

Surgery Upregulates High Mobility Group Box-1 and Disrupts the Blood–Brain Barrier causing Cognitive Dysfunction in Aged Rats

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Keywords

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SUMMARY

Aim: Postoperative cognitive dysfunction (POCD) is a growing and largely underestimated problem without defined etiology. Herein, we sought to determine the relationship between cognitive decline, blood–brain barrier (BBB) permeability, and inflammation, namely high mobility group box-1 (HMGB1), after surgery in aged rats. **Methods:** Aged rats were randomly assigned as surgery group (n = 45, splenectomy under general anesthesia), anesthesia (n = 45, 2% isoflurane for 2 h), and naïve control (n = 15). Markers of inflammation were measured in plasma and brain. Blood–brain barrier ultrastructure and permeability were measured by transmission electron microscope (TEM) and IgG immunohistochemistry. Cognitive function was assessed in a reversal learning version of the Morris water maze (MWM). **Results:** Surgical trauma under general anesthesia caused distinct changes in systemic and central proinflammatory cytokines. Levels of HMGB1 and the receptor for advanced glycation end products (RAGE) were significantly upregulated in the hippocampus of operated animals. Immunohistochemistry and TEM showed BBB disruption induced by surgery and anesthesia. These molecular changes were associated with cognitive impairment in latency with the MWM up to postoperative day 3. **Conclusions:** HMGB1 and RAGE signaling appear pivotal mediators of surgery-induced cognitive decline and may contribute to the changes in BBB permeability after peripheral surgical trauma.

Introduction

Postoperative cognitive dysfunction (POCD) is a common complication following surgery and hospitalization, frequently seen among elderly patients [1]. With a steady increase in the surgical geriatric population, postoperative cognitive decline is rapidly becoming a major global health burden [2]. 25–50% of hospitalized patients experience confusion and cognitive disturbances affecting multiple domains including learning and memory, orientation, language comprehension, abstract thinking, executive functioning, social integration, and concentration following major surgical procedures [3,4]. About 40% of hospitalized patients retain cognitive impairment after 5 years from the operation, and a link between surgery, anesthesia, and permanent dementia has been recently proposed [5–7].

Although the mechanisms underlying the pathophysiology of POCD are not well understood, some risk factors including advanced age, low level of education, comorbidities, duration of anesthesia, second operation, postoperative pain, and hospitalization have been reported from clinical studies, with age the only univocal risk factor [3,8]. Recently, a relation

between cognitive decline and inflammation has been suggested. In preclinical models, surgical trauma has been associated with neuroinflammation, cytokine release, and memory dysfunction [9–13]. In the present study, we have focused on the role of high mobility group box-1 (HMGB1), a classical danger-associated molecular pattern (DAMP) with a pivotal role in mediating acute damage response and subsequent inflammatory processes [14], in response to anesthesia and aseptic surgical trauma. Modulation of HMGB1 and its cognate receptor for advanced glycation end products (RAGE) have been reported in several disease states, ranging from infection to chronic inflammation and cancer [15,16]. Studies have shown that HMGB1 might play a role in neuroinflammation [17,18], yet there is limited evidence on how HMGB1 contributes to CNS inflammation, blood–brain barrier (BBB) dysfunction, and cognitive decline.

In this study, we used a surgical model in aged rats to define the role of HMGB1 in mediating surgery-induced cognitive dysfunction and further define the immune-to-brain signaling, in particular changes in the BBB, that may associate with the process of cognitive decline.

Materials and Methods

Animals

Experiments were performed in accordance with the guidelines for experimental animal use established by the ethics committee of the Central South University. A total of 105 female Sprague Dawley (SD) aged rats (22–23 months old, 450–560 g) were used in the study. Animals were purchased from the Dongchuang Laboratory animal center (Changsha, Hunan, China) and housed in standard cages under controlled laboratory conditions (temperature of $23 \pm 3^\circ\text{C}$, 12-h light/12-h dark cycle) with free access to water and laboratory chow. All rats were allowed to adapt to their new environment for 7 days before beginning the experiments. Rats were randomly divided into seven groups: control (C, $n = 15$), anesthesia only (A1, A3, A7 $n = 15/\text{group}$), anesthesia plus surgery (S1, S3, S7 $n = 15/\text{group}$). Rats were sacrificed at posttreatment days 1, 3, and 7.

Experimental Procedures

Rats were exposed to isoflurane anesthesia (3% isoflurane in 100% oxygen for induction followed by 2% for maintenance) for 2 h with endotracheal intubation with a 14-gauge catheter. Animals breathed spontaneously and were extubated as the loss of righting reflex (LORR) was regained. Gas concentrations and respiratory rate were continuously monitored with a multi-function monitor (Datex-Ohmeda, Helsinki, Finland), rectal temperature was controlled to $37 \pm 0.5^\circ\text{C}$, and heart rate (HR) and mean arterial blood pressure (MAP) were monitored non invasively using a rat tail sphygmomanometer (Midwest Co. Ltd., Beijing, China). Pulse oximeter oxygen saturation (SpO₂) was measured continuously (GE Healthcare, Finland). Animals in the anesthesia plus surgery groups underwent splenectomy under aseptic conditions as previously described [19]. This standardized procedure delivered similar injury to every animal in order to assess remote effects of trauma to the CNS. Briefly, a small incision about 2–3 cm was made in the upper left quadrant through the skin and muscle wall. The spleen was visualized, isolated, and removed. The wound was infiltrated with 0.25% bupivacaine then closed with sterile sutures. Rats in the control group were exposed to 100% oxygen for 2 h in a gas chamber.

