Sevoflurane Postconditioning Ameliorates Neuronal Migration Disorder Through Reelin/Dab1 and Improves Long-term Cognition in Neonatal Rats After Hypoxic-Ischemic Injury

Yahan Zhang1 · Qiushi Gao1 · Ziyi Wu1 · Hang Xue1 · Ping Zhao1

Received: 18 April 2020 / Revised: 9 May 2021 / Accepted: 17 May 2021 © The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract
Sevoflurane postconditioning (SPC) has been widely reported to attenuate brain injury after hypoxia–ischemia encephalopathy (HIE) by inhibiting neural necrosis and autophagy. Moreover, recent reports revealed that sevoflurane facilitated hippocampal reconstruction via regulating migration. Yet, it remains unclear whether the promotion of neural migration by SPC repairs the hippocampal injury after HIE. Here, we hypothesize that SPC exerts a neuroprotective effect by ameliorating neuronal migration disorder after HIE and regulating Reelin expression. Furthermore, the downstream Reelin/Dab1 pathway may be involved. The classical Rice–Vannucci model of hypoxia–ischemia was performed on postnatal day 7 rat pups, which was followed by SPC at 1 minimum alveolar concentration (MAC 2.5%) for 30 min. Piceatannol, causing Reelin aggregation in vivo, was used to detect whether Reelin/Dab1 was involved in the neuroprotection effect of SPC. Hippocampal-dependent learning ability tests were conducted to assess the long-term effects on locomotor activity and spatial learning ability. Our findings suggest that hypoxia–ischemia injury inhibited neurons migrated outward from the basal zone of dentate gyrus, disrupted cytoarchitecture of the dentate gyrus (DG), and led to long-term cognition deficits. However, SPC could relieve the restricted hippocampal neurons and repair the hippocampal-dependent memory function damaged after HIE by attenuating the overactivation of the Reelin/Dab1 pathway. These results demonstrate that SPC plays a pivotal role in ameliorating neuronal migration disorder and maintaining normal cytoarchitecture of the DG via inhibiting overactivated Reelin expression. This process may involve overactivated Reelin/Dab1 signaling pathway and spatial learning ability by regulating the Reelin expression which may associate with its neuroprotection.

Keywords Sevoflurane postconditioning · Hippocampal dentate gyrus · Hypoxia–ischemia · Reelin · Long-term neurocognition

Introduction
Neonatal hypoxic-ischemic encephalopathy (HIE) is one of the prevalent causes of severe neurological morbidity in neonates, which may lead to devastating neurological, cognitive, and behavioral disorders. The incidence of HIE is approximately 1/1000 in live births, and survivors may continue to suffer from severe sequelae (Ma et al. 2019; Zhao et al. 2016). To date, only a small number of clinically promising therapies have been reported, including hypothermia. However, the long-term efficacy of these therapies in terms of alleviating brain damage after HIE is still very low.

The development of hippocampal neurons is a sensitive, multistep process involving progenitor proliferation from the neurogenesis niche dentate gyrus (DG) zone at a low rate. Following migration to the final location, differentiation and synaptogenesis until integration with the existing neuronal circuit, the immature neurons finally come into functional state (Ming and Song 2005; Piatti et al. 2006). There are more than 9000 immature GABA neurons being sent daily from the subgranular zone (SGZ) outward the granular and multiple molecular layers in the DG, and this process continues throughout the life. However, when environmental injuries damaged the vital developmental process mentioned above, neural self-repairment against injury was initiated, including more neuron production from the neurogenic
zone. However, self-repairment is limited and still unable to repair ruined structures (Moskowitz et al. 2010). Previous evidence confirmed that precise neuronal migration is vital for the establishment of hippocampal functional neural circuits. Meanwhile, the classical neuronal migration modes are predominantly guided by nuclear body movement and cytoskeletal remodeling. Hiroki also indicated that neuron migration was associated with dynamic extension/retraction of actin filaments and microtubules (Umeshima et al. 2007; Meseke et al. 2013). Moreover, Chai et al. reported that actin filaments depolymerized by Cofilin and phosphorylated at serine 3 by LIMK1 can inhibit its dynamic extension (Chai et al. 2009). As for microtubule-associated protein (MAP) tau, external stress could cause it hyperphosphorylated, subsequently disrupt microtubule network, and eventually inhibit neuron migration (Song et al. 2013; Liu et al. 2009). Of note, many neuropsychiatric diseases have been reported to be associated with disorders neural migration, including schizophrenia, autism, temporal lobe epilepsy, and neurodegenerative disease (Knuesel 2010; Yang et al. 2012; Folsom and Fatemi 2013). It may be possible to ameliorate neurological disability after brain injury if we could take advantage of certain medicine-targeted migratory promotion of the neonatal brains.

