Abstract

Background: Clinical and animal studies demonstrated that neuroinflammation from anesthesia (sevoflurane) is the main contributor to cause perioperative neurocognitive disorders (PND). Recently, it was reported that microglia respond to hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, which was the target of sevoflurane. Whether HCN channels are involved in the induction of neuroinflammation after sevoflurane exposure is still unclear.

Results: Sevoflurane exposure had increased cognitive dysfunction and anxiety-like behaviors in rats. Rats inhaled with sevoflurane had activated microglia and increased neuroinflammation (IL-1β, IL-6, and TNF-α) in the hippocampus. RNA sequencing identified 132 DEGs (86 up-regulated and 46 down-regulated DEGs [differentially expressed genes]) in the hippocampus of PND rats. RNA-sequencing also uncovered that sevoflurane exposure down-regulates HCN2 expression. Pathway and process enrichment analysis suggests DEGs are mainly enriched in regulation of system process, positive regulation of glutamate secretion, secretion, regulation of synaptic transmission, regulation of nervous system process, behavior, negative regulation of sodium ion transport, and learning or memory. We validated that sevoflurane exposure can down-regulate the levels of PEX5R/Trip8b (an interaction partner and auxiliary subunit of HCN channels) and HCN1-4 channels in the hippocampus of PND rats. We used immunofluorescence staining to identify that HCN2 co-labels with neurons (Neun), astrocytes (GFAP), and microglia (iba1). We observed that the co-labeling of HCN2 with neurons or microglia decreased in the hippocampus and cortex after sevoflurane exposure. Blocking HCN2 by ZD7288 treatment further activated microglia and aggravated sevoflurane exposure-induced anxiety-like behavior, cognitive impairment, and neuroinflammation.

Conclusions: We concluded that sevoflurane exposure can induce an increased level of neuroinflammation, microglial activation, cognitive dysfunction, and anxiety-like behaviors in rats. HCN2 channel, as the target of sevoflurane action, mediates this process. HCN2 might be a target for the treatment and prevention of sevoflurane-induced PND.

Keywords: Microglia, Neuroinflammation, Sevoflurane, HCN2, Perioperative neurocognitive disorders
65 years is about 54% [4]. In cardiac surgery patients, its incidence may be as high as 60% [5]. The high incidence of PND makes it an essential topic in perioperative medical research. Its harmfulness and adverse effects on postoperative recovery have seriously increased the burden on the global medical system and the families of surgical patients.

The pathogenesis of PND is complex, involving numerous pathological and molecular biological mechanisms. Many clinical and animal studies have shown that neuroinflammation may play a critical neuropathological process for PND [6–9]. Nevertheless, the mechanisms for the initiation and induction of neuroinflammation after anesthesia are still unclear.

As a homeostasis maintainer in the CNS (central nervous system), microglia is the resident phagocyte in the CNS, which is the first responder to neuroinflammation or injury, and rapidly adjusts its phenotypic and functional changes according to the brain environment [10, 11]. Many published research has reported that microglia is vital in determining neuroinflammatory response in inducing PND [12, 13]. The activation of microglia facilitates the increase of hippocampal neuroinflammatory cytokine levels (interleukin-1β [IL-1β], IL-6, and TNF-α [tumor necrosis factor α]) and finally causes cognitive impairment [14, 15]. In contrast, effectively depleting CNS microglia by using PLX5622 (colony-stimulating factor 1 receptor [CSF1R] inhibitor) reduces hippocampal levels of inflammatory mediators and remarkably improves cognitive impairment [16]. In addition, other animal studies also demonstrated that suppressing microglial activation in CNS may be a therapeutic target for PND caused by anesthesia or surgery [15, 17]. Thus, finding a target that regulates the biological function of microglia is the key to solving PND.

We and others found that sevoflurane, as a volatile anesthetic, can inhibit hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels, and HCN knockout or selective HCN channel blocker ZD7288 can increase anesthetic properties of sevoflurane [18, 19]. Interestingly, an in vitro experiment has provided the novel discovery that HCN channels can be expressed on primary microglia and contribute to various microglial functions and influence the course of microglial activation [20]. However, in vivo research, whether sevoflurane through HCN channels mediates microglial activation and neuroinflammation triggering PND is unknown.

Based on the above evidence, we assumed that sevoflurane exposure can lead to neuroinflammation and the deficit of learning and memory and that these effects are regulated by HCN channels. We used sevoflurane exposure to induce the PND rats model to examine these hypotheses. Furthermore, we employed molecular biology, neurobehavioral science, transcriptome sequencing, etc. Ultimately, in vivo experimental level, our results will provide crucial evidence for the involvement of HCN channels in PND, which might be a therapeutic target for PND treatment.

Results
Sevoflurane exposure triggered cognitive impairment and anxiety-like behaviors in rats
The schedule of the first experiment and schematic diagram of MWM is shown in Fig. 1A, B. After PND rat models were induced by sevoflurane exposure, PND rats suffered from neurobehavioral changes such as cognitive impairments and anxiety-like behaviors. In probe trials, the number of rats entering the platform quadrant and time spent in the platform quadrant in the PND group was significantly decreased compared with the control group (Fig. 1C, B). On the third day after sevoflurane exposure, the MWM test showed that rats in both PND and control groups had declined as time passed. In the control group, the number of rats entering the platform quadrant and time spent in the platform quadrant evidently decreased on the third day after PND establishment (Fig. 1E, F). Meanwhile, in the PND group, time spent in the platform quadrant of rats was significantly reduced on the third day after PND establishment (Fig. 1G). Over time, the rats in both groups showed a natural decline in the memory capacity formed by the water maze training, which contributed to no significant difference in the MWM test between the two groups on the third day after sevoflurane exposure.

Furthermore, PND rats also exhibited anxiety-like behaviors, and the schematic diagram of OPT and EPM were also displayed in Fig. 2A, B. The OPT has shown that PND rats had fewer vertical scores, the number of entering the central zone, and time spent in the central zone (Fig. 2C–E). EPM tests have also presented that PND rats had fewer entering the opened arms (Fig. 2F, 2G, 2H). The number of entering the platform quadrant of rats in the control group on the first day and third day after sevoflurane exposure. Data are shown as mean ± SD (n = 8). *P < 0.05 or **P < 0.01.

(See figure on next page.)

**Fig. 1** Sevoflurane exposure inducing cognitive dysfunction. A, The schedule of the first experiment. B, The schematic diagram of the MWM test. C, The number of entering the platform quadrant of rats in the MWM test. D, The time spent in the platform quadrant of rats in the MWM test. E, The time spent in the platform quadrant of rats in the control group on the first day and third day after sevoflurane exposure. F, The number of entering the platform quadrant of rats in the control group on the first day and third day after sevoflurane exposure. G, The time spent in the platform quadrant of rats in the PND group on the first day and third day after sevoflurane exposure. H, The number of entering the platform quadrant of rats in the PND group on the first day and third day after sevoflurane exposure. Data are shown as mean ± SD (n = 8). *P < 0.05 or **P < 0.01.
Fig. 1 (See legend on previous page.)
These neurobehavioral experiments showed that sevoflurane exposure induced neurocognitive impairment in rats accompanied by depression and anxiety behaviors.

