell loss, and improved cognitive recovery after injury compared with isoflurane-treated animals. Lipopolysaccharide and interferon-γ induce microglial activation in vitro, leading to proliferation, induction of inflammatory factors (nitric oxide, ROS, TNF-α, and interleukin-1β) and Iba-1 and inducible nitric oxide expression. These effects, as well as microglia-induced neurotoxicity, were significantly attenuated by pretreatment with propofol. Moreover, propofol blocked NADPH oxidase enzymatic activity and reduced the expression of its membrane subunits p22phox and gp91phox both in vitro and in vivo. In addition, the inhibitory effects of propofol on microglial activation were significantly reduced when the p22phox subunit of NADPH oxidase complex was knocked down by siRNA. Together, these findings support the conclusion that propofol reduces microglial activation and associated neurotoxicity in part by limiting the activation of NADPH oxidase.

Previous reports addressing the effects of propofol on microglial function have been conflicting. Yu et al. found that propofol inhibits extracellular pressure-stimulated phagocytosis, proliferation, and secretion of TNF-α, interleukin-1β, and total nitrate in a human microglial cell line (Fig. 9). Small interfering RNA (siRNA) knockdown of p22phox reduces the protective effects of propofol in vitro. Subconfluent BV2 microglia cells were transfected with nontargeting siRNAs (control), siRNA targeting p22phox or gp91phox. After the transfection, cells were grown for 6 h and then exposed to propofol and lipopolysaccharide (LPS) treatment for 24 h. Western blot demonstrated a 50% reduction in p22phox and 48% reduction in gp91phox protein expression as compared with scrambled control-siRNA-transfected cells. N = 4 independent measurements. 

### Statistical analyses

Statistical analyses were performed by two-tailed t test. GAPDH = glyceraldehydes-3-phosphate dehydrogenase.
line. In contrast, Shibakawa et al.40 claimed no observed effects of propofol at concentrations up to 300 μM on the production of nitric oxide or TNF-α from lipopolysaccharide-stimulated glial cells. The blood plasma concentrations of propofol are reportedly 40–60 μM (7.12–10.68 μg/ml) at anesthesia induction and 10–25 μM (1.78–4.45 μg/ml) during anesthesia maintenance.41,42 Our observations are more in agreement with those of Yu et al.39 In microglial cells in vitro, propofol at concentrations as low as 12.5–25 μM significantly inhibited ROS, nitric oxide, TNF-α, and interleukin-1β induction when lipopolysaccharide concentration was 10 ng/ml. With a higher concentration of lipopolysaccharide (50 ng/ml), greater doses of propofol were required to be effective.

The neuroprotective effects of propofol have long been recognized.43 It directly attenuates neuronal death after oxygen and glucose deprivation and prevents the depolarization of the mitochondrial membrane potential in hippocampal neuronal cultures.44 However, the mechanism(s) by which propofol exerts its neuroprotective effects has been unclear. Nitric oxide, ROS, and TNF-α each may contribute to neuronal death in vitro and in vivo.45–52 Nitric oxide can cause neuronal death through the production of the toxic metabolite peroxynitrite or via direct action on lipid membranes of the cells or mitochondria.53,54 ROS induces lipid peroxidation, which results in cell membrane damage and cell death, as well as glutamate release causing excitotoxic cell death.55 TNF-α can cause cell death directly by binding to neuronal TNF receptors linked to death domains that activate caspase-dependent apoptosis,56 but it can also induce glutamate release and enhance excitotoxicity.52 In addition, TNF-α can induce additional release of ROS, by inducing NADPH oxidase activity.57 In our study, lipopolysaccharide-stimulated microglia induced neuronal cell death, which was attenuated
Propofol reduces microglial activation after TBI by propofol treatment. As the treatment reduced each of the above potential neurotoxic factors while robustly reducing neuronal death in vitro, the effects of propofol likely reflect multipotential actions.

NADPH oxidase activation and subsequent ROS formation are important upstream events that can activate microglia and amplify the production of multiple proinflammatory cytokines, such as TNF-α or interleukin-1β. Microglial NADPH oxidase activation and the production of ROS have been implicated as critical regulators of microglia-mediated neurotoxicity. Although propofol has been shown to reduce oxidative stress and damage by functioning as an ROS scavenger, the current study shows that propofol can also inhibit NADPH oxidase to reduce microglial activation and the consequent production of ROS. Notably, propofol down-regulated the expression of the NADPH oxidase membrane components p22phox and gp91phox after lipopolysaccharide stimulation. We speculate that this may be an important mechanism of propofol actions, because siRNA silencing of p22phox significantly attenuated the protective effects of propofol. After TBI, we found that propofol administration significantly reduced the number of activated microglia while reducing expression of p22phox and gp91phox.