Sevoflurane is a widely used inhalational anesthetic in pediatric clinics. Mounting evidence supports the idea that sevoflurane may alleviate neurological deficits by inhibiting neural apoptosis, suppressing overactive autophagy and inhibiting inflammation in time and dose-dependent manner under pathological conditions (Yang et al. 2012; Xue et al. 2019). Besides, evidence proved that sevoflurane postconditioning (SPC) repaired the injury after transient ischemic injury by aligning cortical neuron orientation. Moreover, SPCs have the capacity of raising the self-reparation ability and promoting poststroke neuron regeneration via creating suitable microenvironment for neuron migrating toward the stroke area which was guided by microglial and astrocytes during recovery phase (Ren et al. 2014; Li et al. 2017; Wang et al. 2018). All the evidences above indicated SPC can modulate injured cortex reconstructed after ischemia. However, whether SPC exerts neurological protection on hippocampal neuron recovery after HIE and the associated crucial molecular interactions are still unclear.

Reelin is a large extracellular molecular protein, highly secreted by GABAergic interneurons after birth, which plays a key role in the correct positioning of cortical and hippocampal neurons (Levy et al. 2014; Ogden et al. 2016). Research on Reelin-mutant mice, called the “reeler” phenotype, has contributed to our acknowledgement of the Reelin effect on orchestrating the arrangement of neurons in the cortical layer, hippocampal layer, and its relationship with the assemble and disassemble of microtubules (D’Arcangelo 2005; Kohno 2017). Studies on canonical and non-canonical Reelin signaling pathways have shown that Reelin acted as positional signal during neuron migration process through multiple ways, including binding to the apolipoprotein E receptor 2 (ApoER2 also known as Lrp8) or the very low–density lipoprotein receptor on the migrating neurons, which subsequently activates intracellular Dab1 or N-cadherin to participate in neuron migration, synaptic plasticity modulation, and neurotransmitter release (Levy et al. 2014; Caffrey et al. 2014; Zhao et al. 2004). In cortical neuron migration process, Reelin was reported to modulate cytoskeleton by binding to ApoER2/VLDLR to promote nuclear translocation. Furthermore, previous investigations have revealed that maternal hypoxia had a direct impact on hippocampal neuronal migration by altering Reelin expression and leading to hippocampal lamination malformation (Golan et al. 2009; Komitova et al. 2013). However, it is unclear whether the abnormal expression of Reelin cascade affects microtubule-associated cytoskeleton polymerization after HIE.

Based on the previous evidence, in the present study, we aimed to test the hypothesis that hypoxic and ischemic stress can induce neuron arrangement disorders in DG and thereby cause long-term neurological deficits. SPC may alleviate these neurological impairments by regulating Reelin expression and facilitate long-term spatial learning and cognition in neonatal rats after hypoxic-ischemic injury.

**Materials and Methods**

**Experimental Animals and Grouping**

Postnatal day 7 (PND7) Sprague–Dawley rat pups were used in this study because of peak neurogenesis of the hippocampus (Zhao et al. 2014). Housing conditions of the rats complied with the regulations of the National Animal Experiment Center. Briefly, the pups were housed in a room at 25 ± 2 °C under a 12-h light/dark cycle, with free access to adequate food and water. All animal experimental procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, approved by the Laboratory Animal Care Committee of China Medical University (Shenyang, China; Approval No: 2016PS337K).

**Grouping**

Male pups were selected in this study to exclude estrogen effects, and they were randomly divided into four groups: sham group (Sham), HIE group (HI), HIE with sevoflurane postconditioning group (HI + Sev), and HIE with sevoflurane postconditioning + piceatannol drug group (HI + Sev + G).
Neonatal HIE Model and Drug Administration

Neonatal HIE model protocol was based on a previously described protocol (Xu et al. 2016). Briefly, deeply anesthetized pups (12–20 g body weight) underwent permanent ligation of the left common carotid artery. After recovering for 2 h, the pups were ventilated with 30% O₂ and 70% N at the flow rate of 2 L/min for 2 h in the sham group and 8% O₂ and 92% N in the other groups. SPC at 1 minimum alveolar concentration (MAC 2.5%) was conducted for 30 min immediately after asphyxia, and a gas monitoring device (Datex-Ohmeda Inc., Tewksbury, MA, USA) was used to control sevoflurane concentration.