Plasma Cytokine Measurement (ELISA)

Blood samples were collected transcardially after thoracotomy, following centrifugation at 1346 *g* for 10 min at 4°C . Plasma samples were stored at -80°C before use. Levels of tumor necrosis factor (TNF- α) and HMGB1 were measured by ELISA following manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

Brain Cytokine mRNA Extraction and Reverse-Transcription PCR (RT-PCR)

Hippocampi were quickly dissected and frozen in liquid nitrogen for further use. For PCR, total RNA was extracted from homogenized hippocampal tissues using Trizol (Invitrogen, Carlsbad, CA, USA) following manufacturer's instruction. RT-PCR was performed using standard reagents and protocols (Fermentas, Glen Burnie, MD, USA). Gene-specific primers, forward and reverse,

were as follows: 5'-GAAGCCGAGAGGCCAAATGTCC-3' and 5'-TAGCTCTGTAGGCAGCAATATC-3' for HMGB1, 5'-CCGAGTC-TACCAGATTCCTG-3' and 5'-TTCACGAGTGTTCCTTTGCC-3' for RAGE, 5'-TGACCCCATCTACTCTGACC-3' and 5'-GGCCACTACTTCAGCGTCTC-3' for TNF- α , 5'-CTCCATGAGCTTTGTA-CAAGG-3' and 5'-TGCTGATGTACCAGTTGGGG-3' for IL-1 β , 5'-GTGGGGCGCCCGAGGCACCA-3' and 5'-CTTCCTTAATGTCACG-CACGATT-3' for β -actin. RT-PCR was performed at $95^\circ\text{C}/4$ min and then cycling $94^\circ\text{C}/30$ s, $60^\circ\text{C} \pm 10^\circ\text{C}/30$ s, $72^\circ\text{C}/30$ s, terminated by heating to $72^\circ\text{C}/5$ min. The products were amplified for 30 cycles. Band densities were quantified using Quality One Software (Bio-Rad, Hercules, CA, USA). The amount of mRNA was expressed as a ratio of the densitometric measurement derived from target mRNA to that from β -actin.

Western Blot

Hippocampi were homogenized in cold RIPA buffer and centrifuged at 4°C at 13350 *g* for 5 min. The quantity of protein in the supernatants was determined using a BCA protein assay kit (Wellbio, China) according to the manufacturer's instructions. Equal amounts of protein samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride membranes. Membranes were blocked with 5% skim milk TBS buffer for 1 h and then incubated with primary antibodies: mouse anti-HMGB1 monoclonal (1:1000; Abcam, Cambridge, UK), rabbit anti-RAGE polyclonal (1:500; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), rabbit anti-TNF- α polyclonal (1:1000; Abcam, Cambridge, UK), rabbit anti-IL-1 β polyclonal (1:1000; Santa Cruz Biotechnology Inc.) overnight at 4°C . After thorough washing, membranes were incubated with goat anti-mouse (1:3000; Proteintech Group, Wuhan, China) and goat anti-rabbit (1:3000; Proteintech Group) secondary antibodies at room temperature. Membranes were then treated with enhanced chemiluminescence detection kit (Pierce; Thermo Scientific, Shanghai, China), and the intensity of each band was quantified by densitometry. Relative expression was normalized to β -actin (1:4000; Proteintech Group).

Immunohistochemistry

Blood-brain barrier damage was assessed by immunoglobulin G immunostaining as previously described [20,21]. Five rats in each group were anesthetized with chloral hydrate (10%) and perfused transcardially with 0.01 M PBS (4°C) followed by 200 mL of ice-cold 4% paraformaldehyde. The brain was removed and postfixed in 4% paraformaldehyde overnight, rehydrated, and embedded in paraffin. Sections (5 μm) were deparaffinized, preincubated with 3% H₂O₂ at room temperature for 5 min to block endogenous peroxidase activity, and then washed twice with PBS. Sections were blocked in 3% normal goat serum at room temperature for 1 h followed by incubation with rabbit anti-rat IgG antibody (1:200; Vector Laboratories, Burlingame, CA, USA) at 4°C overnight and incubated with avidin-biotin-HRP complex (ABC) reagent (1:200; Vector Laboratories) for 2 h and developed using DAB method. The sections were rinsed and mounted.

For HMGB1 immunofluorescence, sections were blocked in 3% normal horse serum and incubated with a mouse anti-HMGB1

antibody (1:200; Abcam, Cambridge, UK) at 4°C overnight. Sections were then incubated with horse anti-mouse secondary antibodies (Vector Laboratories) and Cy3-streptavidin (1:100; Jackson Immuno Labs, West Grove, PA, USA) for 2 h. Images were acquired with a Nikon eclipse 80i microscope (Nikon, Tokyo, Japan). Assessments were performed in a blinded fashion.