The transcriptomic analysis uncovered that sevoflurane exposure down-regulated HCN2 expression. GO enrichment analysis indicated that DEGs were mainly enriched in the regulation of system process, positive regulation of glutamate secretion, secretion, regulation of synaptic transmission, regulation of nervous system process, and learning or memory.

To identify possible pathways causing PND, we used RNA sequencing to screen for genes and pathways associated with PND. RNA sequencing identified 132 DEGs (86 up-regulated and 46 down-regulated DEGs, with \( P < 0.05 \) and \( |\log_{2}FC| \) [fold change] > 1) after inducing PND (Fig. 3A–D). In the volcano plot and expression heat map of DEGs, HCN2 marked in Fig. 3A, D was down-regulated after sevoflurane exposure. GO enrichment analysis indicated that the top 11 clusters with enriched representative terms (one per cluster) were shown in Fig. 4A–C. GO Biological Processes enrichment analysis displayed that DEGs were primarily enriched in the regulation of system process, positive regulation of glutamate secretion, secretion, regulation of synaptic transmission, regulation of nervous system process, behavior, regulation of membrane potential, negative regulation of sodium ion transport, and learning or memory (Fig. 4C). GO Cellular Components enrichment analysis showed that DEGs were mainly enriched in axonemal dynein complex, basement membrane, AMPA glutamate receptor complex, dendritic shaft, and parallel fiber to Purkinje cell synapse (Fig. 4B). GO Molecular Functions enrichment analysis presented that DEGs were primarily enriched in cytoskeletal motor activity, minus-end-directed microtubule motor activity, dynein light intermediate chain binding, and calmodulin binding (Fig. 4A).

Protein–protein interaction network and MCODE components were used to identify the gene lists in
Fig. 4D. The functional description of the corresponding components (Fig. 4E) shows sevoflurane exposure induced the change of these hub genes related to pathways of neurodegeneration-multiple diseases, Huntington disease, and amyotrophic lateral sclerosis. The finding suggests that sevoflurane exposure can affect synaptic transmission, secretion, nervous system process, ion transport, and learning or memory. Hub genes enrichment analysis also suggests that sevoflurane anesthesia may be a relevant factor for neurodegeneration-multiple diseases, Huntington disease, and amyotrophic lateral sclerosis. Sevoflurane-induced genetic alteration related to neurodegeneration strongly suggests that there might be the same pathogenic pathway between PND and neurodegeneration, which has clinical relevance for PND patients.

Sevoflurane exposure down-regulated the levels of PEX5R/Trip8b-HCN channels and activated microglia in the hippocampus

According to RNA-sequencing results, regulation of system process, secretion, negative regulation of sodium transport, regulation of synaptic transmission, and regulation of nervous system process may be involved in the pathogenesis of PND. We seek to explore molecular evidence linking these pathogenic pathways/processes to PND. Based on our previous research and other reports, HCN is associated with the sodium or potassium ion transmembrane transport, regulating neuronal excitability and transmission [19, 21, 22], and is also expressed in microglia and regulates microglial function [20]. In vitro, HCN2 expressed with a higher degree was found in primary microglia, and regulation of HCN2 can alter the secretion and functions of microglia [20]. In this study, sevoflurane exposure can significantly downregulate the expression of HCN1-4 mRNA (Fig. 5B-F). The tetraatricopeptide repeat-containing Rab8b-interacting protein (Trip8b or PEX5R) is an interaction partner and auxiliary subunit of HCN channels [23, 24]. PEX5R/Trip8b C and N terminus have a biochemical association with HCN2 channels (Fig. 5A) [25–27]. Our results also found that sevoflurane exposure reduces the PEX5R/Trip8b and HCN2 expression at the transcript and protein levels (Fig. 5C, D, I, J). Meanwhile, we observed that microglia...
in the hippocampus also was activated, with the upregulation of iba1 (the marker for microglial activation), as shown in Fig. 5G, H. Many microglia infiltrating the hippocampus were also observed, as shown in Fig. 5K, L. These results indicated sevoflurane exposure reduced levels of PEX5R/Trip8b and HCN channels and activated microglia in the hippocampus.

**HCN2 was expressed in neurons, astrocytes, and microglia.**

Sevoflurane exposure triggered the down-regulation of HCN2 in microglia and neurons.

We used IF staining to identify that HCN2 co-labels with neurons (Neun), astrocytes (GFAP), and microglia(iba1), which illustrates that HCN2 is widely expressed in different nerve cells of the CNS (Additional file 1: Fig. S1). Then, we found that the co-labeling of HCN2 with microglia reduced in the hippocampus and cortex (Fig. 6A-D). In addition, we also observed that the co-labeling of HCN2 with neurons decreased in the hippocampus and cortex (Fig. 6A-D). Thus, sevoflurane exposure caused extensive down-regulation of HCN2, including on microglia and neurons.

**Sevoflurane exposure caused neuroinflammation in the cortex and hippocampus**

After inducing PND, we used ELISA and HE staining to estimate neuroinflammation. In HE staining, we have found that neurons were intact without morphological alteration, and there are some inflammatory cells (small round nuclei) infiltrating the hippocampus and cortex of PND rats (Fig. 7G). ELISA also displayed that proinflammatory cytokines (IL-6, IL1β, and TNFα) were apparently

---

**Fig. 4** GO enrichment analysis and screening hub genes. **A**, GO molecular Functions enrichment analysis. **B**, GO Cellular Components enrichment analysis. **C**, GO Biological Processes enrichment analysis. From panels A to C, X-axis is log10(P) (the P-value in log base 10) and Y-axis shows enriched terms (one per cluster). Larger log10(P)-values represent more meaningful enrichment entries. **D**, Protein–protein interaction network and MCODE components in the gene lists. **E**, Functional description of the hub genes and GO enrichment analysis. PND group (n = 3) vs. control group (n = 3).
Fig. 5  Sevoflurane exposure down-regulating PEX5R/Trip8b and HCN channels and activating microglia. A, Structure of PEX5R/Trip8b from UniProt Knowledgebase (https://www.uniprot.org/uniprot/Q925N3), which C and N terminus has a biochemical association with HCN channels. B, Sevoflurane down-regulated HCN1 mRNA (n = 4). C, Sevoflurane down-regulated HCN2 mRNA (n = 4). D, Sevoflurane down-regulated PEX5R mRNA (n = 4). E, Sevoflurane down-regulated HCN3 mRNA (n = 4). F, Sevoflurane down-regulated HCN4 mRNA (n = 4). G, Immunoblotting of iba1, PEX5R, and HCN2 in the hippocampus (n = 3). H, Sevoflurane up-regulated iba1 expression. I, Sevoflurane down-regulated PEX5R expression. J, Sevoflurane down-regulated HCN2 expression. K, L, Sevoflurane exposure activated microglia (n = 4). Arrows indicate microglia. Data are shown as mean ± SD. *P < 0.05 or **P < 0.01.
increased in PND rats’ cortex and hippocampus compared with the control group (Fig. 7A, F). This evidence displayed that sevoflurane exposure triggered a neuroinflammatory response.