Drug Administration In Vivo

Piceatannol (30 μM, Sigma-Aldrich, USA), a highly selective inhibitor of ADAMTS-4 and ADAMTS-5, exerts an inhibiting cleavage effect of Reelin into proteolytic fragments at both N- and C-terminal (Lauer-Fields et al. 2008). It was injected into the left lateral ventricle of the pups 30 min before hypoxia in the HI + Sev + G group via 5-μL Hamilton syringes (Wen et al. 2018; Zhang et al. 2018). Meanwhile the rats in HI group received an equal volume of DMSO.

Pulse Labeling Study by Bromodeoxyuridine Injection

5-Bromo-2-deoxyuridine (BrdU) pulse birth-labeling method for detecting neuron migration has been considered as a potential and accepted method (Bartley et al. 2005; Bingham et al. 2005; Golan et al. 2009). To determine the migration of newborn neurons, BrdU (B5002, Sigma-Aldrich) was intraperitoneally injected into dams at the dose of 150 mg/kg per day for 3 days consecutively from PND6 to PND9. The pups were sacrificed, and their brains were harvested for migration detection on PND14 and PND21. For the immunofluorescence assay, the pups were deeply anesthetized with pentobarbital (80 mg/kg), and their brains were harvested for migration detection on PND14 and PND21. Besides, in order to show neuronal migration, DCX-positive cells, representing immature migrating neurons, were labeled and counted in several layers on PND14 and PND21.

Tissue Processing and Immunofluorescence Staining

For the immunofluorescence assay, the pups were deeply anesthetized with pentobarbital (80 mg/kg), and their brains were harvested. Coronal sections of paraffin-fixed brain were cut in series at the thickness of 3.0–3.5 μm. Similar to that for BrdU staining, the sections were treated with 2 N HCl at 37 °C for 30 min for denaturing DNA, followed by neutralization with 0.1 M borate buffer (pH 8.5). The sections were then blocked with 5% fetal bovine serum for 30 min and then incubated overnight with primary antibodies. On the next day, the slices were incubated with one or two types of secondary antibodies for single or double staining for 4 h at room temperature, and cell nuclei were counterstained with DAPI. The following antibodies were used: anti-mouse Reelin (1:200; G10 Abcam, Cambridge, UK), anti-rabbit Dab1 (1:100 Biorbyt, Cambridge, UK), anti-rabbit BrdU (1:100; Abcam), anti-rabbit DCX (1:100; Cell Signaling Technology, Boston, MA, USA), and anti-mouse NeuN (1:100; Cell Signaling Technology, Boston, MA, USA). Cell nuclei were labeled with DAPI and then cover slipped with an antifade reagent (P0128S, Beyotime, China). The ratio of Reelin/Dab1-coexpressing cells to the total Reelin-positive cells was calculated for evaluating Reelin and Dab1 expression. To evaluate the number of Reelin-positive cells at PND14 and PND21, we counted the number of positive cells and then divided this number by the total cell number in a fixed area (1000 × 1000 pixels) using NIS-Elements AR Analysis 4.50.00 software; this procedure was performed for every 50-μm coronal section slice, for a total of 5 sections per brain. To evaluate the combination of Reelin and Dab1 interaction under different drug treatments, the ratio of double-positive cell number to Reelin-positive cell number was calculated on PND14 and PND21. According to previous reports on neuron migration, we calculated the ratio of BrdU/NeuN co-expressing cells to total BrdU-positive cells and compared the percentages in the subgranular cell layer (SGZ) and the granular cell layer (GCL) on PND14 and PND21. Besides, in order to show neuronal migration, DCX-positive cells, representing immature migrating neurons, were labeled and counted in several layers on PND14 and PND21.

Nissl Staining

Nissl staining was performed on PND21 using a staining kit. The coronal sections were stained according to the manufacturer’s instructions (G1430, Solarbio, China). Neural orientation and distribution of the hippocampal neurons of the DG region were captured using a digital microscope camera. The relative ratio of neuronal density of the DG was analyzed with ImageJ software.

