RESEARCH PAPER

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Peripheral and cerebral inflammation induced by repeated anesthesia and surgery do not cause impairment of learning and memory in middle-aged mice

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Postoperative cognitive dysfunction is a postoperative complication of the central nervous system that reduces quality of life and increases mortality in perioperative patients, especially among elderly patients. Many studies have shown that the incidence of postoperative cognitive impairment in adults induced by one-time anesthesia and surgery is very low, while multiple experiences of anesthesia and surgery can induce cognitive impairment in the developing brain. However, the effect of multiple experiences of anesthesia and surgery on cognitive function over a short period in middle-aged mice, i.e., 6 to 8 months old, remains unclear. In this study, we explored whether the cognitive function of mice aged 6–8 months is impaired after multiple operations. Middle-aged mice (6 to 8 months old) healthy male C57BL/6 mice underwent exploratory laparotomy under isoflurane anesthesia. Morris water maze testing was performed after the operations. Blood and brain samples were collected at 6 h, 24 h, and 48 h after the operations. Serum IL-6, IL-1, and S-100β concentrations were detected by ELISA. The expressions of ChAT, AChE, and Aβ in the hippocampus were measured by western blot. Up-regulation of Iba1 and GFAP, respectively, indicated activation of microglia and astrocytes in the hippocampus. Expression of Iba1 and GFAP was examined by immunofluorescence. The present results revealed that serum IL-6, IL-1β, and S-100β concentrations were enhanced after multiple instances of anesthesia and surgery, and microglia and astrocytes in the hippocampus were activated. However, learning and memory were not impaired in the middle-aged mice by multiple experiences of anesthesia and surgery. There were no changes in ChAT, AChE, and Aβ in the hippocampus after multiple experiences of anesthesia/surgery. Taken together, we suggest that although multiple anesthesia/surgery procedures can induce peripheral inflammation, neuroinflammation, and transient cerebral injury, it is insufficient to impair learning and memory in middle-aged mice.

Key words: cognitive function, anesthesia, surgery, inflammation, central cholinergic system, microglia, astrocyte

INTRODUCTION

Postoperative cognitive dysfunction (POCD) has long been noted in clinical practice; it is a postoperative complication of the central nervous system, with the primary clinical symptom being the decline of short- and long-term learning and memory function (Newman et al., 2001; Bilotta et al., 2010; Monk and Price, 2011). Patients with POCD show a significantly lower quality of life, higher mortality, and early retirement from work (Steinmetz et al., 2009). Thus, POCD has become a social problem of particular concern to anesthesiologists, surgeons, and their patients.

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Although the precise mechanisms leading to POCD have not been fully elucidated, accumulating evidence has indicated that neuroinflammation plays a vital role in the pathogenesis of POCD (Wang et al., 2014; Li et al., 2016; Luo et al., 2019; Safavynia and Goldstein, 2019). Surgical trauma or anesthesia can increase the level of proinflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and S-100β calcium-binding protein. Pro-inflammatory cytokines enter the brain and cause neurotoxic symptoms (Balusu et al., 2016). Increased levels of these inflammatory factors are associated with the occurrence of POCD (Cibelli et al., 2010; Mooijaart et al., 2013; Gan et al., 2020: Kavrut Ozturk et al., 2020). Peripheral inflammation is considered to trigger cerebral β-amyloid (Aβ) accumulation and cognitive impairment (Cattaneo et al., 2017; Marottoli et al., 2017). Through the inhibiting accumulation of Aβ, rapamycin can attenuate postoperative cognitive deficits (Shen et al., 2016). Aβ might be a biochemical marker for us in predicting the occurrence of POCD (Xie et al., 2006; Geng et al., 2017; Wu et al., 2018). The central cholinergic system may be another important player in the pathogenesis of POCD. The central cholinergic nerves play a critical role in the formation and maintenance of learning and memory (Chen et al., 2018; Zhang et al., 2018; Xu et al., 2019; Zhu et al., 2021). The described aspects of inflammation, Aβ accumulation, and the cholinergic system are all important in the pathogenesis of POCD. Therefore, this study selected serum IL-6, IL-1β and S-100β, as well as choline acetyltransferase (ChAT), acetylcholinesterase (AChE), and Aβ in the hippocampus, as the main index to explore how multiple operations affect learning and memory in middle-aged mice.

Age is an independent risk factor for POCD (Benson et al., 2017), as many studies have shown that a single one-time surgery did not result in impairment of cognitive function in mice aged 4–6 months (Rosczyk et al., 2008; Wuri et al., 2011). A previous study demonstrated that learning and memory in younger adult mice (2 months old) were not affected after repeated anesthesia and surgery, although mild neuroinflammation was observed (Zhou et al., 2020). Regarding the effect of multiple anesthesias and surgery procedures on learning and memory in middle-aged mice (6 to 8 months old), no data was found. Unfortunately, at this age, in clinical settings, many diseases may require repeated operations. Examples include skin flap transplantation after a fracture or plastic surgery for the skin after a burn injury. Thus, in the present study, we aimed to evaluate whether learning and memory in mice aged 6–8 months was impaired after multiple operations, with the multiple operation subjects undergoing surgery every four days (3 times in total).

METHODS

Animals

6 to 8-month-old male C57BL/6J mice were provided by Hunan SJA Laboratory Animal Co., Ltd. The mice were divided into three groups: control, single anesthesia and surgery, and multiple anesthesia and surgery. Mice were kept under a 12 h light-dark cycle and controlled room conditions (24 ± 2°C; 50 ± 10% humidity). The mice had free access to food and water. All the mice were acclimated for seven days before the experiment began.

This study was approved by the Animal Care and Use Committee of the Second Affiliated Hospital of Jiaxing University. All animal procedures were in accordance with the NIH Guide for Care and Use of Laboratory Animals.

Surgery model

In accordance with previous studies, mice were anesthetized with isoflurane (including 2.0% isoflurane in 0.30 FiO_2) (Feng et al., 2017). The mice underwent an exploratory laparotomy (Li et al., 2016). First, a 3 cm incision was made in the middle of the abdomen. Second, sterile cotton swabs were dipped in wet normal saline, and the abdominal organs, such as liver, spleen, kidney, and small intestine, were explored successively. The exploration of the abdominal cavity lasted for three minutes and the exposure lasted for three minutes. The whole experiment took approximately thirty minutes. Third, around the incision, 0.1% lidocaine infiltration was used as postoperative analgesia, and then the wound was closed by 5-0 Vicryl sutures. The temperatures of the mice were maintained at 36–37°C throughout the duration of the experiment. After surgery, the mice were spontaneously resuscitated. After the single anesthesia and surgery group mice underwent their first operation, the multiple anesthesia and surgery group mice underwent the same operation, for a total of 3 times.