Transmission Electron Microscopy (TEM)

Hippocampi were quickly dissected, fixed in 2.5% glutaraldehyde in 0.1 mol/L cacodylic acid buffer (pH 7.3) overnight, and post-fixed in the 2% Osmic Acid for 2 h. The fixed brain was dehydrated through an ethanol series soaked in the mixed liquor of epoxy resin and acetone for 24 h, 37°C, following embedding in Epon812, DDSA, DMP30 for 24 h, cut into semithin sections, and stained with toluidine blue to find the position. Slice were then cut into ultra-thin sections with an ultramicrotome (LKB-III, Sweden) and mounted on copper grids, stained with uranyl acetate and plumbi nitras. Specimens were observed under a transmission electron microscope (Hitachi H-7500; Hitachi, Tokyo, Japan).

Morris Water Maze (MWM)

Spatial learning and memory were evaluated in the MWM using a computerized video track system (Smart Junior Software, Panlab, Spain) as previously described [11]. Briefly, a transparent round platform of 10 cm in diameter was placed 1 cm below the water surface and located in the center of the SE quadrant in a circular pool. The rats were placed on the platform for 30 s preceding the start of each trial and then released into the water facing the tank wall in one of the three present entry locations. Rats were trained for 6 days with three trials per day (60 s maximum for a trial, 30 s spent on the platform). If the platform was not located within 60 s, the rats were gently guided to the platform and remained for 15 s. On day 7, animals underwent surgery and/or general anesthesia; on posttreatment days 1, 3 and 7, rats were subjected to a reversal test in which the platform was moved to the opposite quadrant of the pool (NW). The time to reach the platform (latency) and distance was recorded by a videotrack system (Logitech, Suzhou, China).

Statistical Analysis

Results are expressed as mean \pm SD, two-way repeated measures ANOVA was used to analyze training behavioral parameters, and a separate two-way ANOVA was used for reversal testing. ELISA, RT-PCR, and Western blot were analyzed with two-way ANOVA, in which time and treatment were dependent variables. *Post hoc* Bonferroni was performed when ANOVA showed significance. All statistical data were analyzed using SPSS 17.0. (SPSS, Shanghai, China) A probability value of 0.05 was considered to be statistically significant.

Results

Surgery Upregulates Systemic Levels of HMGB1

To evaluate the effects of general anesthesia and surgical trauma on aged rats, we measured systemic levels of TNF- α and HMGB1.

No changes in plasma TNF- α were observed on postoperative days 1, 3, and 7 (Figure 1A). However, levels of HMGB1 were significantly upregulated after surgery on postoperative day 1 ($0.204 \pm 0.023 \mu\text{g/L}$, $P = 0.003$), before returning to baseline. Analysis of HMGB1 plasma levels by two-way ANOVA revealed significant effects of time ($P = 0.001$), treatment ($P = 0.004$), and time \times treatment interaction ($P = 0.170$; Figure 1B). Exposure to anesthesia did not affect systemic levels of these cytokines. Animals had no signs of cyanosis, hypoxia, respiratory depression, or hypotension. Heart rates, MAP, and SpO₂ were 343.5 ± 35.09 beats/min, 91.60 ± 12.3 mmHg, and $98.7 \pm 1.13\%$, respectively.

Anesthesia and Surgery Impair the Blood–Brain Barrier of Aged Rats

Next, we investigated the effects of HMGB1 on the brain and the BBB. Proinflammatory cytokines modulate the CNS both *via* direct and indirect pathways [22]. Here, we interrogated the BBB by measuring IgG expression in the brain parenchyma. IgG-positive staining was pivotally observed in the hippocampus and was widely distributed around the CA1 area after surgical treatment, with some leakage visible also in other brain areas such as the hypothalamus (Figure 2 S1). Milder changes were seen at day 1 after exposure to isoflurane compared to naïve rats (Figure 2 A1). To assess the functionality of the neurovascular unit after surgery, we used transmission electron microscopy. Operated rats had impairments in the BBB at postoperative day 1, consisting of swollen vessels and expanded perivascular space. Furthermore, astrocytes end-feet was enlarged, and detachment of the end-feet

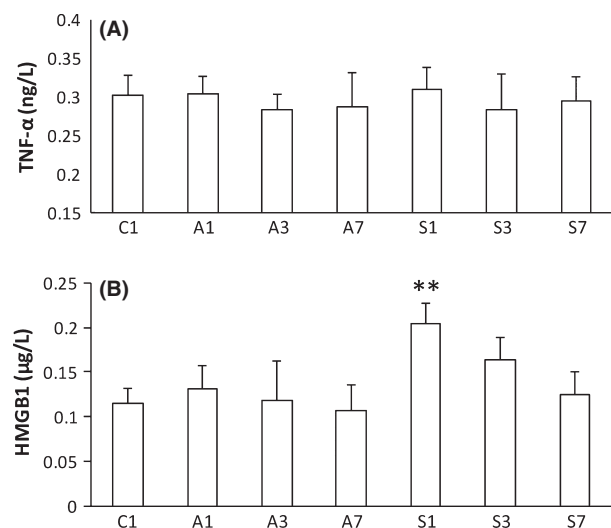


Figure 1 Systemic changes in proinflammatory cytokines measured by ELISA after anesthesia and surgery. (A) No changes from baseline were observed in tumor necrosis factor (TNF- α) at all time points. (B) HMGB1 was significantly upregulated after surgery on postoperative day 1, before returning to baseline. No changes were measured after exposure to isoflurane only. Bars denote means \pm SD, $n = 10$. * $P < 0.05$, ** $P < 0.01$ versus control by two-way ANOVA followed by Bonferroni. C: control; A1, A3, and A7: anesthesia at days 1, 3, and 7; S1, S3, and S7: surgery at days 1, 3, and 7.