Western Blotting Analysis

Hippocampal DG tissue was carefully dissected from the brains of the pups using an anatomical microscope (Nikon SMZ445) on PND14 (n = 5 per group). Frozen hippocampal DG tissue was cut and then lysed on ice for 30 min. The lysate was centrifuged, and the total protein concentration was measured using the BCA Protein Assay Kit (P0010; Beyotime). The proteins were separated by electrophoresis on 8% or 10% SDS-PAGE gel and then electrotransferred to polyvinylidene fluoride membranes (IPVH0010; Millipore, Germany). The target proteins were incubated with ...
the specific primary and second antibodies. The following primary antibodies were used: GAPDH (1:1000; Cell Signaling Technology), Reelin (1:1000; G10 Abcam), Dab1 (1:500; Biorbyt, Cambridge, UK), Phospho-Dab1 (phospho-Tyr198, 1:100; Biorbyt), GSK-3β (1:1000; Cell Signaling Technology), Phospho-GSK-3β (Ser9, 1:500; Cell Signaling Technology), Tau (1:400; Sigma-Aldrich), and Phospho-tau ser396 (1:400; Abcam). The band intensity was quantified using Image PRO PLUS software.

Ethological Tests

Three behavioral tests were conducted on days PND28–34 as previously described (Wang et al. 2018; Xu et al. 2016; Xue et al. 2019).

Open-Field Test

To evaluate anxiety and locomotor behavior, the rats were placed in a 100 cm (W) × 100 cm (D) × 40 cm (H) arena with opaque sidewalls equipped with infrared detectors for 10 min (Noldus Ethovision XT, Netherland), and the movement distance, time spent in the center and border zone, movement velocity, and stool number were recorded for anxiety mood evaluation.

Morris Water Maze Test

Spatial learning and the memory ability of rats were tested using the Morris water maze (MWM) test from PND29 to PND34. Briefly, during the first consecutive 5 days, the rats were placed into a circular, opaque pool (1.6 m diameter, 60 cm height) for 90 s to search for an invisible platform. Once failed, they were allowed to stay on it for another 20 s. During the spatial probe test, the rats were released from the opposite quadrant with the platform removed and permitted to swim for 90 s. The paths of each animal were recorded by infrared detectors, and the data were analyzed by an image analysis software (Noldus Ethovision XT).

8-Arm Radial Maze Test

The 8-arm radial maze (RAM) test was conducted as described previously (Pan et al. 2012). The whole test was divided into 3 parts: acclimatization phase (2 days), training phase (5 days), and choice phase (1 day). During the acclimatization phase, the rats were allowed to explore freely for 10 min with all arms baited after controlling their weight until 85–90% of free-feeding body weight was achieved. During the next 5 days of the training phase, four of the eight arms were baited randomly, and the rats were trained twice a day to find the food reward with an interval of 1 h between the trials. Each trial was terminated within 10 min or before 10 min if the rats found all food rewards. During the choice phase, all arms were unbaited, and the rats were allowed to find the food reward within 10 min. Working memory was tested according to the entries into formerly baited arms while reference memory was tested according to any entry into a non-baited arms. The total travelled distance of rats, reference memory error (RME), and working memory error (WME) were recorded and analyzed using an image analysis software (Noldus Ethovision XT).

Statistical Analysis

Data are presented as mean ± standard deviation (SD). All data were analyzed with SPSS 20.0 or GraphPad Prism 7.0. The data were analyzed using Bartlett’s test for equal variances and the Shapiro–Wilk test for normality. One-way analysis of variance (ANOVA) followed by Newman–Keuls post hoc test was used. Data of escape latency in the MWM were analyzed using two-way ANOVA for repeated measurements. Differences with $p < 0.05$ were considered to be statistically significant.

Results

Neuron Migration Process was Inhibited and DG Neuron Distribution was Disordered after HIE, and SPC Alleviated These Injuries

