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The impact of preoperative stress on age-related cognitive dysfunction after abdominal surgery: a study using a rat model

Natsuki Nakagoshi[1], Fabricio M Locatelli[1], Sonoe Kitamura[1], Seiji Hirota[1] and Takashi Kawano[1*]

Abstract

Objective This study examines the impact of preoperative stress on postoperative neuroinflammation and associated cognitive dysfunction, with a focus on aged individuals. The goal is to determine whether managing preoperative stress can enhance postoperative outcomes and lower the risk of cognitive impairment.

Results In aged rats, preoperative restraint stress significantly worsened neuroinflammation and cognitive deficits following abdominal surgery. Elevated levels of pro-inflammatory cytokines were observed in the hippocampus and medial prefrontal cortex two days post-surgery, and these effects persisted for twenty-eight days. In contrast, adult rats did not show significant changes in neuroinflammation or cognitive function due to preoperative restraint stress. An ex vivo analysis indicated that hippocampal microglia from aged rats exhibited an intensified proinflammatory response to lipopolysaccharide stimulation, further heightened by preoperative restraint stress. These findings suggest that managing preoperative stress could mitigate these adverse effects, leading to better postoperative recovery and cognitive health in elderly patients.

Keywords Preoperative stress, Neuroinflammation, Cognitive dysfunction, Postoperative delirium, Aged rats

Introduction

Postoperative delirium (POD) is a transient mental disturbance that occurs following surgery, characterized by confusion and cognitive decline [1]. Although typically short-lived, POD can delay recovery, prolong hospital stays, and increase the risk of long-term cognitive issues, particularly among elderly patients [2, 3].

Neuroinflammation, driven by microglial activation and cytokine release, plays a central role in POD [4]. Despite this understanding, no specific treatments are currently available, which highlights the importance of preventive measures, especially non-pharmacological strategies [5]. Therefore, identifying modifiable preoperative risk factors is crucial. While some factors, such as advanced age, are immutable, preoperative stress is modifiable and may contribute to POD by triggering neuroinflammation [6, 7].

We previously reported an animal model of POD, optimized for evaluating postoperative neuroinflammation and cognitive function [8]. Building on this foundation, the present study aims to further explore the impact of preoperative stress and aging on neuroinflammation. By expanding on our previous findings, we seek to provide novel insights into the prevention of POD in elderly patients and to refine strategies for mitigating cognitive decline associated with surgery.

*Correspondence:
Takashi Kawano
takashika@kochi-u.ac.jp
[1]Department of Anesthesiology and Intensive Care Medicine, Kochi Medical School, Oko-cho, Kohasu, Nankoku 783-8505, Kochi, Japan

Methods

Animals and experimental design

The study adhered strictly to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and received approval from the Institutional Animal Care and Use Committee of Kochi Medical School (Approval No. P-00076). Male Wistar rats, categorized as adults (2–4 months old) and aged (17–19 months old), were purchased from Alfresa Shinohara Chemicals Corporation (Kochi, Japan) and housed in our laboratory in pairs under standard laboratory conditions with a 12-hour light-dark cycle. Food and water were provided *ad libitum*, except during the restraint stress procedure. After randomization, all animals underwent a standard open-field test to assess baseline activity.

Three sets of experiments were conducted as indicated in Additional file 1: Fig. S1. Experiments 1 and 2 evaluated in vivo cognition and hippocampal cytokines during the acute and long-term postoperative phases. Rats were divided into four groups using a 2×2 design: Preoperative Restraint Stress (PRS) vs. non-PRS, and sham surgery vs. abdominal surgery. Experiment 1 assessed cognitive function two days post-surgery, while Experiment 2 focused on cognitive outcomes 28 days post-surgery. Each group included eight animals, as per our previous studies [8]. In Experiment-3, hippocampal microglia were isolated for ex vivo analysis at baseline (naïve and one-day after PRS) and 28 days after surgery or sham following PRS or non-PRS conditions. The data from the naïve group under baseline conditions were used as the data for the non-PRS/non-Surgery group.

Preoperative restraint stress procedure

On experimental days, rats were acclimated for one hour before procedures. The stress group underwent PRS for 2 h/day for three days, between 12:00 and 14:00, to avoid circadian rhythm influences. PRS involved placing the rat in a plexiglass restrainer to limit limb movement. After each PRS session, rats were returned to their home cages. Control animals were housed in the same room without food or water during the restraint period. After the final PRS treatment, animals were anesthetized and underwent laparotomy or sham procedures.