Behavioral testing

Morris water maze (MWM) testing was performed to assess learning and memory in the mice, following the protocol of a previous study in which the mice rested for two days after operations (Morris et al., 1982; Su et al., 2011). The MWM consisted of a white circular pool, 110 cm in diameter and 60 cm deep, with a circular platform of 10 cm in diameter hidden at 1.0 cm beneath the water's surface. The pool was filled with opaque milky water (23–25°C) to a depth of 35 cm. The pool

was surrounded by invariable visual cues, which were not changed until the end of the experiment. All MWM test subjects were monitored and tracked via a television camera (HIK VISION Co., Ltd., Hangzhou, China) mounted overhead.

The MWM test included a location-based navigation test (training) and a space probe test (testing). The location-based navigation test was performed for 4 days. Each day, mice were put into the maze at different points. Once the mouse found the platform, it was allowed to rest on the platform for 30 s. If the mouse did not find the platform within 60 s, it was guided to the platform to rest for 30 s. Latency to reach the platform, swimming speed, and time spent in each quadrant were calculated from the recorded videos using MWM software (RWD Co., Ltd., Shenzhen, China). The space probe test was completed on the 7th day after the operations. In this test, the platform was removed and mice swam for 60 s. The swimming time in each quadrant was recorded.

Enzyme-linked immunosorbent assay (ELISA)

Blood samples were obtained at 6 h, 24 h, and 48 h after operations. Then, centrifugation was performed at 3000 × g at 4°C for 10 min, and the supernatant (serum) was collected. The supernatants were analyzed for IL-1β and IL- 6 using an ELISA kit (Thermo Fisher Scientific, Vienna, Austria) and S-100β using the ELISA kit (Cloud-Clone Crop, Houston, USA), according to the manufacturer's protocol. The intensity of the color was measured using a spectrophotometer at a wavelength of 450 nm (Epoch, BioTeK).

Immunofluorescence staining

Immunofluorescence staining of the microglial marker, ionized calcium-binding adapter molecule 1 (Iba1), and the astrocyte marker, glial fibrillary acidic protein (GFAP), were used to study microglial and astrocyte morphology. Brains were fixed by 4% paraformaldehyde and dehydrated with 30% sucrose solution. The samples were freeze-mounted in OCT embedding medium. Brain tissue coronal slices (4 μm thick) were obtained in a freezing microtome (Leica, China). The brain tissue slices were washed with PBS and 0.4% Triton X-100, then sections of brain tissue were blocked with 10% normal donkey serum (Servicebio, Wuhan, China). The brain slices were incubated with Iba1 antibody (1:500, Abcam) and GFAP antibody (1:1000, Abcam) overnight at 4°C and then incubated with FITC-labeled secondary antibodies (1:300, Abcam) for 1 h at room temperature. The tissue images were visualized and captured using a confocal fluorescence microscope (Eclipse C1, Nikon). The fluorescence signal intensity was quantified using Image-Pro-Plus 6.0.

Western blot

The hippocampal tissues were homogenized in cell lysis buffer containing complete protease inhibitor mix (Servicebio, Wuhan, China) and then separated by centrifugation (12,000 × g for 20 min, 4°C). The concentration of protein in the supernatants was determined using a bicinchoninic acid (BCA) assay kit (Servicebio, Wuhan, China). Proteins were separated by 10% SDS-PAGE gels and transferred to PVDF membranes (Millipore). Next, the membranes were blocked with 5% TBST for 60 min and then incubated overnight at 4°C with primary antibodies, including acetylcholinesterase antibody (AChE, 1:1000, Abcam), β-amyloid antibody (Aβ, 1:1000, Abcam) and choline acetyltransferase antibody (ChAT, 1:1000, Abcam). Following three washes (5 min each) in TBST, the membranes were exposed to HRP-conjugated secondary antibodies for 60 min at room temperature. The chemiluminescence signal was detected using an ECL kit (Servicebio, Wuhan, China). The membranes were detected using an enhanced chemiluminescence system (CLINX, China), and quantitative analysis of protein expression was performed using AlphaEaseFC (Alpha Innotech).

Statistical analysis

The results are presented as mean ± SEM. Data were analyzed using GraphPad Prism 6.0. The escape latency during location-based navigation and the average swimming speed were analyzed at different time points after anesthesia and surgery using two-way analysis of variance (ANOVA). Data from the space probe test, the levels of IL-6, IL-1β, and S-100β, the quantified fluorescence of microglia and astrocytes, and the concentration of Aβ, AChE, and ChAT were analyzed using one-way ANOVA. $P<0.05$ was considered statistically significant.

RESULTS

Single or multiple anesthesia/surgery procedures had no effects on learning and memory in middle-aged mice

To determine whether single or multiple exposures to anesthesia and surgery caused impairment

of learning and memory in middle-aged mice, we utilized the MWM after one or three operations. The experiment was carried out after mice had been allowed to adapt to the environment for 7 days. An exploratory laparotomy was performed under isoflurane anesthesia; the operation was performed every 4 days for a total of 3 operations. After the final operation, the mice rested for 2 days. The location-based navigation test (training) lasted for 4 days, and the space probe test (test) was carried out on day 15 (Fig. 1). The mean