To better visualize neuron migration from the SGZ to the superficial GCL, the BrdU pulse labeling protocol was used (Supplementary Figure S1). Initially, the migration of the pulse-labeled cells to the destination was detected, and the ratio of BrdU+/NeuN+ cells to the total BrdU-positive cells in each layer was calculated (Fig. 1A: PND14; B, PND21). The ratio of BrdU+/NeuN+ cells in the SGZ layer to BrdU+ cells in the whole layer was enhanced in the HI group as compared to that in the Sham group on PND14 and PND21 respectively. However, the ratio of BrdU+/NeuN+ cells in the GCL layer to BrdU+ cells in the whole layer was decreased as compared to that in the Sham group on PND14 and PND21 respectively. These ratio changes indicated that a large number of labeled neurons were restricted to the SGZ zone rather than migrating outward after HIE attack (Fig. 1C, D: HI versus Sham, $p < 0.05$ and $p < 0.05$ on PND14 and PND21, respectively). However, the ratio of BrdU+/NeuN+ cells in the GCL to the total BrdU+ cells in the whole layer was decreased as compared to that in the Sham group (Fig. 1C, D: HI versus Sham, $p < 0.01$ and $p < 0.01$ on PND14 and PND21, respectively). To exclude the interference of reactive neurogenesis after HIE labeled by BrdU, the total BrdU+ cells were counted, and the data showed no significant difference among all groups on PND14 and PND21 (Supplementary Fig. 2A, B). SPC decreased the elevated number of neuronal cells restricted in the SGZ zone and increased the ratio in the GCL (Fig. 1C, D: HI+Sev versus...
Fig. 1 Neuron migration process was perturbed after HIE and the effect of sevoflurane postconditioning on these deficits on PND14 and PND21. BrdU (red)/NeuN (green) double-positive cell distributed in the SGZ zone and GCL zone on PND14 (A) and PND21 (B), respectively. The ratios of BrdU+/NeuN+ neurons in the SGZ zone or GCL zone to the whole number BrdU+ cells were calculated on PND14 (C) and PND21 (D) separately. DCX-positive neurons (Green) on PND14 (E, G) and PND21 (F, H) separately. Values are mean ± SD (n = 5). One-way ANOVA with Tukey post hoc multiple comparison tests were used for data analysis. *p < 0.05, **p < 0.01 vs Sham group; ^p < 0.05, ^^p < 0.01 vs Hi group. Scale bar = 50 μm. Nissl staining was performed on PND21. Representative photographs of Nissl stained-Dentate gyrus (I). Neuronal density relative ratio (J). Values are mean ± SD (n = 5). One-way ANOVA with Tukey post hoc multiple comparison tests was used for data analysis. ***p < 0.001 vs Sham group; ^^p < 0.01 vs Hi group. Scale bar = 50 μm.
HI, \( p < 0.05, p < 0.05 \) in SGZ layer; Fig. 1C, D: HI + Sev versus HI, \( p < 0.01, p < 0.05 \) in GCL layer on PND14 and PND21, respectively. Consistently with the above results, DCX positive neurons, which represented migrating neural progenitors, increased in the SGZ layer and correspondingly decreased in the GCL layer after HIE attack in the HI group compared to that in the Sham group on PND14 and PND21, respectively (Fig. 1E–H: HI versus Sham, \( p < 0.05 \) and \( p < 0.05 \) in the SGZ layer and \( p < 0.05 \) and \( p < 0.05 \) in the GCL layer on PND14 and PND21, respectively). However, SPC significantly reversed the above results (Fig. 1E–H: HI versus Sham, \( p < 0.05 \) and \( p < 0.05 \) in the SGZ layer and \( p < 0.05 \) and \( p < 0.05 \) in the GCL layer on PND14 and PND21, respectively).

Nissl staining was conducted on PND21 to study the distribution of hippocampal DG neurons (Fig. 1I, J; Additional Fig. 1). As compared to the Sham group, the HI group showed a decreased neuron ratio with rare cytoplasm and few Nissl bodies (Fig. 1J: HI versus Sham, \( p < 0.001 \)). Moreover, neuron suffered from hypoxic, and ischemic stress represents loose distribution without polarity. SPC alleviated Nissl body loss and promoted dentate gyrus neuron reorganization (Fig. 1J: HI + Sev versus HI, \( p < 0.01 \)). Representative photographs of Nissl stained-Dentate gyrus of each group were provided in X200 and X400 magnific pictures and rectangles were used to depict the same zoomed in regions (Additional Fig. 1).

**Reelin Expression Was Enhanced After HIE and SPC Decreased the Expression**

In order to study the Reelin expression level change after HIE and SPC treatment, immunostaining was conducted. Data showed that more Reelin-positive cells in the HI group than those in the Sham group on PND14 and PND21 (Fig. 2A–D: HI versus Sham, \( p < 0.01, p < 0.01 \) on PND14 and PND21, respectively). However, SPC significantly reduced Reelin expression (Fig. 2A–D: HI + Sev versus HI, \( p < 0.01, p < 0.01 \) on PND14 and PND21, respectively).