**ZD7288 treatment aggravated sevoflurane exposure-induced cognitive impairment and anxiety-like behaviors of PND rats**

The schematic diagram of the second experiment is displayed in Fig. 8A. ZD7288 is a selective and effective HCN channel blocker, which was harnessed to inhibit the HCN channel in the brain. After blocking HCN channels in brain, rats in the PND-HCN-B group showed more increased cognitive impairments and anxiety-like behaviors than rats in PND-NS. In probe trials, the number of entering the platform quadrant and time spent in the platform quadrant of rats in the PND-HCN-B group significantly decreased compared with the PND-NS group (Fig. 8B, C). In evaluating anxiety-like behaviors, OPT has shown that rats in the PND-HCN-B group had fewer entering the central zone and time spent in the central zone (Fig. 8F, G). The EPM test also presented that rats in the PND-HCN-B group had fewer entering the opened arms (Fig. 8D, E). These neurobehavioral experimental results show that blocking HCN2 can aggravate sevoflurane exposure-induced cognitive impairment and anxiety-like behaviors, which presents a synergistic effect.
ZD7288 treatment reduced HCN2 expression, increased microglial activation, and aggravated sevoflurane exposure-induced neuroinflammation

After ZD7288 preconditioning PND rats, we employed PCR and WB to estimate the HCN2, iba1, and CD68 (indicative of microglial phagocytosis) expression. ZD7288 treatment down-regulated HCN2 expression at the protein level and transcription level (Fig. 9B, E). ZD7288 treatment in PND rats can increase microglial activation in the hippocampus (Fig. 9D, F). Moreover, CD68 mRNA was also up-regulated after blocking HCN2 in the hippocampus (Fig. 9C).

Then, we used ELISA to detect the neuroinflammatory level. In the PND-HCN-B group, ELISA has presented that PND rats’ proinflammatory cytokines (IL-6, IL1β, and TNFα) were significantly increased in both the cortex and hippocampus compared with rats in the PND-NS group (Fig. 9G, F). Therefore, inhibition of HCN2 can further aggravate sevoflurane exposure-induced neuroinflammation and activation of microglia.

Fig. 7 Sevoflurane inducing PND with neuroinflammation in the cortex and hippocampus. A, Sevoflurane up-regulated IL-6 in the hippocampus (n = 4). B, Sevoflurane up-regulated TNF-α in the hippocampus (n = 4). C, Sevoflurane up-regulated IL-1β in the hippocampus (n = 4). D, Sevoflurane up-regulated IL-6 in the cortex (n = 4). E, Sevoflurane up-regulated TNF-α in the cortex (n = 4). F, Sevoflurane up-regulated IL-1β in the cortex (n = 4). G, HE staining of cortex and hippocampus. Arrows indicate neurons. Triangles indicate inflammatory cells. Data are shown as mean ± SD. *P<0.05 or **P<0.01.
Discussion

Sevoflurane, as a common inhalation anesthetic, is widely used in the field of anesthesiology over the world. HCN channels as the target for sevoflurane were sufficiently established by our team and other researchers [18, 19]. To the best of our knowledge, this study was the first report to demonstrate that sevoflurane acts on its target-HCN2 channel to regulate microglial function and neuroinflammation in vivo, triggering cognitive impairment and anxiety-like behaviors in rats. Our findings have addressed that HCN2 has a significant regulatory effect on microglial activation and neuroinflammation in mediating sevoflurane-induced PND. Moreover, RNA-sequencing uncovered sevoflurane exposure down-regulates HCNI2, and pathway and process enrichment analysis suggests DEGs are mainly enriched in regulation of system process, positive regulation of synaptic transmission, regulation of nervous system process, and learning or memory, which might be associated with PND.

As we know, PND is closely associated with surgery and anesthesia. Our results have illustrated that sole sevoflurane exposure without surgery could induce cognitive dysfunction and anxiety-like behaviors. These behavioral changes are also accompanied by neuroinflammation and microglial activation. Similarly, previous studies also reported that rats only exposed to 2% sevoflurane for five hours or 2.5% sevoflurane for six hours produced cognitive impairment [28, 29]. In this study, rats have exposed to 3% sevoflurane for four hours to establish the PND models, which also caused the cognitive deficiency. Together, we argued that a long time or high dose of sevoflurane exposure could trigger rats' cognitive dysfunction. It may also suggest that reducing the dosage or the duration of sevoflurane exposure can be beneficial for patients at risk of PND. On the third day after PND, we found that PND rats had no difference in the MWM test compared with rats in the control group, which suggests cognitive impairment induced by sevoflurane was time-dependent, and PND symptoms improved with time delay.
Fig. 9 ZD7288 treatment increasing neuroinflammation and microglial activation of PND rats. **A**, Diagrammatic illustration of our findings. HCN2 is extensively expressed in different cells of the cortex and hippocampus, including neurons, microglia, and astrocyte. Sevoflurane down-regulated HCN2 mediating cognitive dysfunction, anxiety-like behaviors, microglial activation, and neuroinflammation. Sevoflurane combined with ZD7288 (HCN2 blocker) produced strong synergies in this process. **B**, ZD7288 treatment down-regulating HCN2 mRNA of PND rats (n = 4). **C**, ZD7288 treatment up-regulating CD68 mRNA of PND rats (n = 4). **D**, Immunoblotting of HCN2 and iba1 (n = 3). **E**, ZD7288 treatment down-regulating HCN2 expression of PND rats. **F**, ZD7288 treatment activating microglia of PND rats. **G**, ZD7288 treatment increasing IL-6, IL-1β, and TNF-α in the hippocampus of PND rats (n = 4). **H**, ZD7288 treatment increasing IL-6, IL-1β, and TNF-α in the cortex of PND rats (n = 4). Data are shown as mean ± SD. *P < 0.05 or **P < 0.01.
Clinical manifestations of PND include cognitive impairment and confusion, anxiety, and personality changes. Our study used OFT and EPM to estimate anxiety-like behaviors of PND, which thoroughly examined neurobehavioral changes in the PND rats model. In this study, sevoflurane exposure triggered anxiety and decreased space exploration capability, consistent with the previous study [30]. These findings suggest that PND rats with cognitive dysfunction also presented anxiety-like behaviors, which should attract more attention from perioperative medicine.