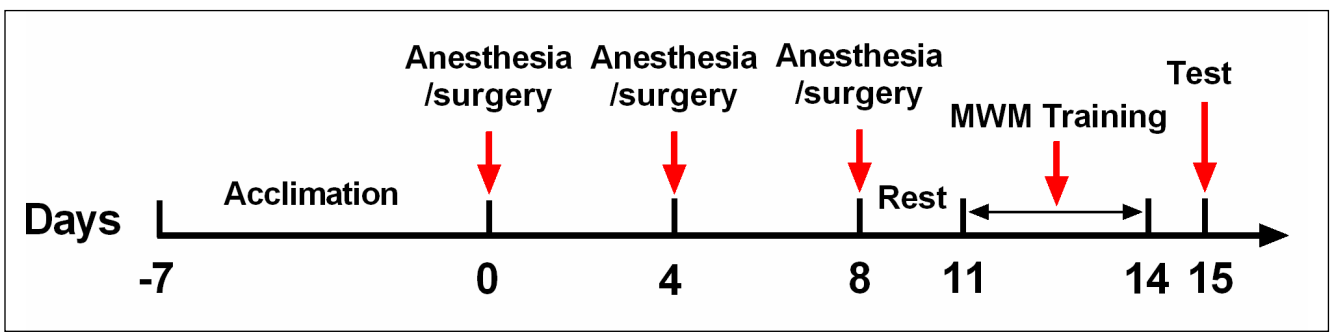

Fig. 1. Experimental protocol. Schematic timeline of the experimental paradigm of the surgery procedure and Morris water maze test. Mice in the multiple anesthesia/surgery group underwent surgery every 4 days for a total of 3 operations. The mice were allowed to rest for 2 days after the three operations, then, training was conducted for 4 days, and probe tests were conducted on day 15.

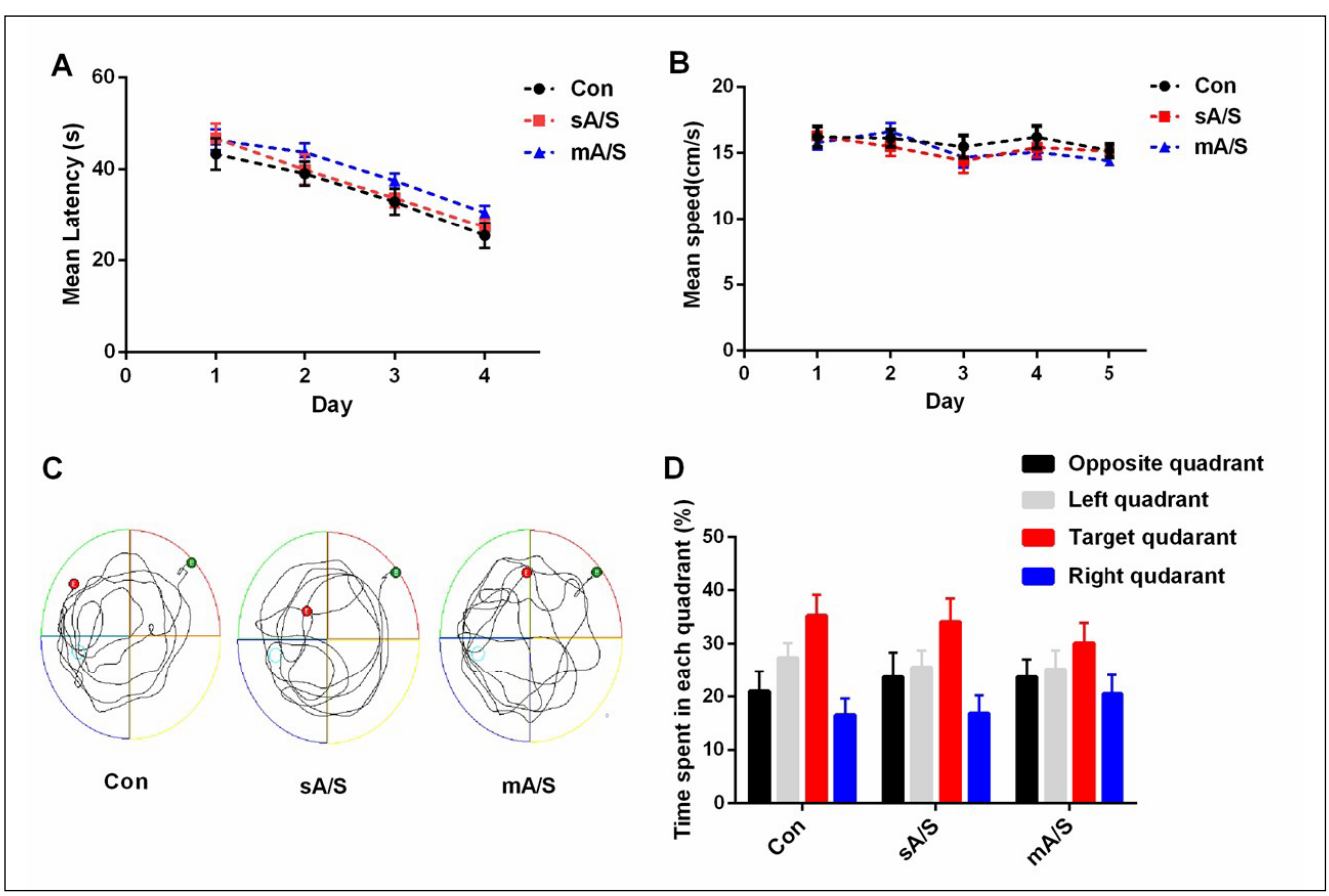

Fig. 2. Multiple anesthesia/surgery procedures did not impair learning and memory in middle-aged mice. (A) Escape latency to reach the hidden platform during the 4-day training. (B) Average swimming speed. (C) Representative exploratory path of three groups of mice in the probe test. (D) Time spent in each quadrant during the probe test. Data are expressed as mean ± SEM (n=12 per group). Labels: (Con) control; (sA/S) single anesthesia/surgery; (mA/S) multiple anesthesia/surgery.

latency to reach platform for the mice from the single and multiple anesthesia and surgery groups was not significantly prolonged during the location-based navigation test than in the control mice (F=0.970, P>0.05, Fig. 2A). Average swimming speed did not differ significantly between the location-based navigation and space probe tests among the three groups (F=0.916, P>0.05, Fig. 2B). In the space probe test, time spent in the target quadrant was comparable among the three groups (F=0.003, P>0.05, Fig. 2D). These findings indicate that single or multiple anesthesia and surgery procedures did not cause impairment of learning and memory in middle-aged mice.