Anesthesia and surgery

Anesthesia was induced in an induction chamber flushed with 2% isoflurane in oxygen at 2 L/min until the animal became recumbent. The animal was then placed on a nose cone connected to a vaporizer to maintain isoflurane (1.0 to 1.5%) in oxygen at 0.5 L/min during the procedure. Abdominal surgery involved a 2-cm midline incision, small intestine manipulation, and closure. For postoperative analgesia, 0.2% ropivacaine (300 μL) was administered *via* wound infiltration. The surgery lasted 10 min. Control rats underwent anesthesia, shaving, and analgesia without surgery.

Trace fear conditioning task and tissue collection

Postoperative cognitive function was assessed using trace and context memory tests following trace fear conditioning on postoperative day 2 in Experiment 1 and on day 28 in Experiment 2. The trace fear conditioning task involved associating a conditioned stimulus (CS) with an unconditioned stimulus (US) across a time gap. The tests were performed 24 h after training. After the behavioral assessment, animals were sacrificed under deep anesthesia with isoflurane inhalation (3–5%) to ensure full unconsciousness. Tissues and plasma were then collected, with the hippocampus and medial prefrontal cortex (mPFC) being harvested and homogenized. The resulting supernatants were stored for subsequent analysis using an Enzyme-Linked Immunosorbent Assay (ELISA) to measure levels of Tumor Necrosis Factor-α (TNF-α) and Interleukin-1β (IL-1β). These cytokines were selected because they are well-established pro-inflammatory markers associated with neuroinflammation and cognitive disorders in neurodegenerative diseases [9, 10]. In Experiment 3, hippocampal microglia were isolated, plated, and stimulated with lipopolysaccharide (LPS) to assess cytokine release as an indicator of microglial immune reactivity.

For more detailed materials and methods, see Additional File 2.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). The Shapiro-Wilk test was used to assess the normality of data distributions. Specifically, differences between groups were analyzed using the Kruskal–Wallis test followed by pairwise Wilcoxon–Mann–Whitney tests with Bonferroni correction for multiple comparisons. Two-way ANOVA was applied to evaluate the effects of age group and experimental conditions on normally distributed data. A p-value < 0.05 was considered statistically significant. Data were processed using SAS (v9.3) and SPSS (v11).

Results

Baseline behavioral and physiological assessments

Before experiments, all animals underwent open-field tests to assess baseline psychomotor states. Habituation and locomotor counts were consistent across groups, indicating similar baseline activity, anxiety, and adaptive behavior, in both adult (Additional file 3: Fig. S2A-D) and aged (Additional file 4: Fig. S3A-D) animals. The PRS paradigm induced a comparable stress response, indicated by elevated plasma corticosterone levels, in both adult and aged rats (Additional file 5: Fig. S4). There were

no differences in arterial oxygen saturation ($p=0.73$), pulse rate ($p=0.84$), or body temperature ($p=0.77$) during anesthesia (Additional file 6: Table S1).

Memory retention tests two days after surgery

During training on the first day post-surgery, freezing responses were similar across groups in both adult ($F_{(3,\ 224)}=0.31$, $p=0.82$) and aged ($F_{(3,\ 224)}=0.46$, $p=0.71$) animals (Additional file 7: Fig. S5A-B), indicating intact fear memory acquisition. Trace memory retention in a novel chamber showed increased freezing after the CS in all groups, Additional file 8, Adult: $p<0.05$ (Fig. S6A); Aged: $p<0.05$ (Fig. S6B). Subsequent pairwise comparisons demonstrated that, while the freezing percentage did not differ between the non-PRS/non-Surgery and PRS/non-Surgery groups, surgical rats had significantly lower freezing compared to non-surgical groups in both adult (Fig. 1A, $p<0.05$) and aged (Fig. 1B, $p<0.05$) animals. Notably, PRS significantly exacerbated this impairment in aged rats ($p<0.05$) but had no effect on adults ($p=0.67$).