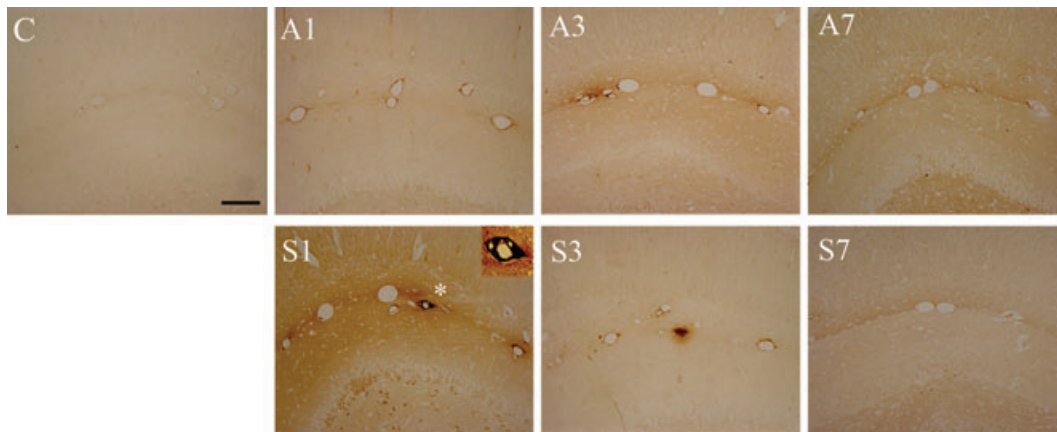


Figure 2 Surgery increases the permeability of the blood–brain barrier (BBB) in aged rats. Hippocampi were harvested at days 1, 3, and 7, and BBB permeability was measured with IgG immunohistochemistry. Picture show CA1 (scale bar = 400 μ m) and photomicrographs were blindly scored. IgG expression was increased around the blood vessels in the hippocampus. Exposure to anesthesia caused mild changes in IgG compared to naïve rats at day 1. Significant IgG-positive staining surrounding the lumen of blood vessels and the brain parenchyma was noted in the surgical group up to postoperative day 3. $n = 5$, C: control; A1, A3, and A7: anesthesia at days 1, 3, and 7; S1, S3, and S7: surgery at days 1, 3, and 7.

plasma membrane from the basal lamina was also observed after surgery (Figure 3S). Mild changes were also noted at day 1 after exposure to anesthesia compared to naïve rats (Figure 3A and C).

Blood–Brain Barrier Damage Leads to Neuroinflammation and *de novo* Synthesis of Cytokine in the Brain

To understand the effects of anesthesia and surgery on the brain, we measured transcription and translation of key proinflammatory cytokines including TNF- α , IL-1 β , and HMGB1. Even though we could not detect changes in systemic TNF- α , both mRNA levels

and protein expression in hippocampal tissues were increased after exposure to anesthesia at day 1 (mRNA: 0.683 ± 0.037 ; protein: 0.952 ± 0.042) or surgery at day 1 (mRNA: 0.855 ± 0.035 ; protein: 1.045 ± 0.055) and day 3 (mRNA: 0.75 ± 0.059 ; protein: 0.907 ± 0.049 ; Figure 4A, $P < 0.001$). Levels of TNF- α and IL-1 β remained elevated in the surgical group up to postoperative day 3 (IL-1 β mRNA: 0.656 ± 0.049 ; protein: 0.906 ± 0.042 ; $P < 0.001$ respectively), then returning to baseline (Figure 4A and B). To understand the specific effects of HMGB1 on the brain, we looked at the receptor for advanced glycation end products (RAGE), a key receptor for HMGB1 signaling. Both HMGB1 and RAGE mRNA levels and protein expression were significantly increased in the