Peripheral inflammation and transient cerebral injury were caused by single and multiple instances of anesthesia and surgery

Single and multiple anesthesia and surgery procedures induced peripheral inflammation. Serum IL-6 levels increased significantly at 6 and 24 h after one-time anesthesia and surgery (F=46.13, P<0.0001, Fig. 3A) and returned to a normal range at 48 h after once anesthesia and surgery. Moreover, Serum IL-6 and IL-1β levels were elevated significantly at 6 h after multiple anesthesia and surgery procedures (F=46.13, P<0.0001 and F=11.67, P<0.01, respectively, Fig. 3A, B). Serum IL-1β was significantly reduced at 24 h after multiple anesthesia and surgery procedures (F=11.67, P<0.05, Fig. 3B).

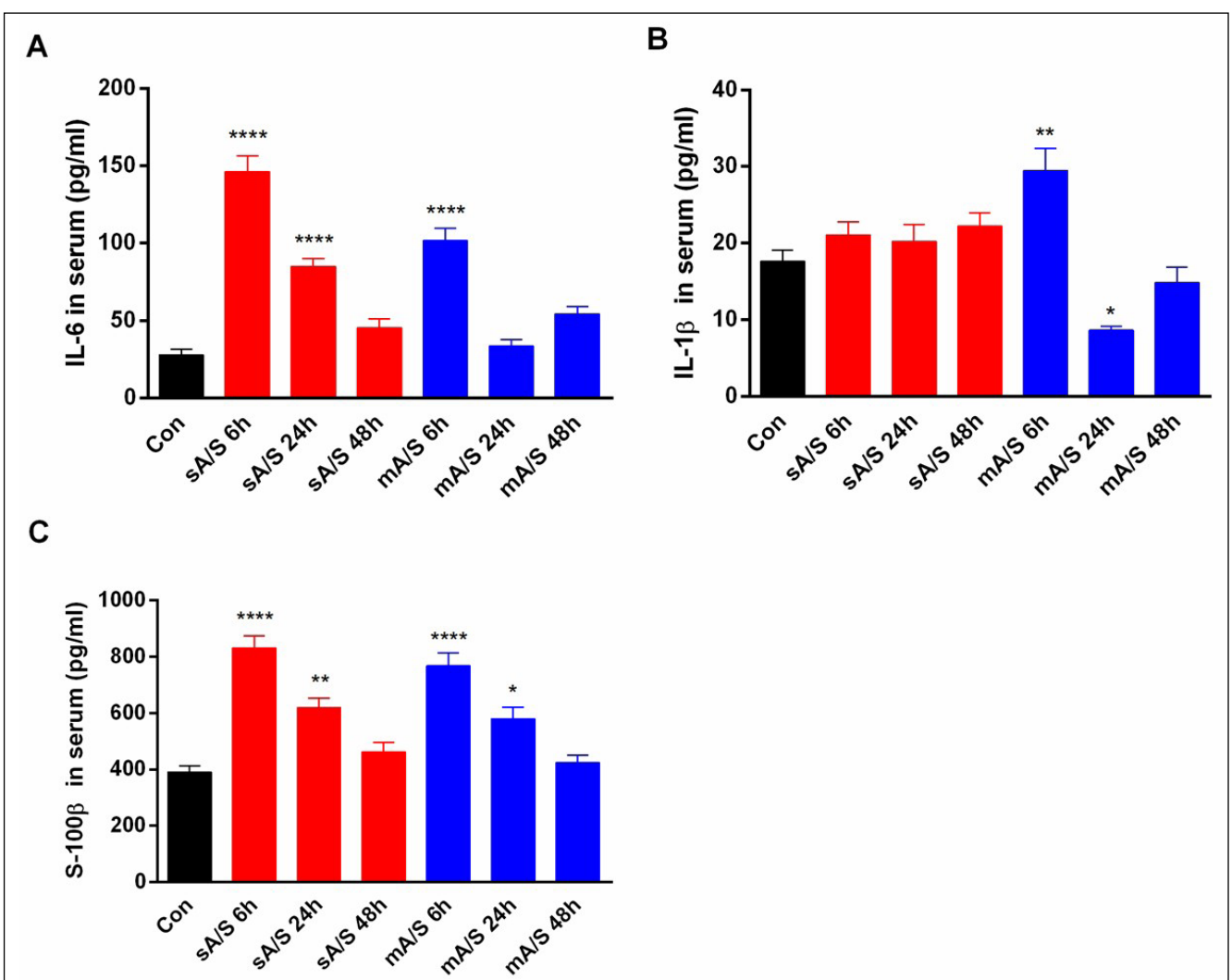

Fig. 3. Single and multiple anesthesia/surgery procedures induced peripheral inflammation and transient cerebral injury. (A-C) The levels of IL-6, IL-1β, and S-100β in blood at 6 h, 24 h, and 48 h after single and multiple anesthesia/surgery procedures. Anesthesia/surgery significantly increased the upregulation of IL-6, IL-1β, and S-100β in serum. Data are expressed as mean ± SEM (n=6 per group) with ****P<0.0001, **P<0.01, and *P<0.05 versus control. Labels: (Con) control; (sA/S) single anesthesia/surgery; (mA/S) multiple anesthesia/surgery.

S-100β is predominantly found in the CNS and its value may reflect neuroinflammation and injury. Single and multiple anesthesia/surgery procedures induced transient cerebral injury. S-100β in serum increased significantly at 6 and 24 h after once and multiple anesthesia and surgery procedures, and it returned to a normal range at 48 h (F=21.53; P<0.0001, P<0.01 and P<0.05, respectively, Fig. 3C).