Context retention testing revealed a significant main effect of group in both adult (Fig. 1C, $p<0.05$) and aged (Fig. 1D, $p<0.05$) animals. In the non-surgical groups of both adult and aged rats, PRS did not affect the percentage of freezing behavior. However, surgical rats exhibited significantly lower freezing behavior compared to control animals (Adults: $p<0.05$; Aged: $p<0.05$). Moreover, PRS further impaired context memory retention in aged PRS/surgical rats compared to the aged non-PRS/surgery group ($p<0.05$). This additional impairment, however, was not observed in adult surgical animals.

Memory retention tests twenty-eight days after surgery

During training, no significant differences were observed in trace fear conditioning acquisition among the groups (Additional file 9: Fig. S7A-B). In adult rats, both trace (Fig. 1E, $p=0.65$) and context (Fig. 1G, $p=0.93$) memory retention were similar across the groups, indicating no surgery-induced cognitive impairment in the late post-operative period. In aged rats, while trace memory was unaffected by PRS (Fig. 1F, $p=0.89$), context memory retention was significantly impaired in the PRS/surgery

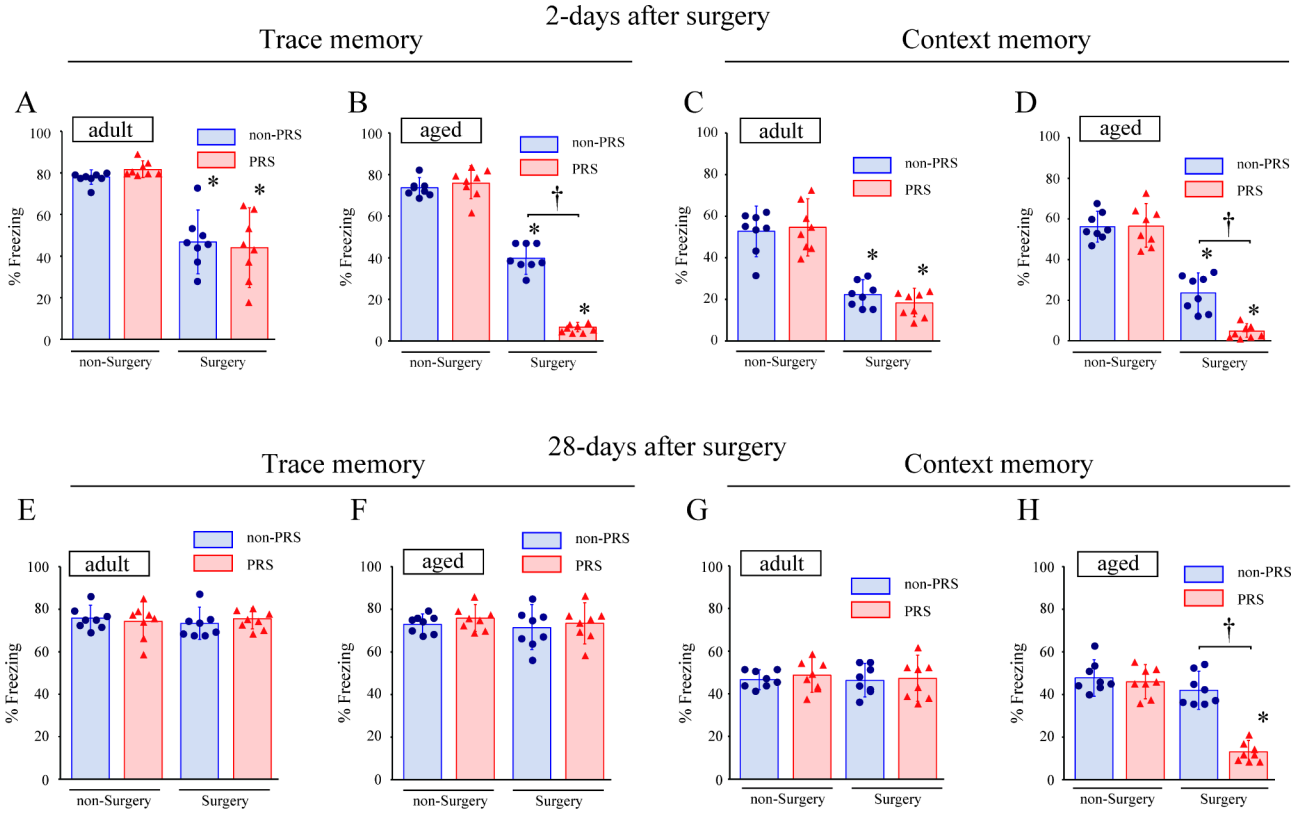

Fig. 1 Freezing Behavior in Adult and Aged Animals During Various Memory Retention Tests Following Trace Fear Conditioning. Panels **A** and **B** illustrate freezing behavior during the trace memory retention test after the tones, while panels **C** and **D** present freezing behavior during the context memory retention test 2 days post-surgery. Panels **E** and **F** show freezing behavior during a subsequent trace memory retention test after the tones, and panels **G** and **H** display freezing behavior during the context memory retention test 28 days post-surgery. Each study group, detailed in the Materials and Methods section, consists of 8 animals ($n=8$). Vertical bars represent the mean and standard deviation. Statistical significance is indicated as $^{*}p<0.05$ compared to the non-PRS/non-surgery group and $\dagger p<0.05$ compared to the non-PRS/surgery group

