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Research Article

Exploring the Impact of Preoperative Sleep Fragmentation on Cognitive Function in Mice: The Role of Microglial Activation and Iron Metabolism

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Background. Perioperative neurocognitive disorders (PND) are a significant concern, particularly for aged individuals. Sleep fragmentation (SF), a common condition in older adults, is considered a risk factor for PND. The present study explored the impact of SF on cognitive function and its association with microglial activation and iron metabolism. *Methods*. Adult and aged C57BL/6J mice were subjected to tibial fracture surgery (TFS) and varying durations of SF. Cognitive function was assessed using the Morris water maze and fear conditioning experiments. Microglial activation was evaluated by measuring CD68 protein expression and inflammatory cytokine levels. Iron metabolism and ferroptosis-related proteins were also examined. *Results*. SF significantly impacted spatial memory and conditioned fear responses in mice, with aged mice showing greater susceptibility. Microglial activation, indicated by changes in CD68 protein expression and inflammatory cytokine levels, was observed in mice exposed to SF. Alterations in iron metabolism, as evidenced by changes in hippocampal iron content and expression of ferroptosis-related proteins, were also observed in these mice. *Conclusion*. SF can lead to significant cognitive impairment, particularly in aged mice, likely mediated through microglial activation and dysregulated iron metabolism. These findings provide novel insights into the pathogenesis of PND and suggest potential targets for intervention. *Significance*. This study illuminates the complex interactions between SF, microglial activation, and cognitive function. It highlights the importance of sleep quality for cognitive health in older adults and points to potential therapeutic strategies for preventing PND, including targeting microglial activation and iron metabolism.

1. Introduction

Perioperative neurocognitive disorders (PND) represent a spectrum of cognitive impairments that can occur in the perioperative period, encompassing postoperative cognitive dysfunction (POCD), postoperative delirium (POD), and long-term cognitive decline [1]. These disorders, previously considered distinct entities, are now recognized as interconnected conditions that can significantly impact patients' quality of life and long-term outcomes postsurgery [2]. As we continue to grapple with the complex etiologies and risk factors of PND, it has become evident that these conditions are not merely a product of surgical stress or anesthesia, but rather result from a multifactorial cascade involving patient's preoperative cognitive status, genetic predisposition, comorbid conditions, surgical factors, and postoperative care practices [3]. In addition to the direct impact on cognitive function, PND can lead to prolonged hospital stays, increased healthcare costs, and higher rates of morbidity and mortality [4]. Moreover, it also contributes to a loss of independence, posing significant challenges to patients' personal lives and social functioning [5]. Despite its

widespread prevalence and impact, PND remains underrecognized and underdiagnosed in clinical practice. Our understanding of the underlying mechanisms is still limited, and further research is urgently needed to identify effective strategies for the prevention, early detection, and management of these disorders. Therefore, it is crucial to shed light on the complexities of PND in order to improve patient outcomes in the perioperative period.

The sleep fragmentation, characterized by repeated awakenings and disruptions in the sleep cycle, has been increasingly recognized as a significant preoperative risk factor for perioperative neurocognitive disorders (PND) in older adults [6]. The intricate relationship between sleep, aging, and cognitive function forms a critical triad in understanding the pathophysiology of PND. As people age, changes in sleep architecture, coupled with the increased prevalence of sleep disorders, contribute to higher levels of sleep fragmentation [7]. In the context of surgery, the role of sleep becomes even more pertinent. Mounting evidence suggests that preoperative sleep fragmentation may predispose older adults to PND, making it a crucial area for investigation. The underlying mechanism is believed to involve the dysregulation of neuroimmunological and neuroendocrine systems that are vital for maintaining cognitive function [8]. Despite this knowledge, the interplay between preoperative sleep fragmentation and PND in older adults is not fully elucidated and remains an active area of research. It is essential to explore this relationship further, as it may open avenues for preventative strategies and interventions that can improve sleep quality, thereby reducing the incidence of PND and enhancing postoperative recovery. In this context, the development and implementation of comprehensive sleep evaluations in the preoperative period could be of particular relevance. Not only would this approach help in identifying patients at risk for PND but could also provide an opportunity for targeted sleep interventions prior to surgery.

Ferroptosis, derived from the Latin word "ferrum" meaning iron, and the Greek term "ptosis" for falling, is a novel type of programmed cell death characterized by iron-dependent accumulation of lipid peroxides [9]. First coined in 2012, ferroptosis represents a distinct form of cell death from other well-known types such as apoptosis, necrosis, and autophagy [10]. Unlike other forms of cell death that often involve the collapse of cellular structure or function, ferroptosis is uniquely dependent on the intracellular balance of iron and lipid peroxidation [11]. It is believed to occur when cells suffer from an iron overload, resulting in the generation of reactive oxygen species (ROS) and subsequent lipid peroxidation, which ultimately leads to cell death [12].

However, despite the mounting evidence suggesting a link between sleep fragmentation, microglial iron metabolism, and the development of PND, the mechanisms underpinning this relationship remain elusive. Our study aims to elucidate these mechanisms and explore the potential of targeting microglial iron metabolism and ferroptosis as a novel strategy for preventing sleep fragmentation-induced PND in older adults. By delving into this relatively unexplored territory, we hope to shed light on the intricate network of processes linking sleep, iron metabolism, and cognitive function, potentially paving the way for new therapeutic strategies to mitigate the risk of PND in the elderly population.