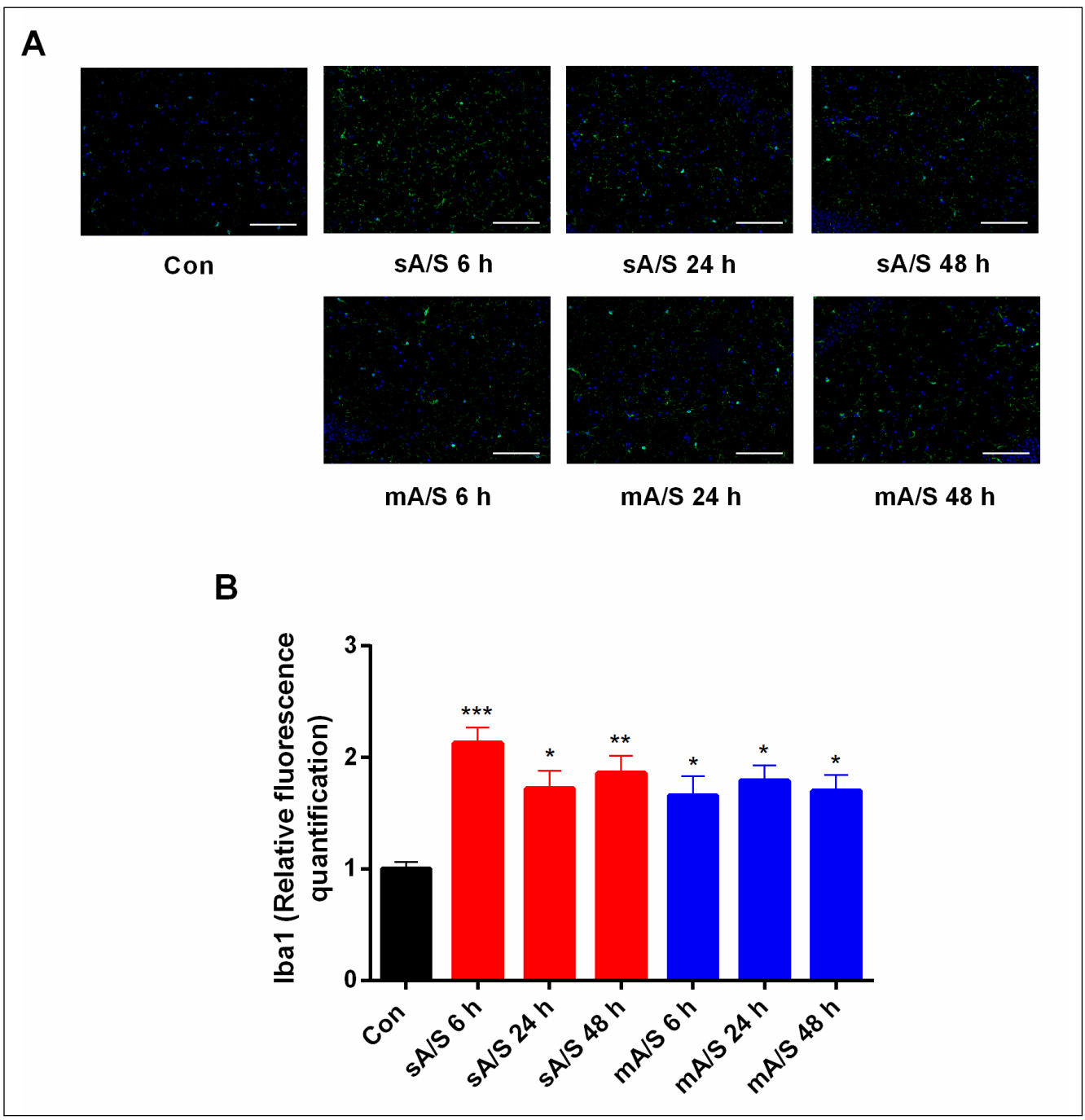

Fig. 4. Single and multiple rounds of anesthesia/surgery induced the activation of microglia in the hippocampus. (A) Representative immunofluorescence staining of the microglial marker Iba1 (in green) in the hippocampus at 6 h, 24 h, and 48 h after operations in middle-aged mice; the nucleus was stained with DAPI (in blue). (B) Fluorescence signal intensity was quantified using Image-Pro-Plus 6.0. Scale bar: 100 μM. Data are expressed as mean ± SEM (n=4 per group) with ***P<0.001, **P<0.01, and *P<0.05 versus control. Labels: (Con) control; (sA/S) single anesthesia/surgery; (mA/S) multiple anesthesia/surgery.

Single and multiple anesthesia and surgery procedures activated hippocampal microglia and astrocytes

The activation of microglia and astrocytes indicates the occurrence of a central inflammatory response. Hippocampal sections were stained for Iba1 and GFAP at 6 h, 24 h, and 48 h after anesthesia and surgery. Iba1 and GFAP immunofluorescent staining showed that there was little activation of hippocampal microglia and astrocytes in the control group (Fig. 4A and Fig. 5A). However, results from single and multiple