group compared to the non-PRS/surgery group (Fig. 1H, $p<0.05$).

Brain cytokine levels after trace fear conditioning

At 2 days post-surgery, TNF-α and IL-1β levels in the hippocampus and mPFC were comparable in non-surgical groups of control and PRS rats in both age groups. However, these cytokines were significantly higher in the hippocampus and mPFC compared to controls in both adult and aged rats (Fig. 2A, C, B and D, all $p<0.05$). PRS did not affect TNF-α or IL-1β production in adult animals (TNF-α: $p=0.78$; IL-1β: $p=0.81$) but significantly exacerbated their levels in aged rats in both the hippocampus ($p<0.05$) and mPFC ($p<0.05$). Plasma IL-1β levels were similar across all groups (Additional file 10: Fig. S8A-B). By 28 days, TNF-α and IL-1β levels in the adult hippocampus and mPFC were comparable among all groups (Fig. 2E and G; hippocampus TNF-α: $p=0.89$, IL-1β: $p=0.91$; mPFC TNF-α: $p=0.46$, IL-1β: $p=0.18$). However, in aged rats, TNF-α and IL-1β levels in the hippocampus (Fig. 2F), but not in the mPFC (Fig. 2H), remained elevated in the PRS/surgery group compared to the non-PRS/surgery group ($p<0.05$).

Considering all age groups, cytokine levels in the hippocampus and mPFC showed a negative correlation with memory retention at 2 days. At 28 days, only aged rats retained a significant association between hippocampal cytokines and memory (Additional file 11: Table S2-3), highlighting the role of neuroinflammation in cognitive deficits after surgery.

Microglial immune reactivity

Under baseline conditions, LPS increased TNF-α production from hippocampal microglia in a concentration-dependent manner in both adult and aged groups (Fig. 3A, $p<0.05$ for each group). However, the LPS-induced TNF-α elevation was more pronounced in aged rats ($F_{(1,\ 40)}=208.0$, $p<0.05$). In adult microglia, the increase in LPS-induced cytokines was similar between the non-PRS and PRS groups (Fig. 3B, $F_{(1,\ 40)}=0.82$, $p=0.37$), whereas in aged microglia, it was significantly amplified in the PRS group (Fig. 3C, $F_{(1,\ 40)}=45.72$, $p<0.05$).