2. Methods

2.1. Animals and Grouping. Adult male C57BL/6J mice, aged between 8–10 weeks, were accommodated in plastic enclosures, with their circadian rhythm regulated by a 12-hour light/dark cycle. Unrestricted access to food and water was ensured throughout the experiment. The surrounding environment was meticulously maintained at a consistent temperature and humidity for the entire duration of research studies. All experimental procedures involving animals were conducted strictly following the guidelines provided in the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Furthermore, the study received formal approval from the Ethics Committee on Laboratory Animal Use at the Second Affiliated Hospital of Nanchang University. In this study, we employed both adult and aged male C57BL/6J mice to investigate the impact of sleep deprivation and pharmaceutical intervention on tibial fracture healing. The mice were stratified into several groups based on age and treatment regimens. For the adult cohort, we established four experimental groups: the adult control (AC) group was maintained under standard conditions; the adult tibial fracture (AT) group underwent a tibial fracture operation with internal fixation; the adult sleep deprivation (AS) group experienced 48 hours of sleep deprivation using a modified multiple platform water method before any procedure; and the adult tibial fracture with preoperative sleep deprivation (ATS) group was subjected to the same sleep deprivation protocol before the tibial fracture operation. Parallel to the adult cohort, the aged mice were divided into four corresponding groups: the control (C) group, the tibial fracture (T) group, the sleep deprivation (S) group, and the tibial fracture with preoperative sleep deprivation (TS) group, each undergoing analogous treatments as their adult counterparts. In a separate set of experiments, the aged mice were allocated into four additional groups to assess the effects of pharmacological agents on fracture healing under sleep-deprived conditions. The TS group was sleep-deprived for 48 hours prior to tibial fracture surgery. The sleep deprivation with minocycline (SM) group received a daily dose of minocycline (30 mg/kg) for 6 days before undergoing sleep deprivation and surgery. The sleep deprivation with deferoxamine (SD) group was administered deferoxamine (100 mg/kg) daily for 6 days followed by sleep deprivation and surgery. Lastly, the sleep deprivation with minocycline and deferoxamine (SMD) group received both minocycline and deferoxamine at the respective dosages before the sleep deprivation and surgical procedure.

2.2. Tibial Fracture Fixation. Under general anesthesia induced by a combination of ketamine (75 mg/kg) and xylazine (10 mg/kg), administered via intraperitoneal injection,

a midline skin incision was made in the adult male C57BL/6J mice over the anterior aspect of the right hindlimb. The soft tissue was carefully dissected to expose the tibia. A controlled fracture was created using a three-point bending apparatus. A 0.38 mm stainless steel fixation pin was inserted retrogradely into the medullary canal of the tibia, across the fracture site, ensuring a stable internal fixation. Following this, the skin incision was sutured with a 5-0 absorbable thread. Postoperatively, the mice were returned to their housing cage and monitored regularly for signs of pain or distress. Analgesics (buprenorphine: 0.05–0.1 mg/kg) were administered as needed for pain relief. All experimental procedures were performed in adherence to the ethical guidelines stated in the guide and received approval from the Institutional Animal Care and Use Committee.

2.3. Sleep Fragmentation. The sleep fragmentation model was established by the improved sleep deprivation method in a multiplatform water environment. In brief, 10 circular platforms (5 cm high and 2 cm in diameter) were placed in a transparent water tank (20 cm × 30 cm × 40 cm), and the distance between each two platforms was 5 cm. The water tank was filled with water to 1 cm below the platform, and the mice were placed on the platform for continuous sleep deprivation for 72 hours (if the mice slept, they would wake up because their heads would touch water or fall into the water), and they were continuously irradiated by fluorescent lamps. Sleep deprivation was performed continuously for 12 hours (i.e., 9 : 00~21 : 00) every day.

2.4. Morris Water Maze Test. Following the internal fixation procedure, the mice were given a recovery period of one week before the commencement of behavioral testing. The Morris water maze (MWM) test was conducted to assess spatial learning and memory. The water maze apparatus consisted of a round pool, 120 cm in diameter and 50 cm deep, filled with water maintained at a temperature of 22 ± 1°C. The pool was divided into four equal quadrants and a hidden platform (10 cm in diameter) was placed in one of the quadrants, submerged 1 cm below the water surface. Visual cues were provided around the maze to assist spatial orientation. Training trials were conducted twice daily for five consecutive days. Each mouse was placed in the water facing the wall of the pool at one of the four starting locations, which were varied randomly. The mice were allowed a maximum of 60 seconds to find the hidden platform. If a mouse failed to find the platform within the given time, it was gently guided to it. Each mouse was allowed to stay on the platform for 15 seconds before being taken out of the pool. On the sixth day, a probe trial was performed by removing the platform. Each mouse was allowed to swim freely for 60 seconds, and the time spent in the target quadrant (where the platform was previously located) was recorded to assess memory retention. The movements of the mice were tracked and recorded using a computerized video system. The parameters analyzed included the latency to find the hidden platform during training trials and the time spent in the target quadrant during the probe trial.

2.5. Fear Conditioning Test. After a recovery period of one week postsurgery, the fear conditioning test was initiated. The fear conditioning setup consisted of a conditioning chamber, equipped with a metal grid floor connected to a shock scrambler. The chamber was cleaned with 70% ethanol between each mouse to remove any scent cues. On the first day, termed the "conditioning day," mice were placed in the chamber for a period of 180 seconds for habituation. After this habituation period, an auditory tone (2000 Hz, 85 dB) was presented for 30 seconds, which coterminated with a 2-second mild foot shock (0.5 mA). This tone-shock pairing was repeated twice with an interval of 60 seconds between the pairings. Mice were returned to their home cage 60 seconds after the last shock. On the second day, termed the "context test," mice were placed back into the same conditioning chamber (without the tone and shock) for 5 minutes to measure fear to the context. Freezing behavior, defined as a complete lack of movement except for respiration, was recorded as an index of fear memory. On the third day, termed the "tone test," the conditioning chamber was altered (altered context) to reduce contextual cues. Mice were placed in the altered chamber for 180 seconds, followed by the same tone used during the conditioning day for another 180 seconds, but without any foot shock. Freezing behavior was again recorded to evaluate the conditioned fear response to the tone. The degree of freezing was measured by a video tracking system, and the percentage of time spent freezing was analyzed for each phase of the experiment.

2.6. Detection of the Inflammatory Cytokines and Oxidative Stress Markers. At designated time points postsurgery, mice were humanely euthanized. Brains were rapidly removed and the hippocampi were carefully dissected on ice. The tissue samples were then homogenized in a suitable buffer to prepare tissue lysates. To measure the levels of inflammatory cytokines, the homogenates were subjected to centrifugation at 10,000 rpm for 10 min at 4°C. The supernatants were collected and stored at −80°C until further use. The concentrations of TNF-α, IL-6, and IL-10 in the supernatants were determined using specific enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's instructions. Absorbance was measured on a microplate reader, and cytokine concentrations were calculated based on the standard curve. To evaluate the levels of oxidative stress markers, the activities of superoxide dismutase (SOD) and the content of malondialdehyde (MDA) in the hippocampal homogenates were measured using specific commercial assay kits according to the manufacturer's instructions. SOD activity was determined based on its ability to inhibit the oxidation of a substrate, with the results expressed in units/mg protein. MDA content, an indicator of

lipid peroxidation, was assessed by measuring the absorbance of the color produced during the reaction with thiobarbituric acid at 532 nm, with results expressed in nmol/mg protein.