Neuroinflammation is regarded as a critical role in mediating PND, and reducing the level of inflammatory mediators can ameliorate PND [6, 7, 31]. In our work, sevoflurane exposure triggered CNS inflammation and up-regulated the proinflammatory cytokines of TNF-α, IL-6, and IL-1β. Notably, ELISA found that proinflammatory cytokines in both cortex and hippocampus were all increased after inducing PND rats by inhalation of sevoflurane. As we know, sevoflurane is inhaled through lung tissue into the bloodstream, travels to the CNS, and interacts with different brain regions. Our results and pharmacological mechanism of sevoflurane in CNS strongly implied that sevoflurane-induced CNS inflammation was extensive and not limited to a specific brain region. The previous study also reported that surgery-induced PND models had marked increases in TNF-α, IL-1β, and IL-6 in the cortex and hippocampus [32]. To sum up, both sevoflurane-induced and surgery-induced PND animal models can produce extensive CNS neuroinflammation.

Microglia is a critical regulator in processing CNS neuroinflammatory changes during PND [16, 33, 34]. Effectively depleting CNS microglia in surgery-induced PND models via inhibiting CSF1R reduced hippocampal levels of inflammatory cytokines and mitigated the development of PND [16]. We also confirmed that microglial activation was associated with neuroinflammation, cognitive dysfunction, and anxiety-like behaviors in sevoflurane-induced PND rats. This finding broadly supports that regulating microglia is a determinant for treating sevoflurane-induced PND.

Subsequently, we tried to find the molecules or channels that sevoflurane interacts with that may be involved with the regulation of microglia. In 2020, a crucial study was the first to report the expression of HCN on primary microglia and explore its function in regulating microglia [20], which provided evidence of linking sevoflurane with microglia. Among HCN channels, HCN2 was reported to be expressed to a higher degree than other HCN subunits in primary microglia [20], which is more closely correlated with microglial function. Likewise, we also observed that HCN2 was expressed in microglia, astrocytes, and neurons, which suggests that HCN2, as a channel, is widely present in the CNS. Therefore, our study focused on examining the role of HCN2 in sevoflurane-induced PND. Based on this finding, in vivo experiment, our results were the first to prove that sevoflurane exposure down-regulated microglial HCN2 channel and activated microglia in the hippocampus. The unexpected finding was that HCN1, HCN2, HCN3, and HCN4 were all down-regulated after sevoflurane exposure.

Furthermore, PEX5R/Trip8b, as an interaction partner and auxiliary subunit of HCN channels [23, 24], was also down-regulated after exposure to sevoflurane. After ZD788 treatment and down-regulation of HCN2 in the hippocampus of PND rats, we found that microglia were activated. Moreover, CD68, the marker of microglial phagocytosis, was also up-regulated. It means that ZD788 treatment mediating down-regulation of HCN2 triggers functional change of microglia, which is consistent with in vitro experiments [20]. Furthermore, PND rats with blocking HCN channels in CNS have aggravated inflammatory responses in the cortex and hippocampus. With further aggravating neuroinflammation and increasing the microglia activation, cognitive impairment and anxiety behaviors were even worse. This neurobehavioral change coincided with marked increases in neuroinflammation intensively implies that combined sevoflurane with HCN channels blocker produces strong synergies in causing PND. Accordingly, we deemed that sevoflurane leads to cognitive impairment, anxiety-like behaviors, and neuroinflammation of PND through regulating PEX5R/Trip8b–HCN2 channel interaction.

There are some limitations in this work that need to be addressed. This study only chose animal experiments to verify our hypothesis and has not carried out in vitro experiments. The reason is that it is difficult to simulate the pathogenesis of PND in vitro. In addition, the treatment of cells with inhaled sevoflurane is still controversial. We used MCODE to identify the hub genes related to PND. These genes were enriched in neurodegeneration–multiple diseases. Thus, these hub genes may be involved in the same pathogenic pathway mediating PND and neurodegeneration, which has clinical relevance for PND patients. At last, future work will be performed on exploring some pathogenic pathways in PND from the perspective of neurodegeneration.

Conclusion
In a word, this study suggests that sevoflurane exposure can induce an increased level of neuroinflammation, microglial activation, cognitive dysfunction, and anxiety-like behaviors in rats. HCN2 channel, as the target of sevoflurane action, mediates this process. HCN2 might be a target for the treatment and prevention of sevoflurane-induced PND. The pathogenesis diagram is shown in Fig. 9A.
Methods

The animal protocol of this animal study was approved by the Institutional Animal Care and Use Committee at Tongji Medical College, Huazhong University of Science and Technology. All animal experiments were performed in accordance with a guide to animal ethics.

Animal groups

The 13–16-month-old male SD (Sprague Dawley) rats were purchased from Biont (Wuhan, China). To verify our hypothesis, we carried out two animal experiments. In the first experiment, the SD rats were randomly assigned to the control group and the PND group (PND). In the PND group, rats have exposed to 3% sevoflurane for four hours to induce PND. Rats in the control group were exposed to oxygen for four hours.

In the second experiment, the PND rats were randomly divided into the following two groups: PND-NS (normal saline) and PND-HCN channels blocker (PND-HCN-B) group. In the PND-HCN-B group, PND rats received a stereotactic intracerebroventricular injection of ZD7288 (HCN channels blocker). In the PND-NS group, PND rats received a stereotactic intracerebroventricular injection of normal saline as solvent control.

PND rat model

In terms of PND models, the establishment of PND models is mainly divided into anesthesia-induced PND and surgery combined anesthesia-induced PND [28, 35, 36]. In order to precisely explore the effect of sevoflurane on HCN channels, we chose to induce the occurrence of PND by long-time and high-concentration exposure to sevoflurane. A long time and 3% sevoflurane in 100% oxygen for rat anesthesia have been reported to effectively trigger cognitive impairment in previous studies [37–39]. In brief, at first, rats were placed into an induction box respectively. Then, the vaporizer was used to input sevoflurane into the induction box, and the concentration of sevoflurane in the induction box was detected through an anesthesia monitor. The sevoflurane exposure concentration was set at 3%, and 2L/min pure oxygen was used as the carrier gas. After 3% sevoflurane exposure for four hours, rats were transferred to a resuscitation box filled with fresh oxygen. The indoor temperature was kept in the range of 23–25 °C.