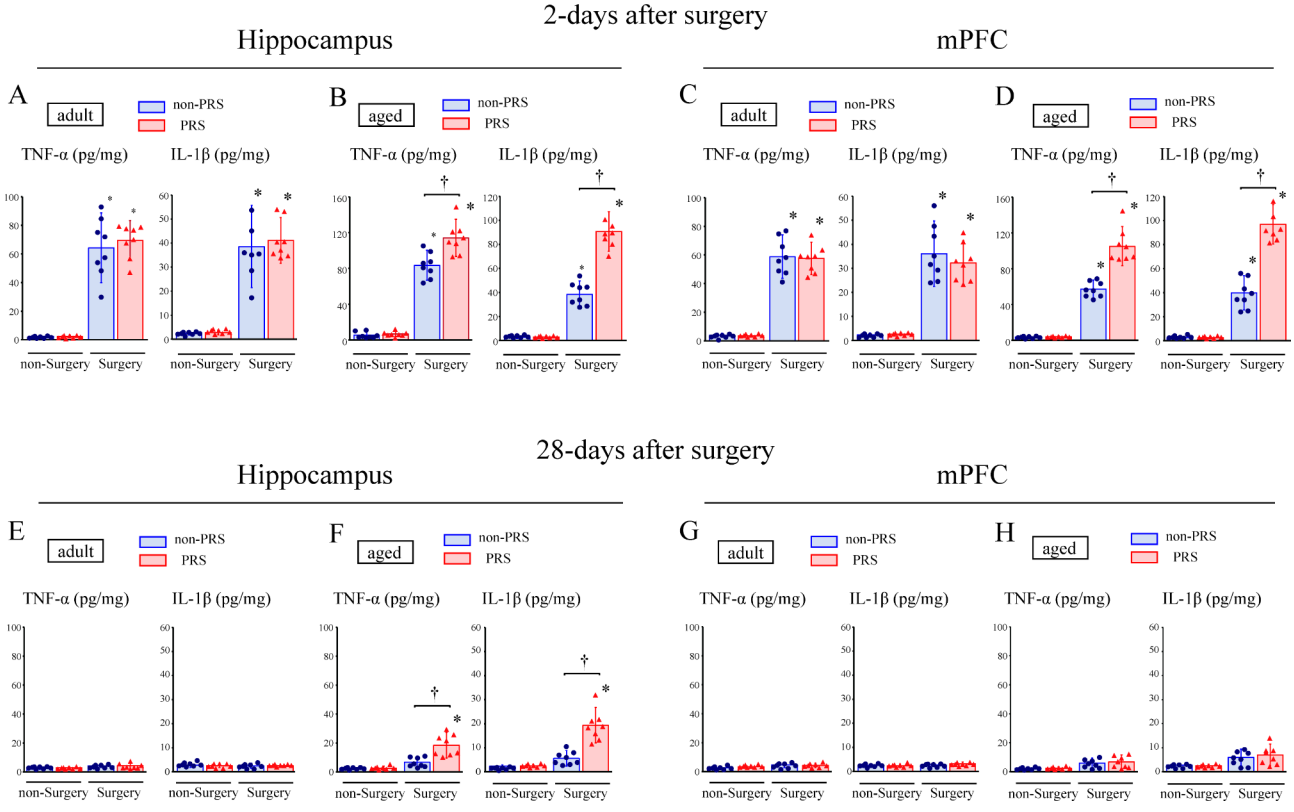

Fig. 2 Tumor Necrosis Factor-α (TNF-α) and Interleukin-1β (IL-1β) levels in the hippocampus and medial prefrontal cortex (mPFC) of adult and aged rats at different time points post-surgery. Panels **A** and **B** display TNF-α and IL-1β levels in the hippocampus, while panels **C** and **D** show TNF-α and IL-1β levels in the mPFC, both at 2 days post-surgery. Panels **E** and **F** present TNF-α and IL-1β levels in the hippocampus, and panels **G** and **H** depict TNF-α and IL-1β levels in the mPFC at 28 days post-surgery. Each study group, as detailed in the Materials and Methods section, consists of 8 animals ($n=8$). Vertical bars represent the mean and standard deviation. Statistical significance is indicated as $^*p<0.05$ compared to the non-PRS/non-surgery group and $\dagger p<0.05$ compared to the non-PRS/surgery group