GABA<sub>A</sub> receptors have been suggested as potential targets for regulating inflammatory responses in various cell types. For example, GABA<sub>A</sub> receptor agonists ameliorate the interleukin-1 production in peritoneal macrophages of mice. Similarly, GABA<sub>A</sub> receptor modulator diazepam can enhance GABA<sub>A</sub> signaling, provoke intracellular acidosis, and impair cytokine production in mice macrophages. Microglia have been characterized as GABA<sub>A</sub>ceptive cells, and the mRNA expression of the GABA<sub>A</sub> receptor α1, α3, and β1 subunits have been identified in human microglia. After 2-h lipopolysaccharide stimulation, bicuculline reduced muscimol-induced reduction of phosphorylation of p38 mitogen–activated protein kinases and p65 NFκB, consistent with Lee’s report in human microglia. Bacterial-derived lipopolysaccharide is a broad proinflammatory agent that stimulates microglia through multiple receptors and signaling pathways, including GABA<sub>A</sub> receptor β type, toll-like receptor 4, CD14, chemokine receptors, and many more. Longer lipopolysaccharide (24 h) treatment showed that muscimol failed to reverse lipopolysaccharide-induced microglial activation—as reflected by nitric oxide, ROS, and TNF-α production—suggesting that GABA<sub>A</sub>-dependent pathways may not be sufficient for regulating inflammatory responses of microglia. Although there is evidence that α, β, and γ subunits all contribute to GABA<sub>A</sub> sensitivity to propofol, we found that propofol inhibition of microglial activation induced by lipopolysaccharide in rat primary
microglia, and BV2 cells was not reversed by GABA<sub>ₐ</sub> receptor antagonists bicuculline or picrotoxin, and the antiinflammatory effect of propofol could not be mimicked by GABA<sub>ₐ</sub> receptor agonist muscimol. A recent study from Ye et al. reported that propofol abolished the lipopolysaccharide proinflammatory cytokine response in BV2 cells, whereas...
inhalation anesthetics including isoflurane had no effect. As GABA_A receptors are known to be a common target for both propofol and isoflurane action,64 our results and those of Ye et al. are consistent and suggest that the GABA system may not be essentially involved in propofol’s antiinflammatory effect. Recent studies have also identified other receptors as potential molecular targets of propofol, including glycine, nicotinic, and M1 muscarinic receptors.65–67 Whether these receptors act as upstream regulators of microglial activation or NADPH oxidase remains to be determined.

Microglial activation and increased inflammatory cytokines have been implicated in the cognitive decline associated with various neurodegenerative diseases.68,69 The activation of microglial cells and neuroinflammation after TBI has been extensively studied by us and many others and has long been linked to changes in cognitive function through both direct and indirect effects on neurons.70 Two recent clinical studies, one using positron emission tomography scanning to delineate microglial activity and the other pathological, have further underscored the importance of microglial activation in chronic neurodegeneration after TBI.5,71 The effects of propofol on microglial activation were marked, as well as highly significant. The impairment in cognitive performance in the MWM task is an indicator of hippocampal damage and the relative contribution of various hippocampal subregions in encoding and retrieval of learning and memory can be quantified by counting neurons in these regions.25,28,72 As there were no significant differences between the two injured treatment groups with respect to TBI-induced neuronal cell loss in various hippocampal subregions, this is consistent with the failure of propofol to improve cognitive performance in the MWM test. In contrast, cognitive outcomes in the novel object recognition test reflect both frontal cortical and hippocampal involvement.25,26,73 Propofol treatment significantly reduced the cortical lesion size and improved neuronal survival in the cortex. Therefore, the degree of cognitive improvement observed in novel object recognition task on treatment with propofol after TBI likely reflects its neuroprotective effects in the cortex. In our rat lateral fluid percussion injury model, the primary injury affects the ipsilateral cortex and adjacent penumbra.23,74 As such, the cortex shows the greatest degree of inflammation and cell death. Changes in the hippocampus and thalamus are more distant and show less inflammatory components. We have identified other approaches that target microglial activation, such as with cell cycle inhibitors that also show protective effects largely restricted to injured cortex.23,74 One major limitation of the study was the application of the general anesthetics before and during the delivery of TBI, which do not ideally reflect clinical situations. But all TBI studies require anesthetic use.

A potential confounding effect in the current study with regard to group differences is that isoflurane itself may have neuroprotective effects; more specifically beneficial effects have been reported by several groups for isoflurane on outcome after TBI.75,76 Isoflurane can also reduce lipopolysaccharide- and interferon-γ–induced microglial activation in vitro, through actions at adenosine triphosphate potassium channels.77,78 However, recent studies have implicated neuroinflammation and microglial activation in the pathogenesis of postoperative cognitive dysfunction,79–81 with a possible link between exposure to volatile anesthetics and exacerbation of neurodegenerative disorders. Interestingly, isoflurane has been associated with cognitive impairment in the absence of any surgical manipulation, whereas propofol has not.82,83 Whether propofol should be preferentially considered for surgery in the elderly or for the treatment of TBI is an important question that requires further studies in other model systems.

References


Anesthesiology 2013; 119:1370-88

Luo et al.


34. Richardson JE, Garcia PS, O’Toole KK, Derry JM, Bell SV, Jenkins A: A conserved tyrosine in the beta2 subunit M4 segment is a determinant of gamma-aminobutyric acid type A receptor sensitivity to propofol. Anesthesiology 2007; 107:412–8


51. Taylor DL, Jones F, Kubota ES, Pocock JM: Stimulation of microglial metabolotropic glutamate receptor mGlu2 triggers...
73. Propofol Reduces Microglial Activation after TBI