2.7. Evaluation of Microglia Activation and Ferroptosis Markers. After euthanasia, hippocampal tissues were collected from the mice postsurgery and homogenized in a suitable RIPA buffer to prepare tissue lysates. The lysates were centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatants were collected for protein quantification and subsequent analysis. The protein concentrations in the supernatants were determined using the Bradford method, which was used for normalization of subsequent assays. The expressions of CD68 protein and ferroptosis-related proteins, HCP1, TfR, Fpn1, DMT1, and GPX4, were evaluated using Western blot analysis. In brief, equal amounts of protein were separated by SDS-PAGE and then transferred onto PVDF membranes. The membranes were blocked and incubated with primary antibodies against HCP1, TfR, Fpn1, DMT1, and GPX4 overnight at 4°C. After washing, the membranes were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies. The protein bands were visualized using an enhanced chemiluminescence detection system.

2.8. Statistical Analysis. All data collected during the study were processed and analyzed using statistical software (such as SPSS and GraphPad Prism). Data are expressed as mean ± standard deviation (SD) unless otherwise specified. Before applying statistical tests, the normality of data distribution was assessed using the Shapiro–Wilk test. For normally distributed data, the differences between the two groups were evaluated using the Student's *t*-test. For multiple group comparisons, one-way ANOVA followed by Tukey's post hoc test was used. If the data were not normally distributed, nonparametric tests were applied. Differences between the two groups were assessed using the Mann–Whitney *U* test. For multiple group comparisons, the Kruskal–Wallis test followed by Dunn's post hoc test was performed. For repeated measures data, such as the fear conditioning test, a two-way repeated measure ANOVA was employed, followed by Bonferroni post hoc tests. The correlation between different variables was determined by the Pearson correlation coefficient for normally distributed data, or the Spearman correlation coefficient for nonnormally distributed data. The results were considered statistically significant at a p value of less than 0.05. All statistical tests were two-tailed.

3. Results

3.1. The Impact of Sleep Fragmentation (SF) on Cognitive Function in Adult and Aged Mice Posttibial Fracture Surgery (TFS). In the results of the study, we assessed the cognitive function of adult and aged C57BL/6J mice following sleep fragmentation (SF) and tibial fracture fixation (TFS) using the Morris water maze and fear conditioning assays. For the Morris water maze, adult mice exhibited a consistent escape latency across all groups, with the adult control (AC group) averaging 18–20 seconds and the tibial fracture with preoperative sleep deprivation (ATS group) showing a slight increase to 24–26 seconds. The number of platform crossings remained similar across adult groups, with a slight decrease in the ATS group. In contrast, aged mice demonstrated a more pronounced effect of treatments on cognitive function. Notably, the aged tibial fracture (T group), sleep deprivation (S group), and tibial fracture with preoperative sleep deprivation (TS group) showed a significant increase in escape latency times during the Morris water maze (76–98 seconds) compared to the control (C group), with the TS group displaying the most substantial delay ($P < 0.05$). Moreover, the number of platform crossings was significantly reduced in these groups, with the TS group showing the least number of crossings ($P < 0.05$). The fear conditioning assay results mirrored these findings. In adult mice, the percentage of postoperative condition-induced freezing time showed minimal differences between groups. However, aged mice in the T, S, and TS groups demonstrated a marked reduction in freezing time compared to controls, with the TS group showing the most significant decrease ($P < 0.05$), indicating a decline in associative memory after SF and TFS (Figure 1).

3.2. Therapeutic Efficacy of Minocycline and Deferoxamine on Cognitive Function in Aged Mice following Sleep Fragmentation and Tibial Fracture Surgery. In this part, we used aged mice to establish a model of TFS, and mice were applied SF for 24 h, 48 h, and 72 h, respectively. Then, we used the microglial inhibitor, minocycline, and/or the iron chelator, deferoxamine (DFO). We analyzed the results of the Morris water maze and fear conditioning experiments at different time points presurgery and postsurgery. Our findings indicate that minocycline and deferoxamine treatments improved cognitive outcomes in aged mice subjected to sleep deprivation and tibial fracture surgery. In the Morris water maze, the escape latency decreased, and the number of platform crossings increased in the minocycline (SM), deferoxamine (SD), and the combined treatment groups (SMD) compared to the sleep-deprived surgery group (TS). The fear conditioning results supported these findings, with treated groups showing higher freezing times, suggesting better memory retention. The combined treatment (SMD) group exhibited the most significant improvements in both tests, highlighting the potential synergistic effects of minocycline and deferoxamine in mitigating cognitive deficits following surgical trauma and sleep deprivation in aged mice (Figure 2).

3.3. Effect of Minocycline and Deferoxamine on Inflammatory Markers in Aged Mice Subjected to Sleep Deprivation and Surgery. The administration of minocycline and deferoxamine in aged mice postsurgery and sleep deprivation showed a notable effect on inflammation. Both treatments reduced the levels of proinflammatory cytokines TNF-α and IL-6 compared to the group that did not receive any drug