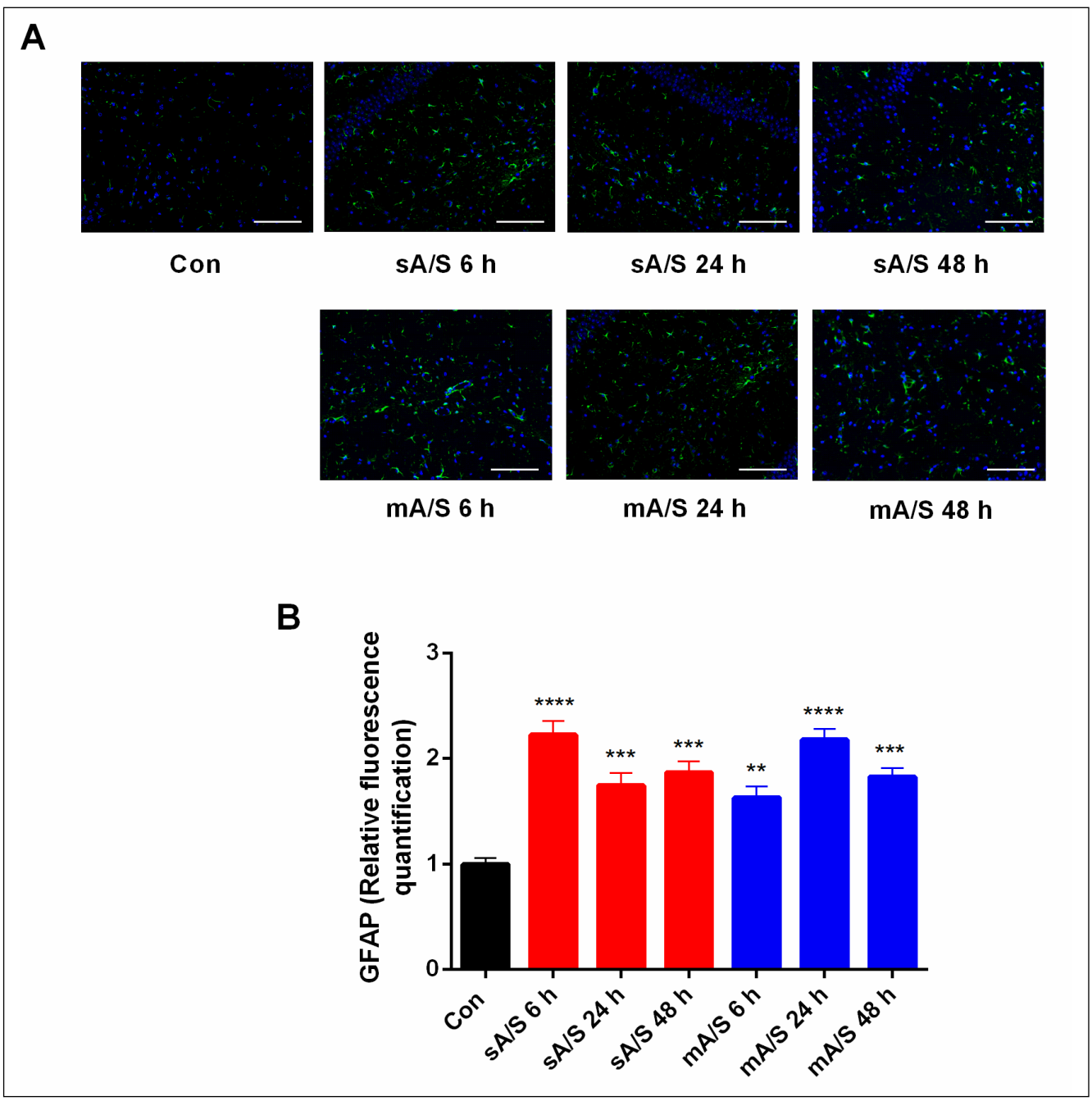

Fig. 5. Single and multiple rounds of anesthesia/surgery induced the activation of astrocytes in the hippocampus. (A) Representative immunofluorescence staining of the astrocytic marker GFAP (in green) in the hippocampus at 6 h, 24 h, and 48 h after operations in middle-aged mice; the nucleus was stained with DAPI (in blue). (B) Fluorescence signal intensity was quantified using Image-Pro-Plus 6.0. Scale bar: 100 μM. Data are expressed as mean ± SEM (n=4 per group) with ****P<0.0001, ***P<0.001, and **P<0.01 versus control. Labels: (Con) control; (sA/S) single anesthesia/surgery; (mA/S) multiple anesthesia/surgery.

anesthesia and surgery group staining showed strong hippocampal microglia and astrocyte activation at 6 h, 24 h, and 48 h after operations (respectively, microglia: F=5.965; P<0.001, P<0.01 and P<0.05, Fig. 4A, B; astrocytes: F=15.60; P<0.0001, P<0.001 and P<0.01, Fig. 5A, B). Collectively, these findings indicate that performing single and multiple anesthesia and surgery procedures causes the release of inflammatory cytokines and a central inflammatory response.

Hippocampal Aβ level was not elevated by single or multiple anesthesia and surgery procedures

A previous study demonstrated that inflammatory cytokines could induce Aβ (Hur et al., 2020). So, in order to determine whether single and multiple anesthesia and surgery procedures affected the levels of hippocampal Aβ, we analyzed Aβ changes in the hippocampus at 6 h, 24 h, and 48 h after operations. No significant difference was observed in the levels of hippocampal Aβ after single or multiple anesthesia and surgery procedures (F=2.448, P>0.05, Fig. 6A, B). These findings indicate that anesthesia and surgery do not elevate levels of this cognition-related protein in middle-aged mice.

The central cholinergic system was not affected by single or multiple anesthesia and surgery procedures in middle-aged mice

To investigate the effects of anesthesia and surgery on the central cholinergic system in middle-aged mice we analyzed ChAT and AChE changes in the hippocampus at 6 h, 24 h, and 48 h after operations. There was no significant difference in the levels of ChAT and AChE in the hippocampus after one-time or multiple anesthesia and surgery procedures (F=2.258, F=0.540, P>0.05, Fig. 7A, B). These findings indicate that anesthesia and surgery do not elevate levels of cognition-related proteins in middle-aged mice.

DISCUSSION

To our knowledge, this is the first study to explore whether multiple rounds of anesthesia and surgery