**SPC Decreased the Association of Reelin and Phosphorylated Dab1 After HIE and Piceatannol Blocked the Effect**

Piceatannol, which can effectively inhibit the activity of the Reelin proteolytic enzyme (ADAMTS-4/5) and preserve
Reelin inactive full-isoform, was used to test whether sevoflurane postconditioning exerted its neuroprotection after HIE via modulating Reelin expression and activating downstream Dab1 mediator. To verify the Reelin/Dab1 intracellular association, immunofluorescence staining of co-expression of Reelin and P-Dab1 was conducted. Our data indicated that the co-expression of Reelin and P-Dab1 was significantly enhanced after HIE, and SPC significantly inhibited this elevation (Fig. 3A–D: HI versus Sham, \( p < 0.01, p < 0.001 \) on PND14 and PND21, respectively). However, yet, the downregulated Reelin and P-Dab1 co-expression level was reversed by piceatannol (Fig. 3A–D HI \(+\) Sev versus HI \(+\) Sev + G, \( p < 0.01, p < 0.05 \) in SGZ; \( p < 0.001, p < 0.01 \) in GCL on PND14 and PND21, respectively).

The Protection of SPC Facilitating Neuron Migration Process After HIE Was Blocked by Piceatannol

Data exclude the interference of reactive neurogenesis after HIE (Supplementary Fig. 3A, B). Consistently with the previous results, there were more BrdU/NeuN-labeled positive cells in the SGZ zones of rats in the HI group than those in the Sham group (Fig. 4A–D, HI versus Sham, \( p < 0.01, p < 0.05 \) in SGZ; \( p < 0.01, p < 0.01 \) in GCL on PND14 and PND21, respectively). This finding indicated that a large number of neurons were restricted to the basal zone of the DG, and this elevated ratio was decreased by SPC (Fig. 4A–D: HI \(+\) Sev versus HI, \( p < 0.001, p < 0.05 \) in SGZ; \( p < 0.01, p < 0.01 \) in GCL on PND14 and PND21, respectively). However, using of Piceatannol can significantly increase the number of neurons blocked in SGZ layer and decrease the neural number in GCL layer separately (Fig. 4C, D: HI \(+\) Sev \(+\) G versus HI \(+\) Sev, \( p < 0.01, p < 0.05 \) in SGZ; \( p < 0.001, p < 0.01 \) in GCL on PND14 and PND21, respectively). Additionally, SPC successfully change the increased number of DCX-positive neurons in the SGZ layer in HI group, and the piceatannol blocked this effect. (Fig. 4E–H: HI versus Sham, \( p < 0.01, p < 0.01 \) in SGZ; \( p < 0.01, p < 0.01 \) in GCL; HI \(+\) Sev versus HI, \( p < 0.01, p < 0.01 \) in SGZ; \( p < 0.01, p < 0.01 \) in GCL on PND14 and PND21, respectively). These findings all indicated that a large number of neurons were restricted to the basal zone of the DG, instead of migrating outward, due to the expression of Reelin full isoform elevated after piceatannol administration.

The Protection of SPC Reorganizing Dentate Gyrus Neuron Distribution was Blocked by Piceatannol

Nissl staining was performed on PND21 to investigate the distribution of hippocampal DG neurons (Fig. 5A). The finding was consistent with the previous result that the HI group
showed a decreased neuron ratio with rare cytoplasm, few Nissl bodies, and disorganized neurons without polarity in the DG as compared to that in the Sham group (Fig. 5B: HI versus Sham, \( p < 0.0001 \)). SPC reorganized neuron loose distribution with proper orientation (Fig. 5B: HI + Sev versus HI, \( p < 0.01 \)). The beneficial effect of SPC was, however, blocked by piceatannol (Fig. 5B: HI + Sev + G versus HI + Sev, \( p < 0.05 \)).