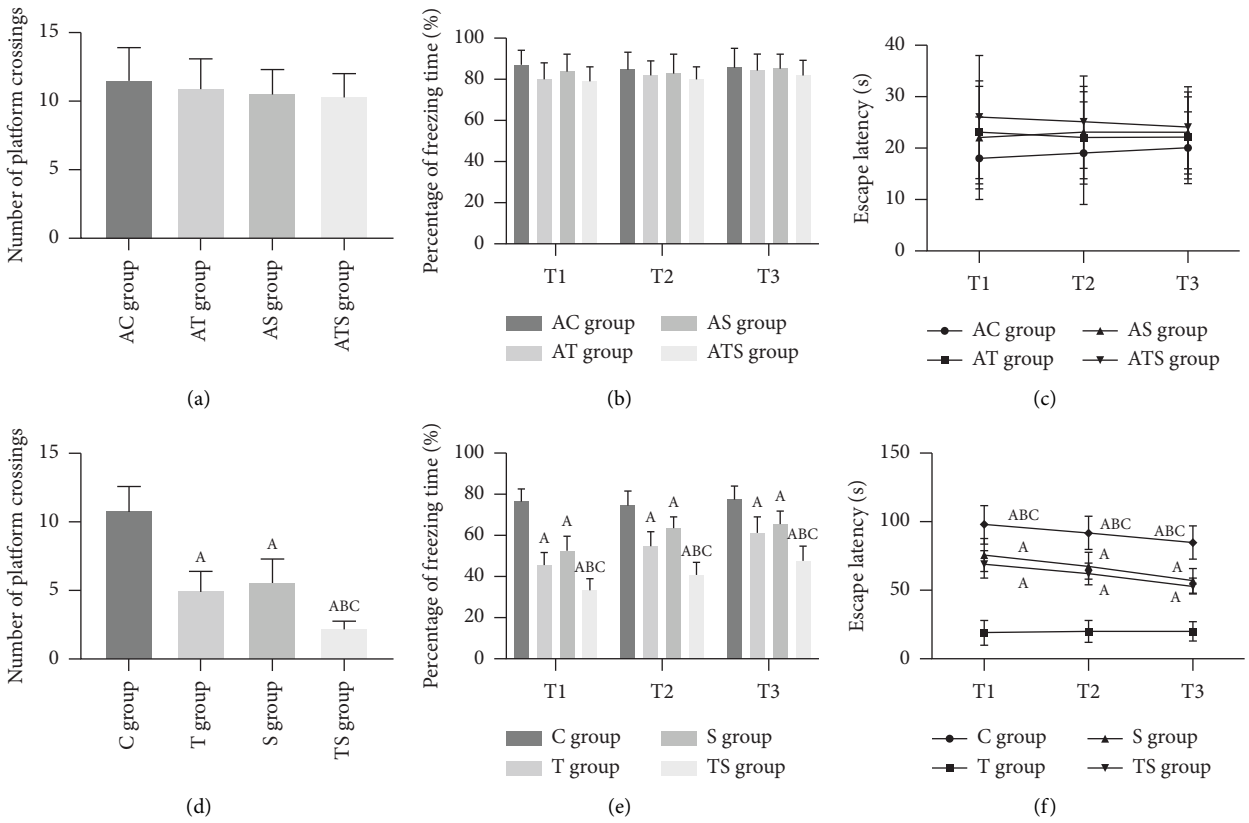

Figure 1: Effects of sleep fragmentation (SF) and tibial fracture surgery (TFS) on cognitive function in adult and aged C57BL/6J mice. (a) Number of platform crossings in the Morris water maze for adult mice across four groups: control (AC group), tibial fracture (AT group), sleep deprivation (AS group), and tibial fracture with preoperative sleep deprivation (ATS group). No significant differences are observed. (b) Percentage of freezing time in fear conditioning for adult mice at three time points (T1, T2, and T3) showing minimal differences between groups. (c) Escape latency in the Morris water maze for adult mice, indicating consistent performance across all groups with a slight increase in the ATS group. (d) Number of platform crossings in the Morris water maze for aged mice, with significant decreases in T group, S group, and TS group compared to the C group, and the lowest crossings in the TS group ($P < 0.05$). (e) Percentage of freezing time in fear conditioning for aged mice, showing a marked reduction in T group, S group, and TS group compared to the C group, especially in the TS group ($P < 0.05$). (f) Escape latency in the Morris water maze for aged mice, demonstrating significantly increased times in T group, S group, and TS group, with the TS group exhibiting the longest delays ($P < 0.05$).

treatment. The combined drug treatment group displayed the lowest levels of these cytokines. In addition, there was an increase in the anti-inflammatory cytokine IL-10 with treatment, suggesting a shift towards a less inflammatory state. The combined treatment group had the highest levels of IL-10, indicating a possible synergistic effect of the two drugs in modulating the inflammatory response postsleep deprivation and surgical trauma (Figure 3).

3.4. Modulation of Oxidative Stress Markers by Treatment in Aged Mice Postsurgery and Sleep Deprivation. The study evaluated the effects of treatments on oxidative stress markers superoxide dismutase (SOD) and malondialdehyde (MDA) in aged mice subjected to sleep deprivation and tibial fracture surgery. The SOD levels were significantly higher in the treatment groups (SM, SD, and SMD) than the TS group at all time points (T1, T2, and T3), indicating an enhanced antioxidant activity. Specifically, the SMD group showed the highest increase in SOD activity, suggesting a synergistic effect of minocycline and deferoxamine. Conversely, MDA levels, an indicator of lipid peroxidation and thus oxidative stress, were markedly lower in the treatment groups than the TS group. This decrease was most pronounced in the SMD group, indicating that the combination of treatments most effectively mitigated oxidative damage. Overall, these results suggest that minocycline and deferoxamine, both individually and in combination, significantly modulate oxidative stress markers, improving antioxidant defense and reducing lipid peroxidation in aged mice postsleep deprivation and surgery. This indicates a potential protective effect against oxidative stress-related damage in postoperative and sleep-deprived conditions (Figure 4).

3.5. Impact of Treatment on CD68 Expression in the Hippocampus of Aged Mice Postsleep Deprivation and Surgery. Our study assessed the impact of treatments on the expression of CD68, a marker of inflammation, in the hippocampus of aged mice subjected to sleep deprivation and tibial fracture surgery. CD68 expression levels were measured at three time points postoperation. The TS group,