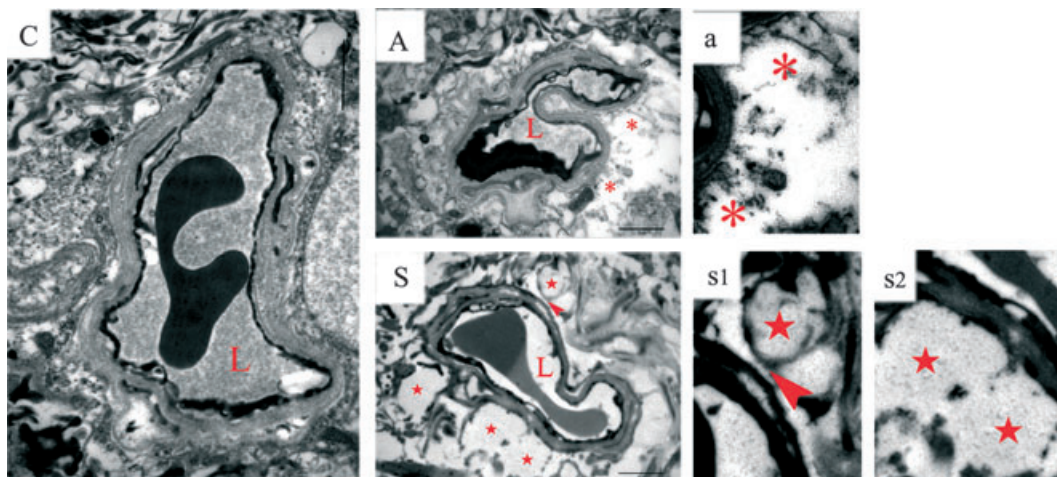


Figure 3 Anesthesia and surgery disrupt the structure of the blood–brain barrier (BBB) at 24 h. Ultrastructure of the neurovascular unit was observed at 24 h after surgery and/or anesthesia using transmission electron microscope. (C) Representative images from a coronal section through the hippocampus of a naïve rat show a normal BBB structure. (A) Exposure to isoflurane for 2 h caused minor changes in the BBB ultrastructure, with mild and localized swelling, expanded perivascular space (*). (S) Swelling in astrocyte end-feet (★) and detachment (arrowhead) was observed after surgery. $n = 5$, C: control; A: anesthesia at day 1. S: surgery at day 1. L: lumen of blood vessel. Scale bar = 2 μ m.

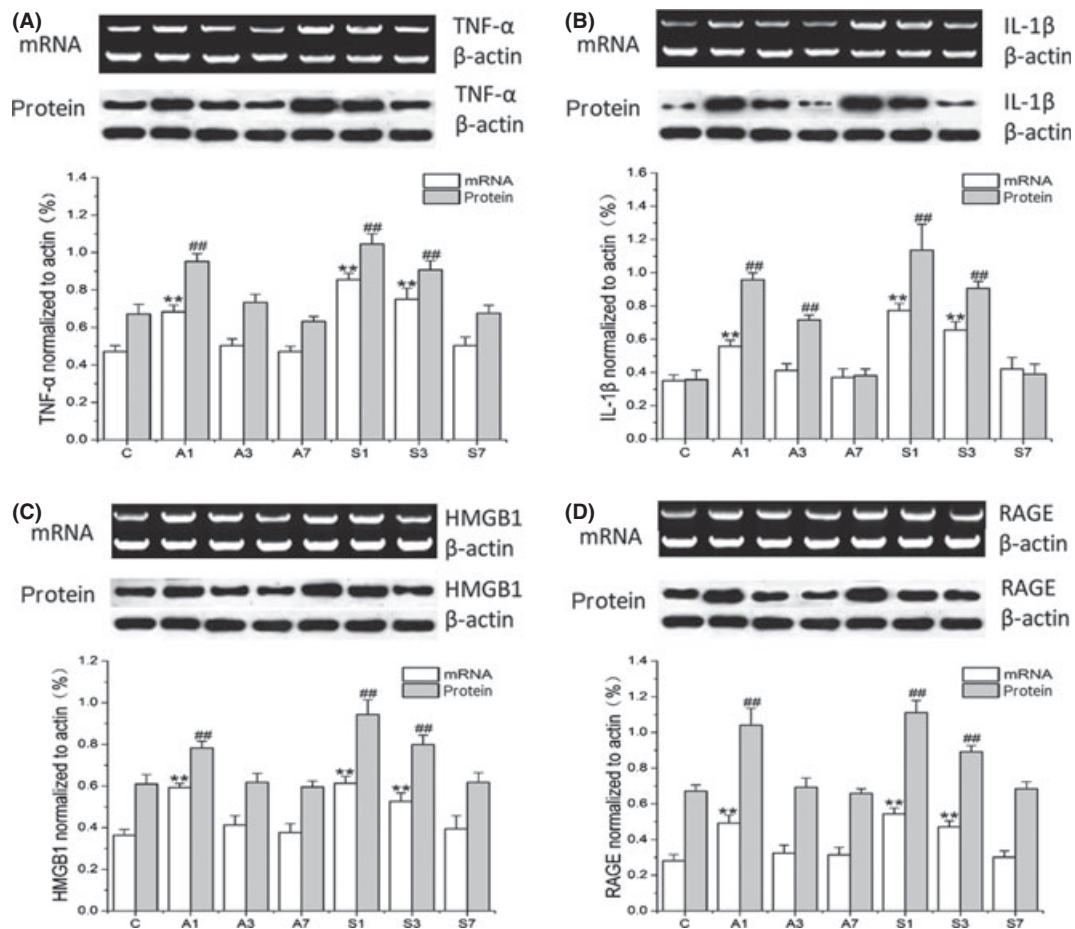


Figure 4 Surgery causes neuroinflammation and activates RAGE signaling. Messenger RNA and protein expressions of key proinflammatory mediators were measured in the hippocampus with reverse-transcription polymerase chain reaction (RT-PCR) and immunoblotting, respectively. High mobility group box-1 (C) and its signaling receptor RAGE (D) were significantly increased after exposure to anesthesia (day 1) or surgery (days 1 and 3). Tumor necrosis factor (TNF- α) (A) and IL-1 β (B) were also upregulated returning to baseline at day 7. Densitometry data were compared to the respective housekeeping gene (β actin). Bars represent mean \pm SD. ** $P < 0.01$ versus control mRNA levels and ## $P < 0.01$ versus control protein levels by two-way ANOVA followed by Bonferroni. $n = 5$; C: control; A1, A3, and A7: anesthesia at days 1, 3, and 7; S1, S3, and S7: surgery at days 1, 3, and 7.