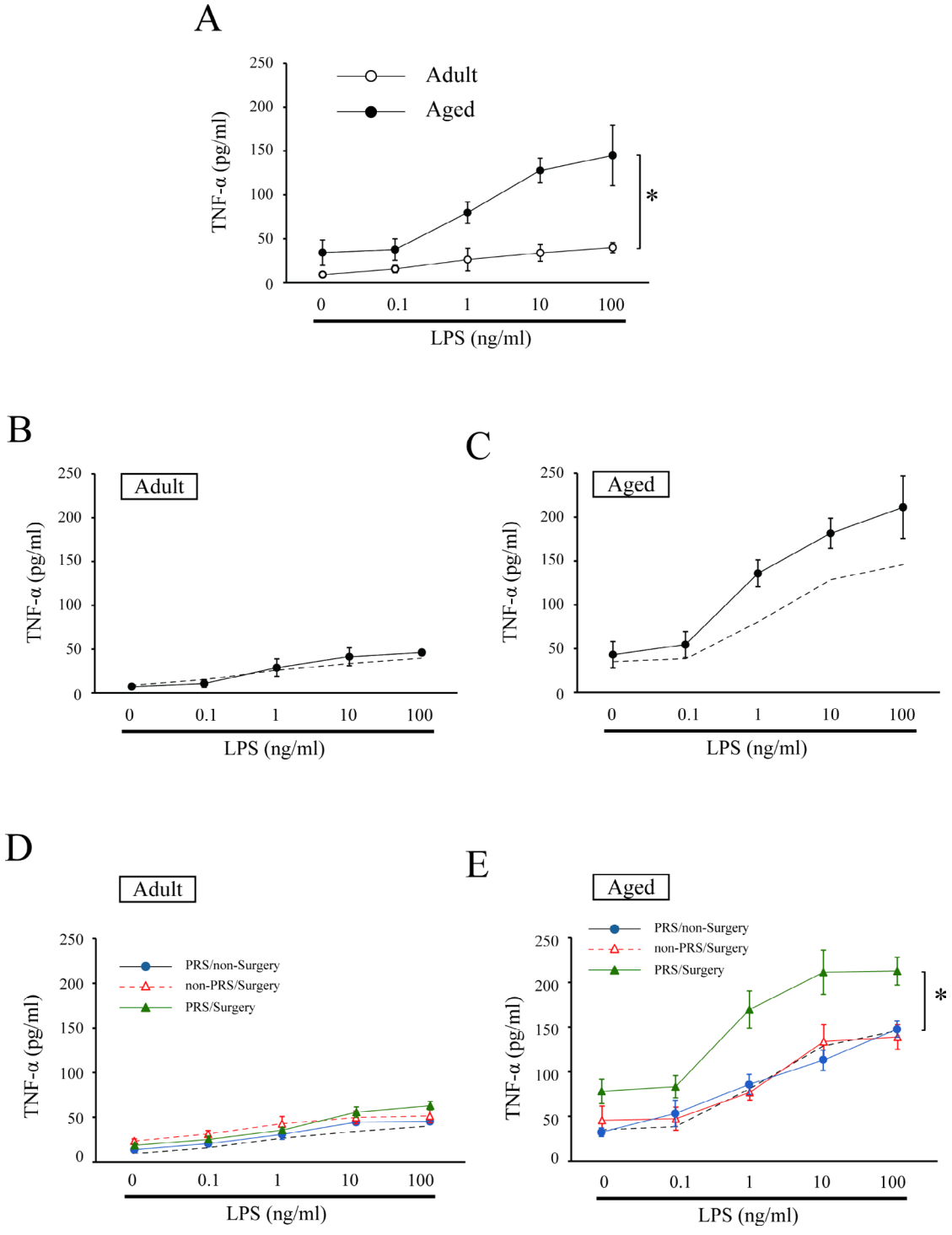

Fig. 3 Concentration-response effects of *ex vivo* lipopolysaccharide (LPS) stimulation on tumor necrosis factor (TNF)-α production in hippocampal microglia. Microglia were isolated from the hippocampi of adult and aged rats under various conditions: without any intervention (**A**, baseline), following preoperative restraint stress (PRS) in adult (**B**) and aged (**C**) rats, and 28 days post-surgery in adult (**D**) and aged (**E**) rats. To highlight changes after PRS relative to baseline, the baseline response (A) is represented as a dashed line in panels B, C, D, and E. Primary microglia were stimulated with varying concentrations of LPS (0.1, 1, 10, or 100 ng/ml) or media alone, and TNF-α levels were measured from supernatants collected 24 h later. Each bar represents the mean ± standard deviation ($n = 5$ per group)

At 28 days post-surgery, the response of microglia from adult hippocampus in the PRS/surgery group to LPS was comparable to other groups (Fig. 3D, $F_{(2, 60)}=0.39$, $p=0.68$). In aged microglia, the immune reactivity was significantly aggravated in the PRS/surgery group compared to baseline (Fig. 3E, $F_{(1, 40)}=143.22$, $p<0.05$).

Discussion

Our findings support the idea that aged microglia exhibit heightened inflammatory responsiveness [11], which may lead to maladaptive neuroinflammation and cognitive impairments such as POD. Recent studies suggest that microglia possess immune memory, influencing subsequent immune responses [11, 12]. This memory is hypothesized to induce sustained neuroinflammation, which may underlie the pathogenesis of certain neurodegenerative diseases [13, 14]. Our results indicate that aged microglia, subjected to strong immune stimulation from surgery with PRS, show increased inflammatory sensitivity in the chronic postoperative phase, correlating with observed cognitive deficits 28 days after surgery. Thus, immune memory in aged microglia may link POD to long-term cognitive decline, suggesting that mitigating neuroinflammation during POD is crucial for preventing such impairments, particularly in the elderly.