Intracerebroventricular injections

ZD7288 is a selective HCN channel blocker [40, 41], which was harnessed to inhibit the HCN channel in the brain. During the second experiment, the rats were anesthetized with continuous 3% sevoflurane anesthesia on an animal anesthesia mask and then fixed onto a stereotaxic frame (RWD, China). Rats in PND-HCN-B and PND-NS groups were all placed in a stereotactic head frame for intracerebroventricular injections via a micro-injection pump. The injection site was located as: AP (anteroposterior) = 1.2 mm, ML (mediolateral) = 1.8 mm, and DV (dorsoventral) = 4.0 mm. Regarding the dosage of ZD7288 in intracerebroventricular injections, the previous study reported that ZD7288 regulated synaptic transmission and generated a dose-dependent inhibition of LTP induction [42]. In detail, low doses (ranging from 0.5 to 10 μg) of ZD7288 have no effect on basal synaptic transmission (fEPSP), and the high dose of 25 μg ZD7288 significantly suppresses basal synaptic transmission [42]. Accordingly, for the sake of avoiding the impact of ZD7288 on LTP and synaptic transmission, we select the dosage of 10 μg ZD7288 (Sigma-Aldrich, America) for intracerebroventricular injections. Rats in PND-HCN-B received an intracerebroventricular injection of 10 μg ZD7288 in 5 μl sterile saline (2 μg/μl of ZD7288) at 0.5 h before sevoflurane exposure. Additionally, rats in the PND-NS group only received an intracerebroventricular injection of 5 μl sterile saline. PND modeling was performed 0.5 h after intraventricular injection.

Morris water maze (MWM)

MWM test was employed to examine the cognition of rats, as described by the previous report [43]. Clinically, PND mainly occurs within 1 week after surgery and anesthesia [4]. We used the MWM to track cognitive changes. During all neurobehavioral experiments, experimenters were blind to group assignment and outcome assignment. In brief, rats were given three trails: visible platform, hidden platform, and probe trial. On day 1, each rat was trained to swim 4 times a day with a visible platform in the pool. From day 2 to day 5, each rat was trained to swim 4 times a day with a hidden platform in the pool. During swimming training, If the rat finds the platform before the 60 s cut-off, it is allowed to stay on the platform for 5 s and then return to its home cage. If the rat does not find the platform, it was placed on the platform to stay there for 20 s before returning it to its home cage. During the probe trial, the platform was removed from the pool, time spent in the platform quadrant and the number of entering the platform quadrant for each rat were recorded in the software. The probe trial was performed on the first day and third day after inducing the PND rat model.

Open field test (OFT)

Exploratory activity, autonomous animal, and anxiety-like behavior were examined by using an open-field apparatus (100 × 100 × 100 cm) [44, 45]. The center area was set as a square 20 cm away from the wall of the open-field apparatus. Each rat was placed in the center area
of the open-field apparatus. The time spent in the central area, number of entering the central area, and vertical score (number of the animal standing on both feet) were recorded for 5 min. Since the cognitive tests showed no difference between groups on day 3 after sevoflurane exposure, we focused only on the behavioral change on day 1 after sevoflurane exposure. Thus, the OFT was implemented on the first day after inducing the PND rat model.

**Elevated plus maze (EPM) test**
Anxiety-like behavior was also measured by the EPM test [44]. The EPM apparatus mainly consists of two closed arms and two open arms. The closed arms in the apparatus were enclosed by a black wall. Each rat was placed in the central zone of the EPM facing one of the open arms. Time of staying in the arms and number of entering arms was measured for 5 min. The EPM test was implemented on the first day after inducing the PND rat model.

**Brain tissue harvest**
According to the results of the MWM test, we chose the time to collect brain tissue. Since the cognitive tests were different only on day 1 after sevoflurane exposure, we harvested the brain tissues on the first day after inducing the PND rat model. After deep anesthesia with sevoflurane, the animal brain tissue was collected immediately for WB (western blotting), RT-PCR (real-time polymerase chain reaction), ELISA (enzyme-linked immunosorbent assay), and RNA-sequencing, which were stored in a refrigerator at −80 °C. Then, we also used saline flush and paraformaldehyde fixation of brain tissue for immunostaining. Brain frozen sections were prepared for HE (hematoxylin and eosin) and immunofluorescent staining, which were stored in a refrigerator at −20 °C.

**RNA-sequencing**
The hippocampus was used for RNA-sequencing. The cDNA library preparation was according to the BGI (China)’s standard procedures. In short, the fragment was end-repaired, dA-tailed, adaptor ligated, and then with a 4-cycle PCR program. The libraries were sequenced following the BGI’s protocols for RNA-Seq on the Illumina HiSeq 2500 platform by using the 50-bp pair-end sequencing strategy. Transcript expression levels were measured by using fragments per kilobase per million reads (FPKM). Pathway and process enrichment analysis has been carried out with the following ontology source: GO Biological Processes, GO Molecular Functions, and GO Cellular Components by Metascape (https://metascape.org/gp/index.html#/main/step1) [46]. All DEGs (differentially expressed genes) in the genome have been used as the enrichment background and were displayed in the volcano plot. Terms with a P-value < 0.01, a minimum count of 3, and an enrichment factor >1.5 are collected and grouped into clusters according to their membership similarities. To further investigate the relationships between the terms, a subset of enriched terms has been selected and rendered as a network plot, where terms with a similarity >0.3 are connected by edges. Protein–protein interaction enrichment analysis has been performed with the following databases: STRING [47], BioGrid [48], OmniPath [49], and InWeb.IM [50] by using Metascape. If the network contains between 3 and 500 proteins, the Molecular Complex Detection (MCODE) algorithm has been applied to examine densely connected network components. Pathway and process enrichment analysis has been applied to each MCODE component independently, and the terms by P-value have been reserved as the functional description of the corresponding components. Expression heatmaps were produced by Dor. Tom from BGI (a web-based multi-omics visualization tool: https://biosys.bgi.com/#/report/mrna/en/help).

**RT-PCR**
Total RNA of the hippocampus was extracted and retrotranscribed into cDNA by using an EntiLink™ 1st Strand cDNA Synthesis Kit (ELK Biotechnology, China) according to manufacturer’s instructions. RT-PCR was performed using a StepOne™ Real-Time PCR machine (Thermo Fisher Scientific, America) and an EnTurbo™ SYBR Green PCR SuperMix kit (ELK Biotechnology, China). The reaction system was prepared according to the instruction. The cycling parameters were 95 °C for 3 min, then 40 cycles at 95 °C for 10 s, followed by 58 °C for 30 s and 72 °C for 30 s. All gene primer pairs are shown in Table 1.