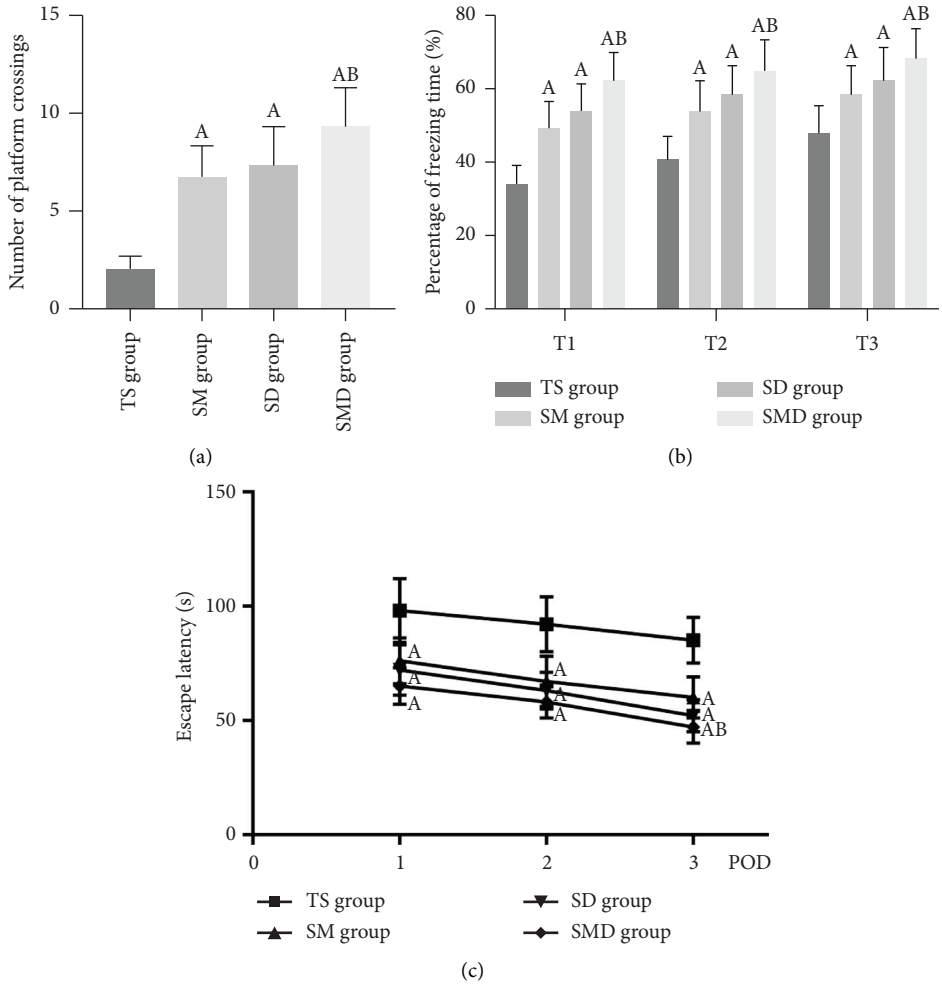

Figure 2: Effects of minocycline and deferoxamine on cognitive function in aged mice after sleep fragmentation and tibial fracture surgery. (a) Number of platform crossings in the Morris water maze for aged mice in the tibial fracture with preoperative sleep deprivation (TS) group, and those treated with minocycline (SM group), deferoxamine (SD group), and combined minocycline and deferoxamine (SMD group). The treatment groups show an increase in crossings compared to the TS group, with significant differences indicated by "A" ($P < 0.05$ compared to the TS group) and "AB" ($P < 0.05$ compared to SM and SD groups). (b) Percentage of freezing time in fear conditioning at three postoperative days (PODs) for the same groups. The SM, SD, and SMD groups exhibit higher freezing percentages than the TS group, indicating improved memory retention. Letters denote statistical significance as follows: "A" ($P < 0.05$ compared to the TS group) and "AB" ($P < 0.05$ compared to SM and SD groups). (c) Escape latency in the Morris water maze over three postoperative days. All treatment groups showed a decrease in escape latency compared to the TS group, with "A" denoting $P < 0.05$ compared to the TS group and "AB" denoting $P < 0.05$ compared to the SM and SD groups.

which received no treatment postsurgery and sleep deprivation, showed relatively high levels of CD68, with values decreasing slightly over time from 1.48 ± 0.19 at T1 to 1.36 ± 0.17 at T3. In contrast, treatment groups exhibited a significant reduction in CD68 expression. The SM group (minocycline treatment) and SD group (deferoxamine treatment) showed marked decreases in CD68 levels at all time points, indicating a reduction in inflammatory activity. The most significant decrease in CD68 expression was observed in the SMD group, which received both minocycline and deferoxamine. This group showed the lowest levels of CD68 at all time points, with the most considerable reduction by T3 (0.68 ± 0.14), suggesting a synergistic effect of the combined treatment in reducing inflammation. These results demonstrate that minocycline and deferoxamine, particularly in combination, effectively reduce inflammatory marker CD68 in the hippocampus, which might contribute to mitigating inflammation-related damage or cognitive decline associated with surgical trauma and sleep deprivation in aged mice (Figure 5).

3.6. Reduction of Hippocampal Iron Content in Aged Mice following Treatment with Minocycline and Deferoxamine. The results revealed a significant reduction in hippocampal iron content across all treatment groups compared to the sleep deprivation and surgery group (TS). In the TS group, iron content decreased slightly over time from $2.28 \pm 0.10\,\mu g/g$ at T1 to $2.16 \pm 0.12\,\mu g/g$ at T3. The minocycline treatment group (SM) and the deferoxamine