hippocampus of operated rats and anesthesia exposure after day 1 (Figure 4C and D, $P < 0.001$). RAGE and HMGB1 levels in operated rats remained upregulated at day 3 before returning to baseline. The variations of HMGB1 mRNA and protein levels were 0.592 ± 0.023 and 0.782 ± 0.033 at day 1 after anesthesia; 0.613 ± 0.033 and 0.943 ± 0.071 at day 1 after surgery, respectively; and 0.526 ± 0.041 and 0.799 ± 0.045 at day 3 after surgery, respectively. Levels of RAGE mRNA expression and protein were 0.491 ± 0.045 and 1.04 ± 0.096 at day 1 after anesthesia; 0.544 ± 0.033 and 1.111 ± 0.069 at day 1 after surgery; and 0.47 ± 0.035 and 0.891 ± 0.036 at day 3 after surgery, respectively. To further corroborate the increase of HMGB1 in the brain, we used immunofluorescence. HMGB1-positive staining around the perivascular space and brain parenchyma was observed in the hippocampus of operated and anesthetized rats at day 1. Operated rats displayed higher HMGB1 in CA1 up to postoperative day 3, returning to baseline by day 7 (Figure 5).

Anesthesia and Surgery Cause Distinct Neurocognitive Impairments

To elucidate the effect of surgery and anesthesia on spatial learning and reference memory, we used the Morris water maze (Figure 6A). No significant differences in latency and swimming speed were reported throughout the groups, and all animals progressively improved over time during the acquisition phase (Figure 6B and C). Repeated measures ANOVA revealed that the latency significantly improved over time ($P < 0.001$). A separate two-way ANOVA examined the effects of time and treatment on spatial memory performance. Analysis of the latency to the platform revealed significant effects of time ($P < 0.001$), treatment ($P < 0.001$), and time \times treatment interaction ($P = 0.037$). No changes were reported in swimming speed ($P = 0.605$; Figure 6D and E). Notably, latency was impaired at day 1 after anesthesia (35.99 ± 7.70 s, $P = 0.001$) and up to postoperative day 3 in the

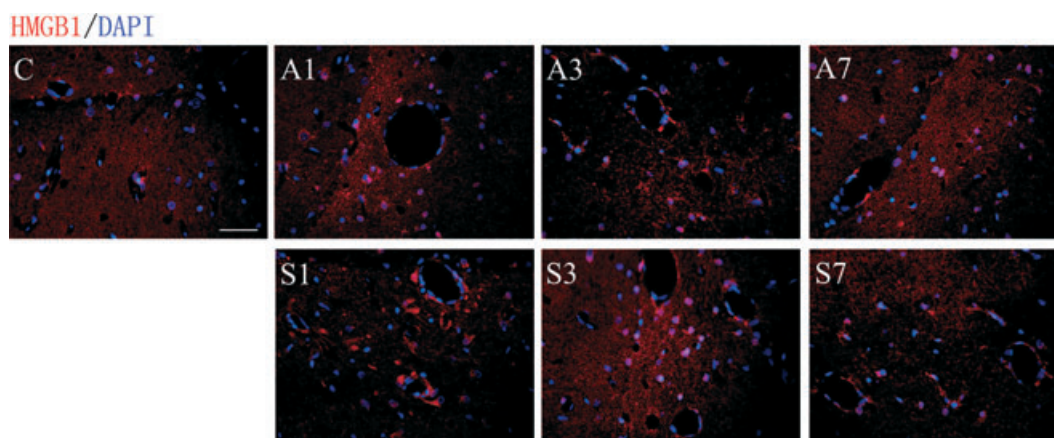


Figure 5 Expression of HMGB1 in the CA1 region of hippocampus in operated rats. HMGB1 was measured using immunofluorescence at days 1, 3, and 7 after surgery and anesthesia. (C) Representative photomicrographs show a low expression of HMGB1 in naïve rats. (A1) Isoflurane induced HMGB1 in the CA1 region of the hippocampus at day 1, returning to baseline thereafter. (S1, S3) Operated rats displayed higher HMGB1 staining in the brain parenchyma and around the vasculature up to postoperative day 3, returning to baseline at day 7. $n = 5$, C: control; A1, A3, and A7: anesthesia at days 1, 3, and 7; S1, S3, and S7: surgery at days 1, 3, and 7. Scale bar = 100 μm

surgical group (44.54 ± 6.63 s at day 1 and 38.30 ± 6.25 s at day 3, $P < 0.001$ respectively; Figure 6D and E).

Discussion

In the present study, we have explored the role of HMGB1 in the development of postoperative cognitive decline. General anesthesia, but more evidently surgical trauma, caused distinct changes in levels of HMGB1 leading to BBB disruption, subsequent neuroinflammation, and memory impairment in aged rats. Overall, these results suggest a novel role for DAMPs signaling in mediating surgery-induced cognitive decline after aseptic trauma and anesthesia (Figure 6F).