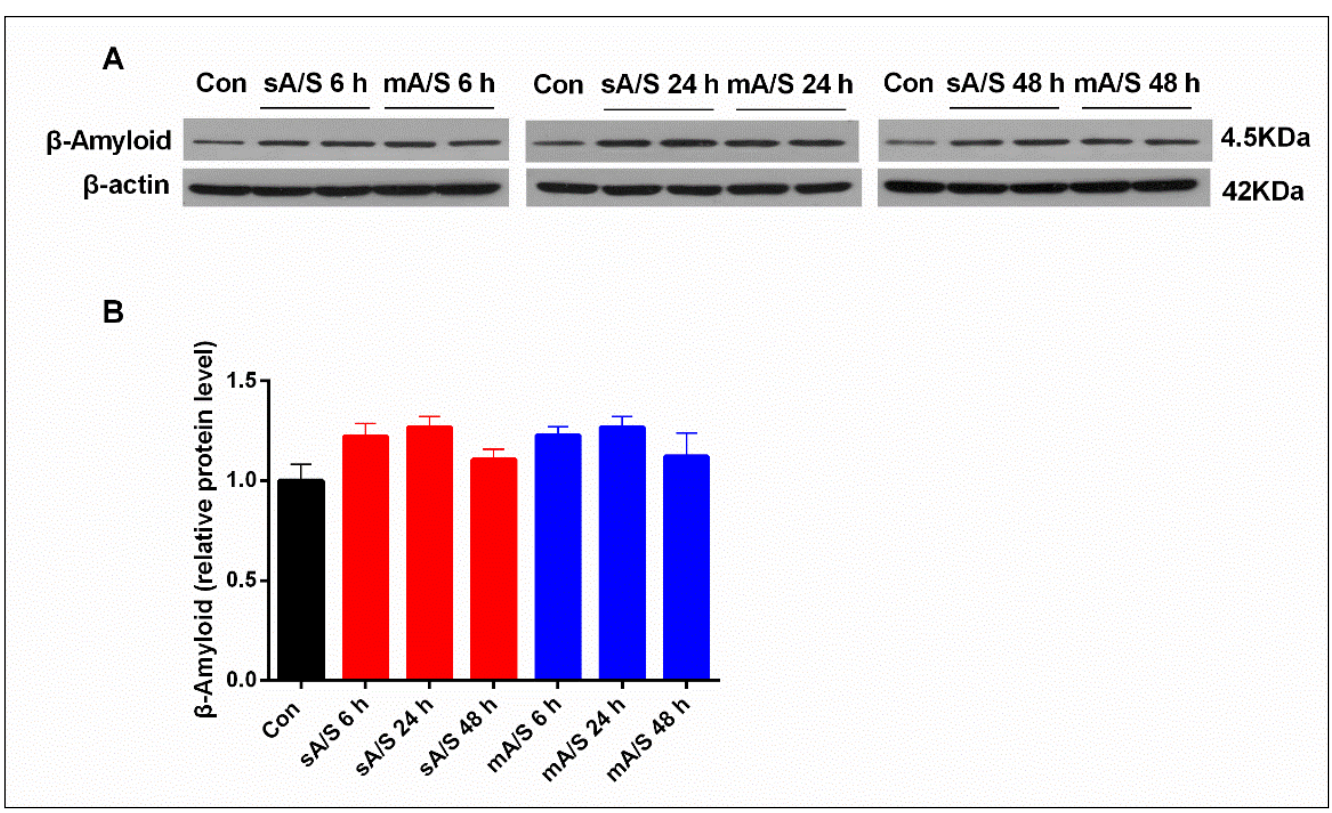

Fig. 6. Single and multiple rounds of anesthesia/surgery did not cause a rise in the level of hippocampal Aβ. (A) Representative bands of western blot for β-amyloid in hippocampus. (B) Quantification of β-amyloid protein levels in hippocampus; β-actin was used for normalizing protein levels. Data are expressed as mean ± SEM (n=4 for each group). Labels: (Con) control; (sA/S) single anesthesia/surgery; (mA/S) multiple anesthesia/surgery.

could cause learning and memory dysfunction in middle-aged mice. In the present study, we tested learning and memory in middle-aged mice and evaluated for changes in peripheral and cerebral inflammation, Aβ, and the central cholinergic system in the hippocampus after multiple rounds of anesthesia and surgery. The findings showed that multiple anesthesia and surgery procedures did not induce a learning and memory deficit in middle-aged mice, although microglia and astrocytes were activated and inflammatory cytokines were increased after anesthesia and surgery.

Multiple rounds of anesthesia and surgery can cause brain damage in patients at different developmental stages. Animal and clinical studies have demonstrated the effect of multiple anesthesia exposures on the developing brain (Coleman et al., 2017; Jiang et al., 2018; Makaryus et al., 2018; Oba et al., 2019). Previous studies showed that multiple exposures to anesthesia and surgery did not induce learning and memory impairment in young brain (2-month-old mice) (Zhou et al., 2020). In the current study, we found that a central inflammatory response was induced by multiple anesthesia and surgery procedures, however, it did not impair learning and memory in middle-aged mice (6 to 8-month-old mice).

Animal and clinical studies have shown that neuroinflammation plays an important role in the occurrence of POCD (Xu et al., 2014; Zhang et al., 2014; Hovens et al., 2016; Hirsch et al., 2016; Berger et al., 2019; Yan et al., 2019). Surgical stress causes inflammation and releases a significant amount of proinflammatory cytokines. These proinflammatory cytokines travel through the damaged blood-brain barrier to result in neuroinflammation. Several studies showed that peripheral immune cells invaded the central nervous system (Degos et al., 2013; Hovens et al., 2015) and delayed resolution of the postoperative neuroinflammatory response (Vacas et al., 2013; Terrando et al., 2011).

These are consistent with our observation; our results showed that the concentration of IL-6 and IL-1β in the serum were has risen at 6 h after anesthesia and surgery and returned to a normal level at 48 h after