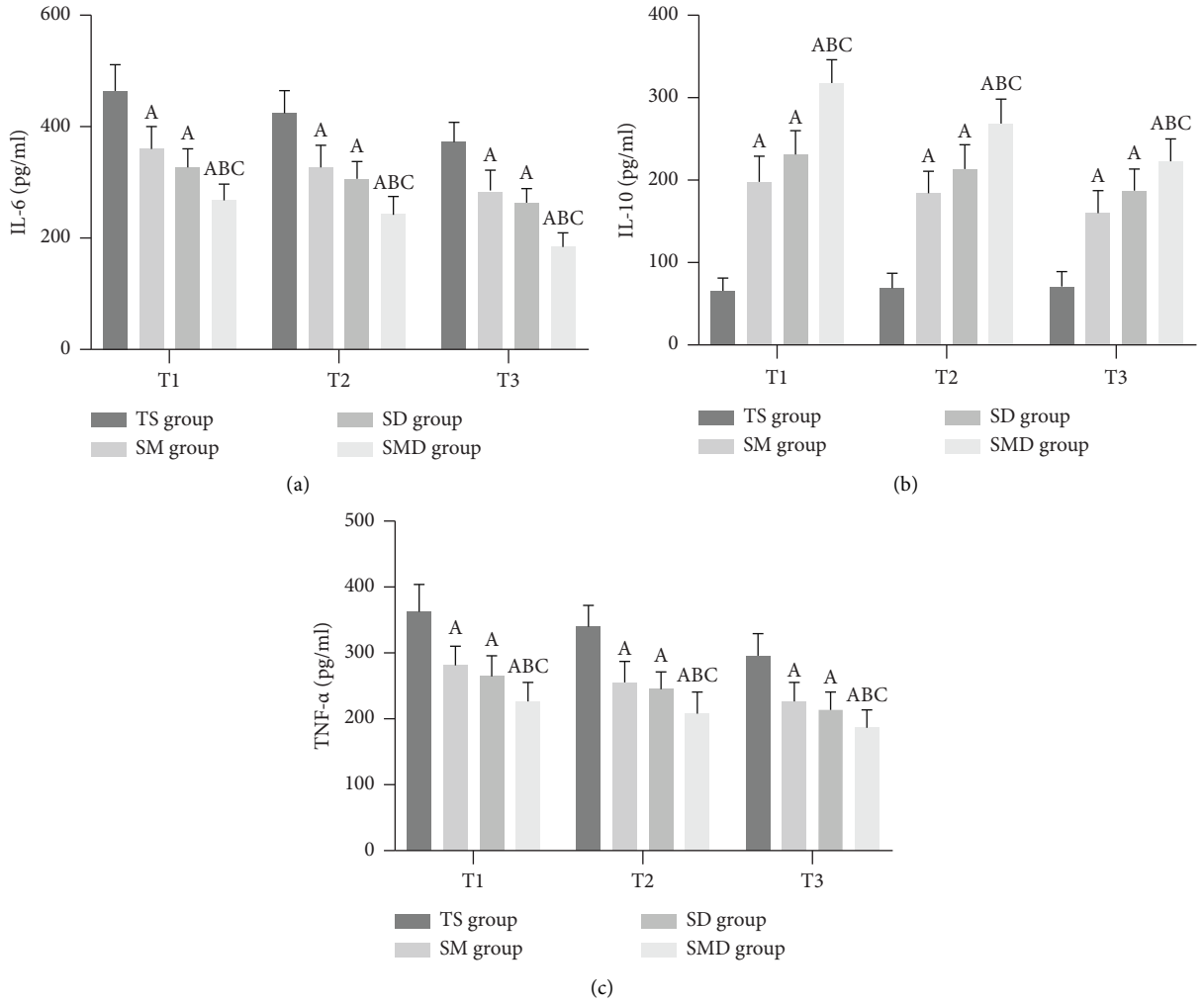

Figure 3: Influence of minocycline and deferoxamine on inflammatory markers in aged mice postsleep deprivation and surgery. (a) Levels of IL-6 across four treatment groups at three time points (T1, T2, and T3). The TS group shows the highest levels, while the SM, SD, and SMD groups exhibit reduced IL-6 levels posttreatment. Statistically significant differences are denoted by "A" for $P < 0.05$ compared to the TS group, "AB" for $P < 0.05$ compared to the SM group, and "ABC" for $P < 0.05$ compared to the SD group. (b) Concentrations of IL-10 in the same groups, indicating increased levels in the SM, SD, and SMD groups compared to the TS group, with the SMD group showing the highest levels. The significance indicators are the same as in (a). (c) TNF-α levels displaying a similar trend to IL-6, with all treatment groups showing a decrease compared to the TS group, and the SMD group having the lowest levels. Statistical annotations follow the same pattern as in (a).

treatment group (SD) showed marked decreases in iron levels at all time points, with the SM group reaching $1.49 \pm 0.09\,\mu g/g$ and the SD group reaching $1.45 \pm 0.12\,\mu g/g$ by T3. The most significant reduction in iron content was observed in the group receiving combined minocycline and deferoxamine treatment (SMD). This group showed the lowest levels of iron at all time points, with a substantial decrease to $1.15 \pm 0.10\,\mu g/g$ by T3, indicating that the combination of treatments was most effective in reducing hippocampal iron content (Figure 6).

3.7. Effects of Pharmacological Interventions on Iron Regulation and Oxidative Stress in the Hippocampus Postsurgery and Sleep Deprivation. Related to iron metabolism and oxidative stress in the hippocampal tissue of mice postsurgery and sleep deprivation, the proteins examined were HCP1, TfR, Fpn1, DMT1, hepcidin, and GPX4. The untreated surgery and sleep deprivation group (TS) displayed baseline expression levels of these proteins. Posttreatment, both minocycline (SM) and deferoxamine (SD) groups showed significantly lower expression of HCP1, TfR, Fpn1, DMT1, and Hepcidin, indicating a decrease in iron accumulation and regulation markers. The combined treatment group (SMD) had the lowest expression of these proteins, suggesting an enhanced effect when both pharmacological agents are used together. For the oxidative stress marker GPX4, the SM and SD groups exhibited an increase in expression, with the SMD group demonstrating the highest expression levels. This suggests that the treatments effectively bolster the antioxidative defense mechanisms in the hippocampus (Figure 7). These results, reinforced by the Western blot analysis, illustrated that minocycline and deferoxamine, particularly in combination, can modulate

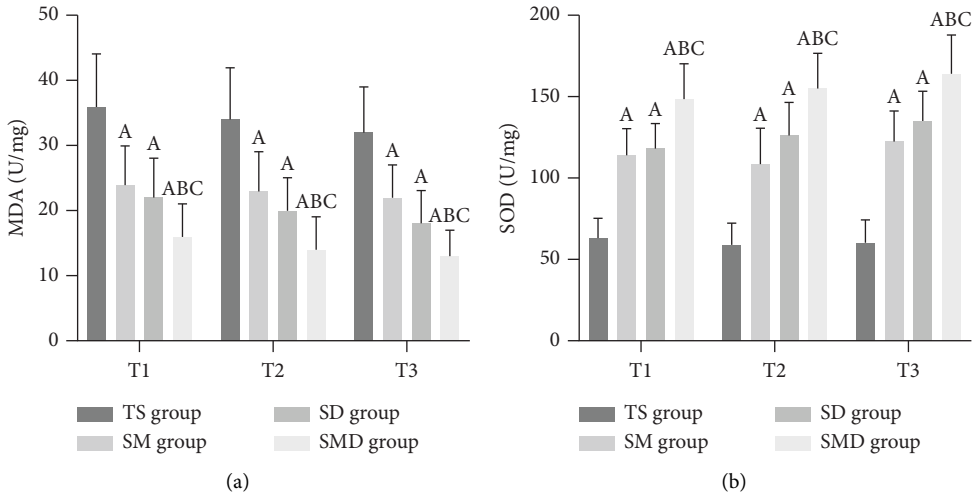

Figure 4: Effects of treatment on oxidative stress markers in aged mice postsleep deprivation and surgery. (a) Malondialdehyde (MDA) levels, indicating lipid peroxidation, across the TS, SM, SD, and SMD groups at three time points (T1, T2, and T3). MDA levels are lower in the treatment groups than in TS, with the SMD group showing the most significant reduction. Significance is noted by "A" for $P < 0.05$ compared to the TS group, "AB" for $P < 0.05$ compared to the SM group, and "ABC" for $P < 0.05$ compared to the SD group. (b) Superoxide dismutase (SOD) activity levels in the same groups. Elevated SOD activity is observed in the treatment groups, with the highest levels in the SMD group, indicating increased antioxidant capacity. Statistical annotations are consistent with those in part (a).