**WB**
Total protein was extracted from the hippocampus on the first day after inducing the PND rat model. The sample was added to lysis buffer (RIPA) with protease and phosphatase inhibitors. The tissue homogenizer was used to produce hippocampal homogenates. Afterward, homogenates were centrifuged at 4 °C at 12,000 rpm for 15 min, and the supernatants were then collected in EP tubes. The concentration of protein in EP tubes was measured by a BCA protein assay Kit (Biosharp, China). Next, protein samples were separated by electrophoresis on 8–12% sodium dodecyl sulfate–polyacrylamide.
gels and transferred to PVDF membranes (Millipore, America). The PVDF membranes were blocked with 5% nonfat milk in 0.1% tris-buffered saline with Tween 20 (TBST), followed by incubation overnight with a primary antibody. The following primary antibodies in the study were used, including anti-HCN2 (1/1000, Invitrogen, America), anti-PEX5R (1/1000, Santa Cruz Biotechnology, America), and anti-iba1 (1/1000, Abcam, United Kingdom). After washing with TBST, the PVDF membranes were incubated with HRP-labeled secondary antibody at RT (room temperature) for one hour. The following second antibodies in the study were used, including anti-rabbit (1/10000, Abcam, United Kingdom) and anti-mouse (1/10000, Abcam, United Kingdom). Then, ECL-A buffer and ECL-B buffer (Biosharp, China) were added for imaging. β-actin (1/5000, Abcam, United Kingdom) was employed as a loading control. The WB images were quantified using Image J software.

Immunofluorescence (IF) staining
Briefly, microwave antigen retrieval was implemented in sodium citrate buffer (10 mM sodium citrate) at 95 to 100 °C for 10 min. Sections were blocked with 5% bovine serum albumin in phosphate-buffered saline (PBS) containing 0.3% Triton-X 100 for 1 h at RT. After washing with PBS, the brain slices were then incubated overnight at 4 °C with primary antibodies, including anti-HCN2 (1/1000, Invitrogen, America), anti-Neun (1/200, Abcam, United Kingdom) and anti-mouse (1/1000, Abcam, United Kingdom). Then, ECL-A buffer and ECL-B buffer (Biosharp, China) were added for imaging. β-actin (1/5000, Abcam, United Kingdom) was employed as a loading control. The WB images were quantified using Image J software.

Table 1  Primers of RT-PCR

| RNA         | Sequences forward/reversed 5′-3′          |
|-------------|----------------------------------------|
| β-actin     | Forward CCGTACGGATAAGACCTTC            |
|             | Reversed TACAGCCGACGCTGTAATCTC         |
| PEX5R/Trip8b| Forward CACCACGGTGTCATGGAGG            |
|             | Reversed GCATACAGAGCTCCTAAGGGT         |
| HCN1        | Forward AGAAATGGTAATGCCAGGAGC          |
|             | Reversed GGTATTCCTCCAGAAGCTCTGG        |
| HCN2        | Forward GAGGCGATCTGATACCTGGATC         |
|             | Reversed GCCGACGACAATAACAGAC           |
| HCN3        | Forward AGACATGGCTCGTGGGTATTCG         |
|             | Reversed TGACTCTGCTAGCAGACGGG          |
| HCN4        | Forward CACCCGTAGCGATGCTGAG            |
|             | Reversed GTACCGTGTCGTCAGTACGTG         |
| CD68        | Forward CAGTGGAGCTTCCTCAGGGCA          |
|             | Reversed GTAACGCAGGGAGAATGGAGC         |

Statistical analysis
Results are reported as mean±SD (standard deviation) to describe data. The data between the two groups were analyzed using the t-test or rank-sum test. In RNA-sequencing results, DEGs were selected by meeting the two criteria: |log 2(fold change, FC)|> 1 and adjusted P< 0.05. The fold change of each gene was calculated by comparing the standardized read counts of the PND group to the control group (fold change = standardized read counts of the PND group/stANDARDized read counts of the control group). A P< 0.05 was considered as a significant difference. All data analyses were performed with Stata 16.0 (Stata Corporation, America).

Abbreviations
PND: Perioperative neurocognitive disorders; HCN: Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels; IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor α; CSF1R: Colony-stimulating factor 1 receptor; BD: Sprague Dawley; PND-NS: PND-Normal saline; PND-HCN-B: PND-HCN channel blocker; AP: Anteroposterior; ML: Mediolateral; DV: Dorsoventral; MWM: Morris water maze; OFT: Open field test; EPM: Elevated plus maze; WB: Western blotting; RT-PCR: Real-time polymerase chain reaction; ELISA: Enzyme-linked immuno sorbent assay; HE: Hematoxylin and eosin; FPKM: Fragments per kilobase per million reads; DEGs: Differentially expressed genes; GO: Gene Ontology database; KEGG: Kyoto Encyclopedia of Genes and Genomes database; FC: Fold change; PEX5R/Trip8b: Tetratricopeptide repeat-containing Rab8b-interacting protein; SD: Standard deviation; IQR: Interquartile range.

ELISA
Cytokines (TNF-α, IL-6, and IL-1β) in the hippocampus and cortex were quantified by using ELISA kits (Bioswamp, China). According to the manufacturer’s instructions, an ELISA was performed. A microplate reader (Thermo Fisher Scientific, America) with absorption at 450 nm was used to measure absorbance values of samples, and cytokines concentration was detected according to the standard curve produced at the same time.

HE staining
Brain slices were stained with an H&E kit (Solarbio, China) according to the manufacturers’ instructions.

Statistical analysis
Results are reported as mean±SD (standard deviation) to describe data. The data between the two groups were analyzed using the t-test or rank-sum test. In RNA-sequencing results, DEGs were selected by meeting the two criteria: |log 2(fold change, FC)|> 1 and adjusted P< 0.05. The fold change of each gene was calculated by comparing the standardized read counts of the PND group to the control group (fold change = standardized read counts of the PND group/stANDARDized read counts of the control group). A P< 0.05 was considered as a significant difference. All data analyses were performed with Stata 16.0 (Stata Corporation, America).

Abbreviations
PND: Perioperative neurocognitive disorders; HCN: Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels; IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor α; CSF1R: Colony-stimulating factor 1 receptor; BD: Sprague Dawley; PND-NS: PND-Normal saline; PND-HCN-B: PND-HCN channel blocker; AP: Anteroposterior; ML: Mediolateral; DV: Dorsoventral; MWM: Morris water maze; OFT: Open field test; EPM: Elevated plus maze; WB: Western blotting; RT-PCR: Real-time polymerase chain reaction; ELISA: Enzyme-linked immuno sorbent assay; HE: Hematoxylin and eosin; FPKM: Fragments per kilobase per million reads; DEGs: Differentially expressed genes; GO: Gene Ontology database; KEGG: Kyoto Encyclopedia of Genes and Genomes database; FC: Fold change; PEX5R/Trip8b: Tetratricopeptide repeat-containing Rab8b-interacting protein; SD: Standard deviation; IQR: Interquartile range.
Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13578-022-00892-6.