Postoperative cognitive dysfunction is currently a poorly understood disorder with undefined etiology and no treatment. Anesthesia has been historically associated with cognitive decline and neurotoxicity, for example during development [23]. Isoflurane, a commonly used anesthetic agent, was shown to increase cytokine expression and cell injury in brain areas such as the hippocampus, thus causing cognitive dysfunction in adult rats [24,25]. In the present study, we found that a single exposure to a clinically relevant concentration of isoflurane with airway intubation impaired learning and memory, suggesting that anesthesia *per se* may play a role in the development of cognitive decline, especially in aged animals. In a limited study of 438 patients, no significant changes in the incidence of POCD were reported when comparing general versus regional anesthesia; however, regional anesthesia decreased mortality and acute cognitive failure in elderly patients [26]. Aside anesthesia, cytokine release, and neuroinflammation have been associated with *sickness behavior* and cognitive disturbances, for example after infection and more recently following aseptic surgery [27,28]. Cytokines have been described as a “double-edged sword” with a pivotal role in protecting against harmful organism/pathogens and supporting homeostasis, but with potentially lethal effects when they become uncontrolled and dysregulated, for example in septic shock [29]. Proinflammatory

molecules are promptly released in the systemic circulation after tissue damage and trauma; TNF- α systemic levels peak within minutes after orthopedic surgery, [10] but following splenectomy, we did not observe significant changes that persisted over days (Figure 1A). Yet, we found increased transcription and translation in the hippocampus of operated rats up to postoperative day 3, confirming a pivotal role for cytokines, including TNF- α and IL-1 β in mediating cognitive dysfunction (Figure 4A and B). Furthermore, levels of HMGB1 and its cell-surface receptor RAGE were also increased (Figure 4C and D), suggesting a possible role for DAMPs signaling in mediating neuroinflammation, BBB disruption, and cognitive decline after surgery.

High mobility group box-1 is a highly conserved nuclear protein with a pivotal role in mediating acute damage response [30]. In stroke models, HMGB1 was induced in activated astrocytes and microvascular structure, inducing alterations in endothelial cell function and increasing BBB permeability [31]. Astrocytes and endothelial cells integrity play a key role in maintaining a patented BBB by controlling its permeability and overall integrity. Administration of HMGB1 neutralizing antibody in brain ischemia-reperfusion injury also limits the damage on the BBB and may represent a viable therapeutic target also after peripheral trauma [32]. Recently, Chavan *et al.* described a role for HMGB1 in mediating cognitive decline among sepsis survivor [33]; changes in systemic HMGB1 and cognitive impairments were also observed after lipopolysaccharide administration in mice [34]. HMGB1 can interact with various receptors including RAGE but also toll-like receptor (TLR)-2 and TLR-4 to mediate chemotaxis and release of proinflammatory cytokines [35]. Interestingly, HMGB1 has been reported to cause lethality in TLR4-defective mice [36], but on the other hand, TLR4-dependent signaling does not appear to be required for the establishment of cognitive decline after aseptic surgery [10]. Here, we described a key role for RAGE signaling and its upregulation in the brain and hippocampal tissue suggesting a pivotal role in causing neuroinflammation and cognitive dysfunction. Remarkably, RAGE has been described as