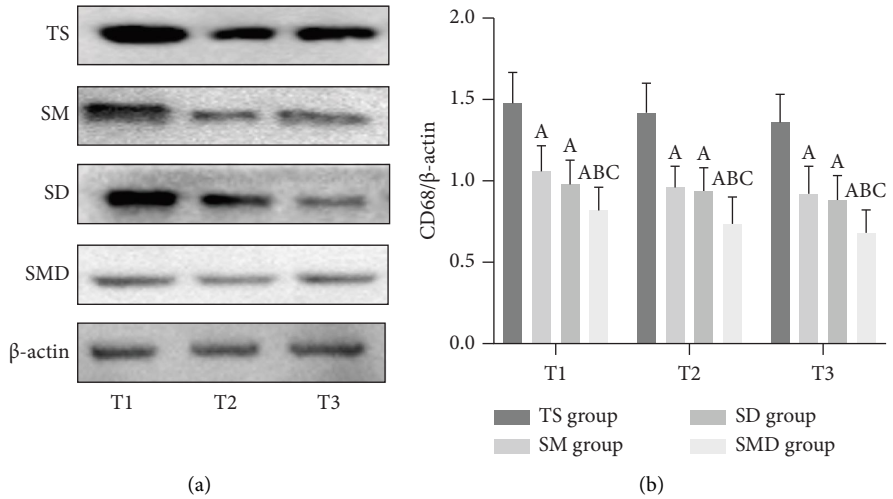

Figure 5: Changes in CD68 expression in the hippocampus of aged mice following treatment postsleep deprivation and surgery. (a) Representative Western blot bands for protein CD68 along with β-actin as a loading control in the TS, SM, SD, and SMD groups. (b) Expression of CD68, normalized to β-actin, in the hippocampus of aged mice across four groups: tibial fracture with preoperative sleep deprivation (TS group), minocycline treatment (SM group), deferoxamine treatment (SD group), and combined minocycline and deferoxamine treatment (SMD group) at three postoperative time points (T1, T2, and T3). The TS group shows the highest expression levels, with a slight downward trend over time. The SM, SD, and SMD groups all exhibit significant reductions in CD68 expression compared to the TS group, with the SMD group showing the greatest decrease, especially by T3. Statistical significance is denoted by "A" ($P < 0.05$ compared to the TS group), "AB" ($P < 0.05$ compared to the SM group), and "ABC" ($P < 0.05$ compared to the SD group).

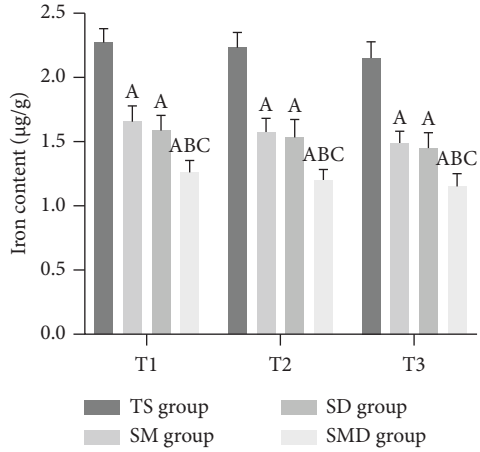

Figure 6: Effect of minocycline and deferoxamine on the hippocampal iron content in aged mice posttreatment. The graph represents the hippocampal iron content (μg/g) in aged mice across four groups: tibial fracture with preoperative sleep deprivation (TS group), minocycline treatment (SM group), deferoxamine treatment (SD group), and combined treatment (SMD group) at three time points (T1, T2, and T3). There is a trend of decreasing iron content in all treatment groups compared to the TS group. The greatest reduction in iron content is seen in the SMD group at all time points, with statistical significance indicated by "A" ($P < 0.05$ compared to the TS group), "AB" ($P < 0.05$ compared to SM group), and "ABC" ($P < 0.05$ compared to the SD group).