Additional file 1: Figure S1. Co-labeling of HCN2 with microglia, astrocytes, and neurons. Arrows indicate co-labeling of HCN2 with cells.

Acknowledgements
We thank Prof. Jiangwen Yin at Shhezi University for the fruitful discussions and kind help.

Author contributions
FX and YFW designed the study. FX and YFW were major contributors in writing and reviewing the manuscript. All authors completed the animal study and analyzed the data. All authors read and approved the final manuscript.

Funding
This study was supported, in part, by the National Key Research and Development Program of China (2018YFC2001802) and the National Natural Science Foundation of China (82071251).

Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
The animal protocol of this animal study was approved by the Institutional Animal Care and Use Committee at Tongji Medical College, Huazhong University of Science and Technology (IACUC number: 2399). All animal experiments were performed in accordance with a guide to animal ethics.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1. Department of Anesthesiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei, China. 2. Department of Anesthesiology, Affiliated Jinling Hospital, Medical School of Nanjing University, Nanjing 210018, China.

Received: 1 July 2022 Accepted: 29 August 2022 Published online: 14 September 2022

References
1. Moller JT, Cluitmans P, Rasmussen LS, Houx P, Rasmussen H, Canet J, et al. Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study ISPOCD investigators International study of post-operative cognitive dysfunction. Lancet. 1998;351(9068):857–61.
2. Boone MD, Sites B, von Recklinghausen FM, Mueller A, Taenzer AH, Shafei S. Economic burden of postoperative neurocognitive disorders among US medicare patients. JAMA Netw Open. 2020;3(7):e2008931.
3. Eevere L, Silbert B, Knopman DS, Scott DA, Dekosky ST, Rasmussen LS, et al. Recommendations for the nomenclature of cognitive change associated with anaesthesia and surgery-2018. Br J Anaesth. 2018;121(5):1105–12.
4. Androssova G, Krause R, Winterer G, Schneider R. Biomarkers of postoperative delirium and cognitive dysfunction. Front Aging Neurosci. 2015;7:112.
5. Needham M, Webb CE, Bryden DC. Postoperative cognitive dysfunction and dementia: what we need to know and do. Br J Anaesth. 2017;119(1):i115–25.
6. Lai Z, Shan W, Li J, Min J, Zeng X, Zuo Z. Appropriate exercise level attenuates gut dysbiosis and valeric acid increase to improve neuroplasticity and cognitive function after surgery in mice. Mol Psychiatry. 2021;26(12):7167–87.
7. Terrando N, Monaco M, Ma D, Foxwell BM, Feldmann M, Maze M. Tumor necrosis factor-alpha triggers a cytokine cascade yielding postoperative cognitive decline. Proc Natl Acad Sci USA. 2010;107(47):20518–22.
8. Kawano T, Eguchi S, Iwata T, Kumaori N, Yokoyama M. Impact of preoperative environmental enrichment on prevention of development of cognitive impairment following abdominal surgery in a rat model. Anesthesiology. 2015;123(1):160–70.
9. Geng YJ, Wu QH, Zhang RQ. Effect of propofol, sevoflurane, and isoflurane on postoperative cognitive dysfunction following laparoscopic cholecystectomy in elderly patients: a randomized controlled trial. J Clin Anesth. 2017;38:165–71.
10. Madore C, Yin Z, Leibowitz J, Butovsky O. Microglia, lifestyle stress, and neurodegeneration. Immunity. 2020;52(2):222–40.
11. Aguzzi A, Barres BA, Bennett ML. Microglia: scapegoat, saboteur, or something else? Science. 2013;339(6116):156–61.
12. Chen YN, Sha HH, Wang YW, Zhou Q, Bhuyan P, Li NN, et al. Histamine 2/3 receptor agonists alleviate perioperative neurocognitive disorders by inhibiting microglia activation through the PI3K/AKT/FoxO1 pathway in aged rats. J Neuroinflamm. 2020;17(1):217.
13. Fan W, Mai L, Zhu X, Huang F, He H. The role of microglia in perioperative neurocognitive disorders. Front Cell Neurosci. 2020;14:261.
14. Safavinya SA, Goldstein PA. The role of neuroinflammation in postoperative cognitive dysfunction: moving from hypothesis to treatment. Front Psychiatry. 2018;9:752.
15. Liu Q, Sun YM, Huang H, Chen C, Wan J, Ma LH, et al. Sirtuin 3 protects against anesthesia/surgery-induced cognitive decline in aged mice by suppressing hippocampal neuroinflammation. J Neuroinflamm. 2021;18(1):41.
16. Feng X, Valldearosca M, Uchida Y, Lutrín D, Maze M, Kolwad SK. Microglia mediate postoperative hippocampal inflammation and cognitive decline in mice. JCI Insight. 2017;2(7):e91229.
17. Zhang D, Li N, Wang Y, Lu W, Zhang Y, Chen Y, et al. Methane ameliorates post-operative cognitive dysfunction by inhibiting microglia NF-κB/ MAPKs pathway and promoting IL-10 expression in aged mice. Int Immunopharmacol. 2019;71:52–60.
18. Scherwin S, Kopp C, Pircher E, Schneider G, Kreuzer M, Haseneder R, et al. Attenuation of native hyperpolarization-activated, cyclic nucleotide-gated channel function by the volatile anesthetic sevoflurane in mouse thalamocortical relay neurons. Front Cell Neurosci. 2020;14:606687.
19. Zhou C, Liang P, Liu J, Ke B, Wang X, Li F, et al. HCN channels contribute to the effects of amnesia and hypnosis but not immobility of volatile anesthetics. Anesth Analg. 2015;121(3):661–6.
20. Vay SU, Flitsch LJ, Rabenstein M, Monieré H, Jakovcevski I, Andjus P, et al. The impact of hyperpolarization-activated cyclic nucleotide-gated (HCN) and voltage-gated potassium KCNQ/Kv7 channels on primary microglia function. J Neuroinflamm. 2020;17(1):100.
21. Rätkai A, Tárnaok K, Aouad HE, Micska B, Schlett K, Szucs A. Homeostatic plasticity and burst activity are mediated by hyperpolarization-activated cation currents and T-type calcium channels in neuronal cultures. Sci Rep. 2021;11(1):3236.
22. Huang Z, Luan R, Kadurin I, Ubele BN, Renger JJ, Dolphin AC, et al. Presynaptic HCN1 channels regulate Cav3.2 activity and neurotransmission at select cortical synapses. Nat Neurosci. 2011;14(4):478–86.
23. Foote KM, Lyman KA, Han Y, Michalidis IE, Heuermann RJ, Mandikian D, et al. Phosphorylation of the HCN channel auxiliary subunit TRIPb is altered in an animal model of temporal lobe epilepsy and modulates channel function. J Biol Chem. 2019;294(43):15743–50.
24. Han Y, Heuermann RJ, Lyman KA, Fisher D, Ismail QA, Cherkovich DM. HCN-channel dendritic targeting requires bipartite interaction with TRIPb and regulates antidepressant-like behavioral effects. Mol Psychiatry. 2017;22(3):458–65.
25. Zolles G, Wenzel D, Böldl W, Schulte U, Hofmann A, Müller CS, et al. Association with the auxiliary subunit PEX5R/TRIP8b controls responsiveness of HCN channels to cAMP and adrenergic stimulation. Neuron. 2009;62(6):814–25.
Sotoudeh N, Namavar MR, Bagheri F, Zarifkar A. The medial prefrontal cortex to the medial amygdala connections may affect the anxiety level in aged rats. BMC Anesthesiol. 2018;18(1):42.