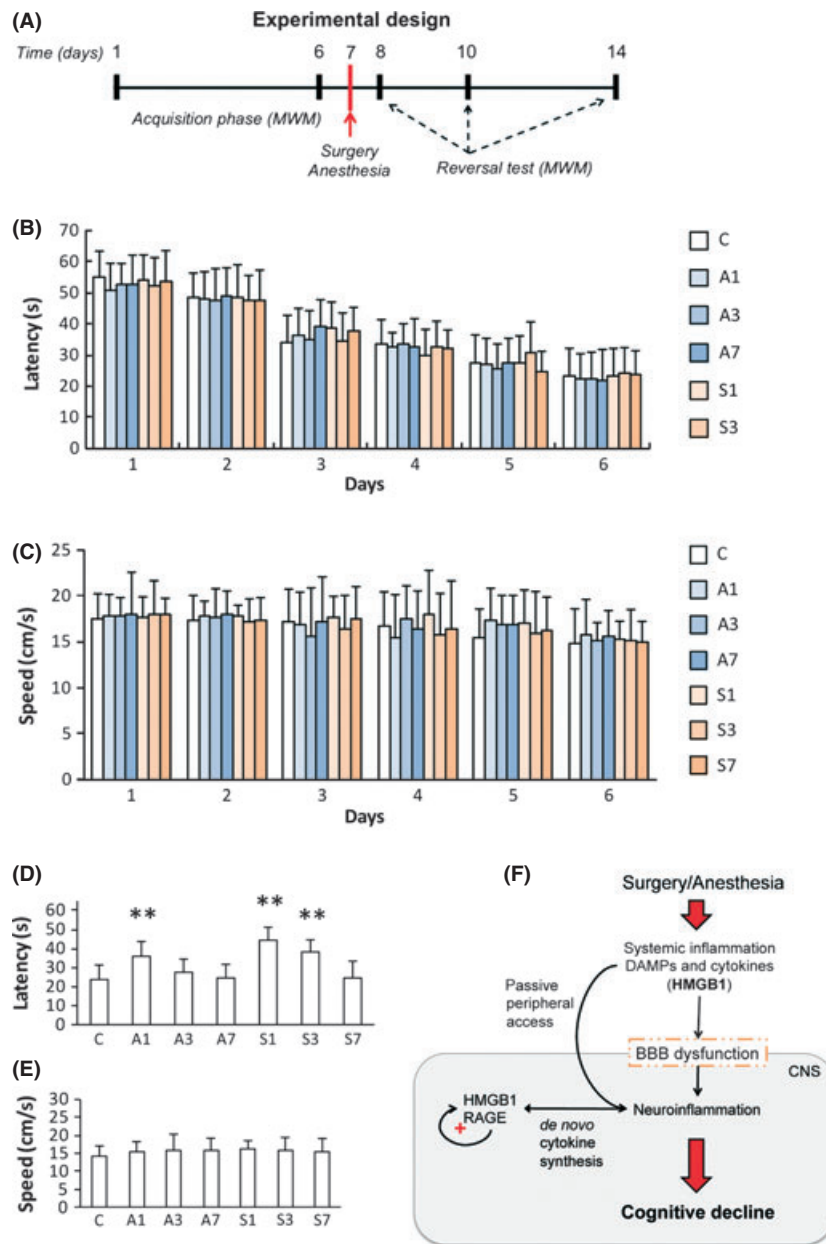


Figure 6 Spatial memory and learning are impaired after anesthesia and surgery. **(A)** Diagram of the experimental design for the neurocognitive assessment with the Morris water maze. **(B)** The time required for each animal to reach the hidden platform (latency) and **(C)** speed during the 6 days of training in the acquisition phase. **(D)** Anesthesia induced spatial learning and memory impairment in the reversal test at day 1. Similarly, operated rats required significantly longer time to locate the submerged platform at postoperative days 1 and 3. **(E)** Average swimming speed during reversal testing did not change across groups. **(F)** Working model: surgical trauma with general anesthesia evokes a systemic inflammatory response in aged rats. Damage-associated molecular pattern (DAMPs), including HMGB1, are released in the systemic circulation contributing to the permeability of the blood–brain barrier (BBB). Opening of the BBB allows peripheral cytokines and alarmins to enter the brain and the CNS, causing neuroinflammation. In the hippocampus, HMGB1 activates RAGE signaling thus perpetuating the inflammatory response and further contributing to neuroinflammation, including *de novo* synthesis of cytokines like TNF- α and IL-1 β . Activation of this positive feedback loop accounts for memory dysfunction and possibly localized synaptic/neuronal deficits. Bars denote mean \pm SD. ** $P < 0.01$ versus control by two-way ANOVA followed by Bonferroni. $n = 15$, C: control; A1, A3, and A7: anesthesia at days 1, 3, and 7; S1, S3, and S7: surgery at days 1, 3, and 7.

an amplifier of the immune and inflammatory response [37]; hence, it may also play a key role in the pathophysiology of neuroinflammation and cognitive decline.

Animal studies consistently show that systemic proinflammatory cytokines communicate with the brain, possibly by impairing BBB function or *via* neuronal signaling [38–40]. The BBB is a

complex cellular gateway that regulates the transport of molecules into the brain and CNS; loss of BBB function is an hallmark of many neurological diseases such as Alzheimer's disease (AD) and multiple sclerosis (MS) [41]. A recent review indicates that aging, hypertension, diabetes, cholesterol, and inflammation can impair and disrupt the BBB, ultimately resulting in neurodegeneration and cognitive decline [42,43]. Recent studies have also implicated that aging deteriorates the structure and function of the BBB and together with an proinflammation systemic milieu may account for the neuronal loss and cognitive decline during aging [44,45].

To date, there is little direct evidence whether HMGB1 and BBB dysfunction could contribute to the cognitive impairment, especially when the injury is on a peripheral organ and not directly to the brain. Our results indicate that surgical trauma, and to a smaller extent anesthesia, induced spatial and learning impairment in aged rats (Figure 6D). It is possible that other factors, including general anesthesia, adjuvate this process by opening the BBB, thus facilitating neuroinflammatory processes and cognitive decline as we observed after day 1 (Figure 3) and up to day 3 (Figure 5). Other groups have reported a progressive breakdown of the BBB after isoflurane exposure [46,47]. The effects of anesthetics, surgical trauma, and preexisting comorbidities may actively contribute to the perioperative cognitive failure as often seen in our intensive care units (ICU) [48].

Some limitations of our study must be pointed out. The relationship between HMGB1 and cognitive dysfunction cannot be completely ascertained as we did not selectively block this molecule, for example using monoclonal neutralizing antibodies. Also, the mechanisms whereby the BBB is impaired after surgery require further understanding, and in this context, neuronal path-

ways may also contribute to the neuroinflammatory response. Recently, infiltration of peripheral macrophages through a damaged BBB was observed after orthopedic surgery, and stimulation of the cholinergic antiinflammatory pathway improved cognitive outcome [40]. Indeed, animal models cannot completely reproduce the clinical situation and its complexity; it remains essential to confirm whether similar changes occur in patients after surgery.

Conclusion

We have demonstrated that proinflammatory cytokines including HMGB1, TNF- α , IL- β , and RAGE signaling were upregulated by anesthesia and surgical trauma. This appears to contribute to the neuronal damage and cognitive dysfunction in aged rats. Increased BBB permeability seems a key hallmark in the development of neuroinflammation and cognitive decline. HMGB1 may represent a novel viable target to interrupt and modulate the inflammatory response underlying the pathogenesis of POCD.

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Conflict of Interest

The authors have no conflict of interest.

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