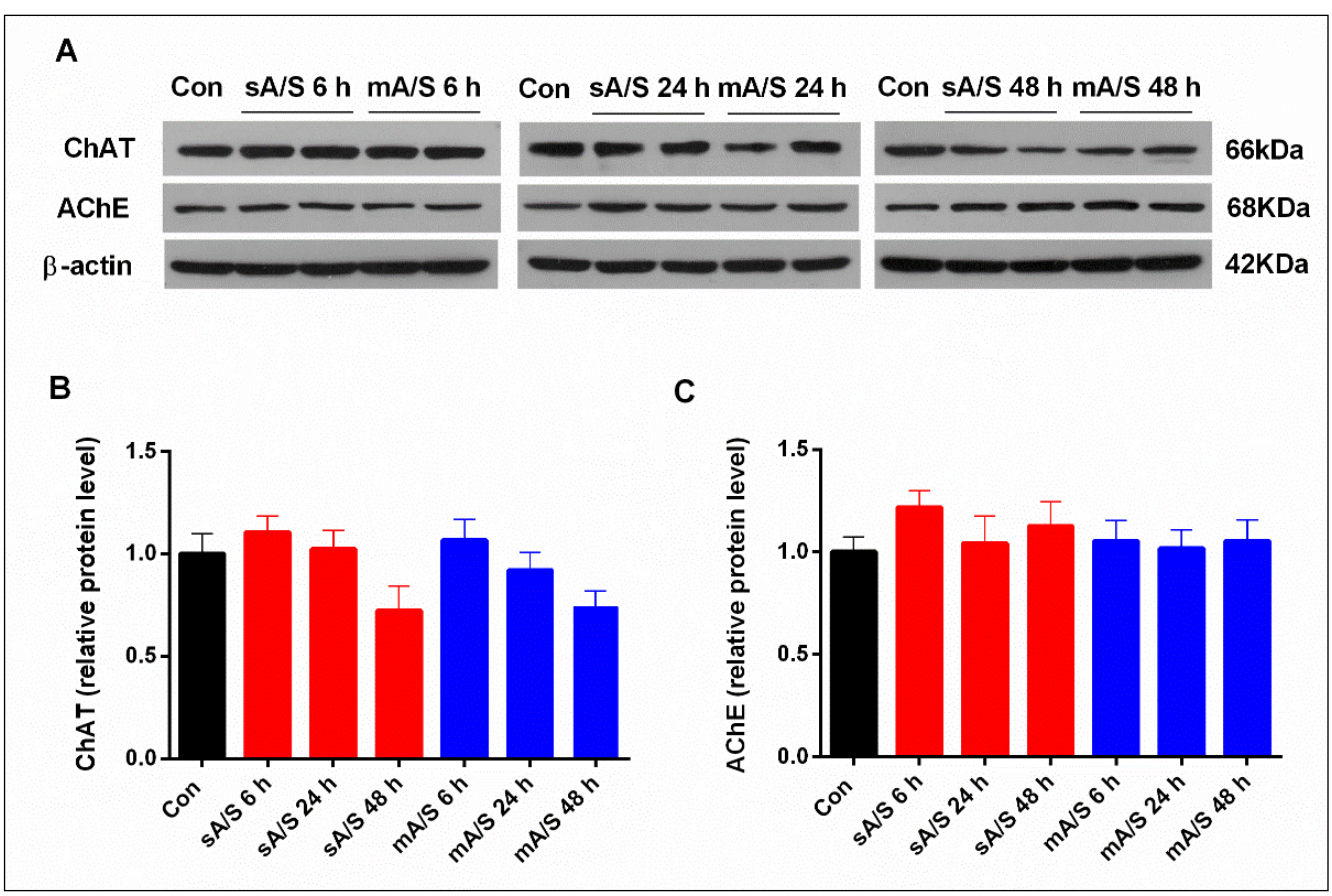

Fig. 7. Single and multiple anesthesia/surgery procedures had no effects on the central cholinergic system. (A) Representative bands of western blot for ChAT and AChE in hippocampus. (B-C) Quantification of ChAT and AChE protein levels in hippocampus. β-actin was used for normalizing protein levels. Data are expressed as mean ± SEM (*n*=4 for each group). Labels: (Con) control; (sA/S) single anesthesia/surgery; (mA/S) multiple anesthesia/surgery.

anesthesia and surgery. Meanwhile, microglia and astrocytes were activated in the hippocampus at 6 h, 24 h, and 48 h after multiple anesthesia and surgery procedures. This suggests that neuroinflammatory responses occur in the brain and delay resolution. In a study using aged mice, anesthesia and surgery induced a decrease in cognitive function and increased levels of IL-1β and IL-6 in the brain and activated astrocytes, while pioglitazone significantly improved cognitive function and inhibited central inflammatory response (Zhang et al., 2017). This indicates that the neuroinflammatory pathogenesis of POCD may be related to age. Accumulating evidence shows that age-related neuroinflammatory changes may be associated with postoperative cognitive impairment (Hovens et al., 2015; Xu et al., 2014; Yang et al., 2017). Our findings showed that multiple rounds of anesthesia and surgery caused neuroinflammation but did not impair learning and memory in middle-aged mice.

Based on the research results of others and the results of this study, we speculate on what may explain this phenomenon. First of all, the immunological system in middle-aged mice can resolve inflammation to inhibit brain injury. Our results regarding S-100β, a serum marker of brain injury, showed that the serum concentration rose at 6 h after anesthesia and surgery and reduced to a normal level at 48 h after anesthesia and surgery. Second, although multiple rounds of anesthesia and surgery caused neuroinflammation, the inflammatory response may not have been severe enough to cause a learning and memory impairment in the middle-aged mice. For example, IL-1β and TNF-α can inhibit microglial phagocytosis and increase Aβ deposition in Alzheimer's disease (Pan et al., 2011); and Hur and colleagues (2020) found that neuroinflammation can induce the expression of IFITM3 in the brain, and IFITM3 increased γ-secretase activity, thereby, increasing Aβ deposition. Interestingly, our results showed that microglia and astrocytes were overactivated in the hippocampus at 6 h, 24 h, and 48 h after multiple anesthesia and surgery procedures, nevertheless, there was no change in Aβ levels in the hippocampus. Third, it is possible that the first two operations preconditioned the host's immune system so that it could respond more effectively to subsequent infections. Mice underwent three consecutive intraperitoneal injections of *E. coli* (euflammation), then an acute inflammatory response was induced by administering E.coli or LPS (Liu et al., 2016). Euflammation can effectively inhibit peripheral and central inflammatory responses induced by injections of LPS or *E. coli*, indicating that the effect of peripheral inflammation on the central nervous inflammatory system is dynamically regulated by the dynamics of peripheral inflammation. Therefore, supposing that the prior two rounds of anesthesia/surgery produced a preconditioning effect, a decrease in serum inflammatory factors might activate anti-inflammatory signaling pathways. The final anesthesia/surgery procedure could be an acute immune response. Our results showed that serum IL-1β was significantly reduced at 24 h after operations.

The brain's cholinergic system modulates memory and hippocampal plasticity (Maurer et al., 2017). The brain's cholinergic system plays an important role in the protection of cognitive function. Degeneration of the brain's cholinergic system has been revealed to be related to age-related cognitive decline (Bartus et al., 1982). Wu et al. (2017) found that the level of acetylcholine in the hippocampus was decreased in aged rats after anesthesia and surgery, correlating with a decline in cognitive function. Moreover, Chen et al. (2018) demonstrated that tacrine(10)-hupyridone could inhibit the activity of AChE and prevent short- and long-term cognitive impairment induced by surgery in aged mice. In the current study, the cholinergic system did not deteriorate after multiple anesthesia and surgery procedures; it was consistent with normal learning and memory. A stable central cholinergic system might also be a factor in why multiple rounds of anesthesia and surgery did not induce a learning and memory deficit in middle-aged mice.

The present study has several limitations to be addressed. We tested only at 7 days after anesthesia and surgery, so the long-term effect on learning and memory remains unknown. To prove this point, the MWM test should be used to further evaluate learning and memory 24–31 days postoperatively. The sample size of the biological data was also limited in this study. Additionally, other surgical procedures and anesthesia methods are needed to further investigate the role of surgery and anesthesia in the central inflammatory response and assess for any learning and memory changes that may follow.

CONCLUSION

In conclusion, it was shown that although multiple anesthesia/surgery procedures caused peripheral and cerebral inflammation, the inflammatory response did not lead to impairment of learning and memory in the middle-aged mice. In clinical practice, some patients may need multiple rounds of anesthesia and surgery for certain diseases or situations, requiring multiple surgeries in a short period of time. These findings provide theoretical guidance for related future clinical research.

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