Zoebeiri M, Chaudhary R, Datunashvili M, Heuermann RJ, Lutjohann A, Narayanan V, et al. Modulation of thalamocortical oscillations by TRIPb6, an auxiliary subunit for HCN channels. Brain Struct Funct. 2018;223(3):1537–64.

Santoro B, Hu L, Liu H, Saponaro A, Pian P, Piskorski RW, et al. TRIPb6 regulates HCN1 channel trafficking and gating through two distinct C-terinal interaction sites. J Neurosci. 2011;31(11):4074–86.

Ge X, Zuo Y, Xie J, Li X, Li Y, Thirupathi A, et al. A new mechanism of POCD caused by sevoflurane in mice: cognitive impairment induced by cross-dysfunction of iron and glucose metabolism. Aging (Albany NY). 2021;9(18):22375–9.

Wei W, Sun Z, He S, Zhang W, Chen S. Protective role of desmopressin against sevoflurane-induced postoperative cognitive dysfunction via the microRNA-129/TLR4 axis. J Clin Neurosci. 2021;92:89–97.

Peng S, Li P, Liu P, Yan H, Wang J, Lu W, et al. Cistanches alleviates sevoflurane-induced cognitive dysfunction by regulating PPARγ-dependent antioxidant and anti-inflammatory in rats. J Cell Mol Med. 2020;24(2):1345–59.

Wang Z, Meng S, Cao L, Chen Y, Zuo Z, Peng S. Critical role of NLRP3-caspase-1 pathway in age-dependent isoflurane-induced microglial inflammatory response and cognitive impairment. J Neuroinflamm. 2018;15(1):109.

Zhang Z, Li X, Li F, An L. Berberine alleviates postoperative cognitive dysfunction by suppressing neuroinflammation in aged mice. Int Immunopharmacol. 2016;38:426–33.

Zhang X, Dong H, Li N, Zhang S, Sun J, Zhang S, et al. Activated brain mast cells contribute to postoperative cognitive dysfunction by evoking microglia activation and neuronal apoptosis. J Neuroinflamm. 2016;13(1):127.

Li J, Shi C, Ding Z, Jin W. Glycogen synthase kinase 3β promotes postoperative cognitive dysfunction by inducing the M1 polarization and migration of microglia. Mediators Inflamm. 2020;2020:7860829.

Zuo W, Zhao J, Zhang J, Fang Z, Deng J, Fan Z, et al. MD2 contributes to the pathogenesis of perioperative neurocognitive disorder via the regulation of δ2GABA(A) receptors in aged mice. J Neuroinflamm. 2018;15(1):204.

Lai Z, Min J, Li J, Shen W, Yu W, Zuo Z. Surgery trauma severity but not anesthesia length contributes to postoperative cognitive dysfunction in mice. J Alzheimer's Dis. 2021;80(1):245–57.

Wang Y, Qian M, Qu Y, Yang N, Mu B, Liu K, et al. Genome-wide screen of the hippocampus in aged rats identifies mitochondria, metabolism and aging processes implicated in sevoflurane anesthesia. Front Aging Neurosci. 2020;12:122.

Wang M, Zuo Y, Li X, Li Y, Thirupathi A, Yu P, et al. Effect of sevoflurane on iron homeostasis and toxicity in the brain of mice. Brain Res. 2021;1757:147328.

Zhang Q, Li Y, Bao Y, Yin C, Xin X, Guo Y, et al. Pretreatment with nimodipine reduces incidence of POCD by decreasing calciumine mediated hippocampal neuroapoptosis in aged rats. BMC Anesthesiol. 2018;18(1):42.

Zhang X-X, Min X-C, Xu X-L, Zheng M, Guo L-J. ZD7288, a selective hyperpolarization-activated cyclic nucleotide-gated channel blocker, inhibits hippocampal synaptic plasticity. Neural Regen Res. 2016;11(5):779–86.

Huang W, Zuo W, Chen L, Wang L, Treuflik F, Ru R, et al. Aldehyde dehydrogenase excitation of lateral habenular neurons via multiple cellular mechanisms. J Neurosci. 2021;41(36):7532–45.

He W, Zheng C, Fu G, Xu X, Lu Q, Guo L. ZD7288-induced suppression of long-term potentiation was attenuated by exogenous NMDA at the Schaffer collateral–CA1 synapse in the rat in vivo. Eur J Pharmacol. 2010;63(1–3):10–6.

Bromley-Brits K, Deng Y, Song W. Morris water maze test for learning and memory deficits in Alzheimer's disease model mice. J Vis Exp. 2011. https://doi.org/10.3791/2920.

Moradi Vastegani S, Hajipour S, Sarkaki A, Basir Z, Parisa Navabi S, Farbood Y, et al. Curcumin mitigates lipopolysaccharide-induced anxiety/depression-like behaviors, blood-brain barrier dysfunction and brain edema by decreasing cerebral oxidative stress in male rats. Neurosci Lett. 2022;782:136697.

Sotoudeh N, Namavar MR, Bagheri F, Zafarifar A. The medial prefrontal cortex to the medial amygdala connections may affect the anxiety level in aged rats. Brain Behav. 2022. https://doi.org/10.1002/brb3.2616.

Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun. 2019;10(1):1523.

Szkarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019;47(D1):D607–13.

Stark C, Breitkreutz BJ, Reguly T, Boucher L, Breitkreutz A, Tyers M. BioGRID: a general repository for interaction datasets. Nucleic Acids Res. 2006. https://doi.org/10.1093/nar/gkj109.

Türei D, Korcsmáros T, Saez-Rodriguez J. OmniPath: guidelines and gateway for literature-curated signaling pathway resources. Nat Methods. 2016;13(12):966–7.

Li T, Wemersson R, Hansen RB, Horn H, Mercer J, Slodkowicz G, et al. A scored human protein–protein interaction network to catalyze genomic interpretation. Nat Methods. 2017;14(1):61–4.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.