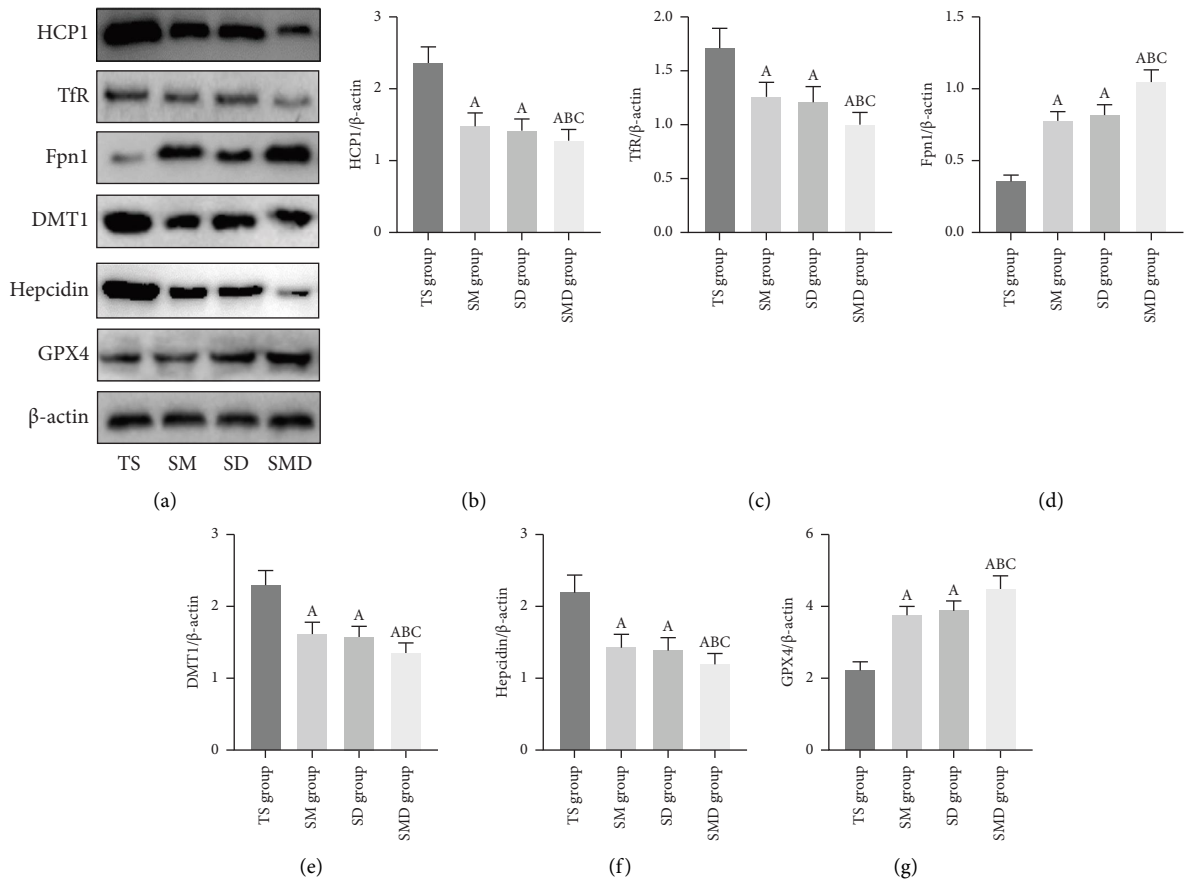

Figure 7: Western blot analysis of iron regulation and oxidative stress proteins in the hippocampus of treated mice. (a) Representative Western blot bands for iron regulation proteins HCP1, transferrin receptor (TfR), ferroportin 1 (Fpn1), divalent metal transporter 1 (DMT1), hepcidin, and oxidative stress protein glutathione peroxidase 4 (GPX4), along with β-actin as a loading control in the TS, SM, SD, and SMD groups. (b–g) Densitometric analysis of HCP1, TfR, Fpn1, DMT1, hepcidin, and GPX4 and normalized to β-actin levels. The graphs show changes in protein expression levels posttreatment with minocycline (SM), deferoxamine (SD), and their combination (SMD) compared to the untreated surgery and sleep deprivation group (TS). Significant decreases in iron regulation proteins are noted in the treatment groups (SM, SD, and SMD) with the lowest expression in the SMD group, while GPX4 expression is increased, indicating enhanced antioxidative response. Significance is denoted by "A" ($P < 0.05$ compared to the TS group), "AB" ($P < 0.05$ compared to the SM group), and "ABC" ($P < 0.05$ compared to the SD group).

the proteins involved in iron homeostasis and oxidative stress, potentially contributing to neuroprotection in the context of surgical trauma and sleep deprivation.

4. Discussion

This study set out to investigate the impacts of sleep fragmentation (SF) on cognitive function in adult and aged mice, with a focus on the role of microglial activation and alterations in iron metabolism and ferroptosis. The results illuminate the intricate network of processes linking sleep fragmentation, microglial activation, and cognitive impairment, providing novel insights into the pathogenesis of perioperative neurocognitive disorders (PND).

Sleep fragmentation was found to significantly impact spatial memory and conditioned fear responses in mice, with aged mice displaying increased susceptibility to these effects compared to adult mice. This finding aligns with a growing body of evidence linking sleep disruption to cognitive decline, particularly in older individuals. Sleep is known to play a crucial role in memory consolidation, and disturbances in sleep architecture could interfere with these processes, leading to cognitive impairment. The finding that aged mice are more affected by sleep fragmentation than adult mice could be attributed to age-related changes in sleep architecture, increased vulnerability to sleep disturbances, and reduced cognitive reserve in older individuals.

A key finding in this study was the abnormal activation of microglia due to preoperative sleep fragmentation. Microglia, the resident immune cells of the central nervous system, play pivotal roles in maintaining brain homeostasis and responding to injury or disease. When activated, microglia can release proinflammatory cytokines that can induce neuroinflammation, which has been implicated in cognitive dysfunction. The alterations in inflammatory cytokines observed in this study further support the role of neuroinflammation in sleep fragmentation-induced cognitive impairment. However, the precise mechanisms underlying microglial activation due to sleep fragmentation warrant further investigation.

Another important observation was the alterations in iron metabolism and ferroptosis following sleep fragmentation. Iron is vital for various cellular functions, but excessive iron can lead to the production of reactive oxygen species and oxidative stress, culminating in ferroptosis. The significant reduction in iron content in the hippocampal tissue of mice exposed to sleep fragmentation, coupled with changes in the expression of ferroptosis-related proteins, indicates a link between sleep fragmentation, dysregulated iron metabolism, and ferroptosis. These findings underscore the potential of targeting iron metabolism and ferroptosis as a novel strategy for mitigating sleep fragmentation-induced cognitive impairment.

One limitation of this study is that it was conducted in mice, and the findings may not be directly translatable to humans due to differences in sleep architecture and brain structure. Future studies in humans are needed to validate these findings. Moreover, while this study provides evidence of a link between sleep fragmentation, microglial activation, and cognitive impairment, it does not elucidate the causal relationships between these factors. Further research is needed to dissect these relationships and uncover the underlying mechanisms.

In conclusion, this study sheds light on the complex interplay between sleep fragmentation, microglial activation, and cognitive impairment. It highlights the potential of targeting microglial activation and ferroptosis as preventative strategies for PND in older adults exposed to sleep fragmentation. These findings add to our understanding of the pathogenesis of PND and pave the way for future research aimed at developing effective interventions to improve sleep quality and cognitive outcomes in the perioperative period.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yong Chen, Peng Yao, Yujuan You, Yanhui Hu, and Enjun Lei conceptualized and designed the study. Xianliang Xing, Xifeng Wang, and Weijian Zhou acquired and analyzed the data.Yong Chen drafted the manuscript. Yanhui Hu and Enjun Lei revised the manuscript. All authors read and agreed to the final version of the manuscript. Yong Chen and Peng Yao contributed equally to this work.

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