We utilized the restraint stress model with a 3-day period to simulate preoperative conditions, as it reliably induces a response in rodents that mirrors the anxiety and tension commonly experienced by surgical patients. This model has been shown to effectively stimulate the Hypothalamic-Pituitary-Adrenal (HPA) axis [15, 16], and our results further support its validity, as evidenced by elevated plasma corticosterone levels in both adult and aged rats.

Excessive stress leads to chronic microglial activation and persistent neuroinflammation, contributing to psychiatric disorders [6, 7, 17]. Our findings reveal that, while the PRS paradigm alone did not independently cause neuroinflammation, it exacerbated surgery-induced inflammation in aged rats, highlighting the heightened sensitivity of microglia to stress with aging. This increased microglial vulnerability appears central to stress responses in aged individuals. Notably, in the non-PRS/surgery group, the heightened sensitivity of aged microglia resolved during the chronic phase, whereas in the PRS/surgery group, it persisted, suggesting that strong immune stimulation during the acute phase had induced immune memory, resulting in prolonged cognitive impairment. These results emphasize that preoperative stress significantly impacts long-term postoperative cognitive outcomes, particularly in aged individuals.

The clinical implications of these findings are substantial, underscoring the importance of managing preoperative stress in elderly patients to improve their prognosis. Effective stress management may reduce the immune reactivity of microglia, thereby attenuating acute postoperative neuroinflammation. This, in turn, could prevent long-term cognitive decline, highlighting the critical role of preoperative interventions in safeguarding cognitive health among aged surgical patients. Elderly patients often face compounded stressors before surgery, and implementing strategies such as counseling, information provision, and relaxation techniques can effectively mitigate this burden. Furthermore, given the variability in stress-coping abilities among the elderly [18, 19], it is crucial to develop accurate methods to assess and optimize preoperative stress. Future research should prioritize clinical trials to validate these findings in humans and explore practical interventions for stress management. Such efforts could enhance postoperative recovery and cognitive health, ultimately improving the quality of life for elderly surgical patients.

Limitations

This study has several limitations. First, the use of rats may not fully replicate human physiology due to species differences. Additionally, the stress model employed may not capture the full complexity of preoperative stress experienced by humans. The study focused exclusively on memory and attention, leaving other POD symptoms, such as agitation and altered sleep-wake cycles, unaddressed. Furthermore, the use of only male rats limits the generalizability of the findings to both sexes. While this approach aligns with previous studies to ensure consistency and comparability, it may have overlooked potential sex differences. The study also measured TNF-α and IL-1β as key pro-inflammatory markers but did not investigate other cytokines that could play significant roles in neuroinflammation and cognitive impairments. Lastly, environmental factors and the reliance on a single surgical model may affect the generalizability of the results. Despite these limitations, the study highlights a potential link between preoperative stress and cognitive decline, underscoring the need for further research to translate these findings into clinical practice.

Abbreviations

POD	Postoperative Delirium
PRS	Preoperative Restraint Stress
IL-1β	Interleukin-1β
TNF-α	Tumor Necrosis Factor-α
mPFC	Medial Prefrontal Cortex
LPS	Lipopolysaccharide
ELISA	Enzyme-Linked Immunosorbent Assay
SD	Standard Deviation
ANOVA	Analysis of Variance
CS	Conditioned Stimulus
US	Unconditioned Stimulus

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-024-07023-z.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

Supplementary Material 11

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Not applicable.

Author contributions
N.N. conducted the investigation, curated and analyzed the data, wrote the main manuscript, and prepared all figures and additional files. F.L. contributed to the investigation, data curation, and analysis. S.H. contributed to the investigation, data curation, and analysis. S.K. contributed to the investigation, data curation, and analysis. T.K. conducted the investigation, curated and analyzed the data, reviewed and edited the main manuscript, and secured funding.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study adhered strictly to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and received approval from the Institutional Animal Care and Use Committee of Kochi Medical School (Approval No. P-00076).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

Consent to participate
Not applicable.

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