**The Reelin/Dab1 Cascade may be Involved in the Protective Effect of SPC and Piceatannol Blocked this Effect**

The staining data indicate that HIE may result in disorder of neuron migration and activation of intracellular P-Dab1 association with Reelin. Here, we tested the role of the canonical Reelin downstream signaling pathway involving Dab1, GSK-3\( \beta \), and the microtubule-associated protein Tau. The HI group showed significant upregulation of Reelin expression in the 388 kDa fragment full isoform, rather than 180 kDa, and phosphorylation of Dab1 (Tyr-198), GSK-3\( \beta \) (Ser9), and Tau (Ser396) as compared to that in the Sham group (Fig. 6A–H: HI versus Sham, \( p < 0.001 \), \( p < 0.01 \), \( p < 0.01 \), \( p < 0.05 \), respectively). However, these increased expressions were significantly attenuated by SPC (Fig. 6A–H: HI + Sev versus HI, \( p < 0.001 \), \( p < 0.05 \), \( p < 0.05 \), \( p < 0.01 \), respectively). Piceatannol partially blocked the reduction induced by SPC (Fig. 6A–D, HI + Sev + G versus HI + Sev, \( p < 0.05 \), \( p < 0.01 \), \( p < 0.05 \), \( p < 0.05 \), respectively). Moreover, in order to exclude the influence of piceatannol on all signal mediator expression, we compare the difference between HI group and HI + G group. However, there was no significant between these 2 groups of the above mediators (Additional Fig. 2). Representative western blotting photographs and their corresponding quantitative analysis of Reelin, P-Dab1/Dab1, P-GSK-3\( \beta \)/GSK-3\( \beta \) and P-Tau/Tau expression level in HI and HI + G groups in order to clarify the influence of Piceatannol exerted on rats in HI Group (Additional Fig. 2).
SPC Promotes Hippocampal Spatial Learning and Memory in HIE Rats and Piceatannol Blocked This Effect

To exclude anxiety and locomotor ability interference produced by several treatments, we conducted an open-field test experiment on PND28. However, there were no differences in the test results among the four groups (Supplementary Figure S4).

In the MWM test, no significant difference in swimming velocities was observed among the four groups (Fig. 7A: \( p > 0.05 \) among all groups). Rats in the HI group showed significantly increased escape latency as compared to rats in the sham group (Fig. 7B: HI versus Sham, \( p < 0.001, p < 0.001, p < 0.001, \) and \( p < 0.01 \) on the 2nd, 3rd, 4th, and 5th day, respectively). SPC attenuated this phenomenon (Fig. 7B: HI + Sev versus HI, \( p < 0.01, p < 0.001, \) and \( p < 0.01 \) on the 3rd, 4th, and 5th day, respectively). Rats in the HI + Sev + G group treated with piceatannol needed more time to find the platform than rats who were administered SPC alone (Fig. 7B: HI + Sev + G versus HI + Sev, \( p < 0.01 \) and \( p < 0.05 \) on the 4th and 5th day, respectively). In the spatial probe test, the HI group rats crossed the platform less often than the Sham group rats (Fig. 7C: HI versus Sham, \( p < 0.01 \)). Rats in the HI + Sev group crossed the platform more often than rats in the HI group (Fig. 7C: HI + Sev versus HI, \( p < 0.05 \)). Piceatannol notably blocked the protective effect of SPC on spatial learning ability (Fig. 7C: HI + Sev + G versus HI + Sev, \( p < 0.05 \)).

**SPC Facilitates DG-Dependent Spatial Learning and Memory in HIE Rats and Piceatannol Blocked This Effect**

The 8-arm radial maze test was conducted in rats of the four groups from PND28 to PND34, and the path parameters were presented (Fig. 8A). The distance traveled during the reward seeking process was increased in the HI group but significantly decreased in the HI + Sev group. Rats in the HI + Sev + G group traveled a greater distance to find the rewards than rats in the HI + Sev group (Fig. 8B: HI versus Sham, \( p < 0.001, p < 0.0001; \) HI + Sev versus HI, \( p < 0.05, p < 0.05; \) HI + Sev + G versus HI + Sev, \( p < 0.05, p < 0.05 \)). SPC abolished the increased RME in the HI group as compared to that in the Sham group (Fig. 8C: HI versus Sham, \( \text{****} p < 0.0001; \) HI versus Sham, \( \text{^^} p < 0.01; \) HI + Sev versus HI, \( & p < 0.05; \) HI + Sev + G versus HI + Sev, \( \& & p < 0.01; \) HI + Sev + G versus HI + Sev, \( \& & & p < 0.001).\)

![Fig. 4](image)

Fig. 4 The protection of sevoflurane postconditioning facilitating neuron migration process after HIE was blocked by piceatannol. BrdU (red)/NeuN (green) double-positive cell distributed in the SGZ zone and GCL zone on PND14 (A) and PND21 (B), respectively. The ratio of BrdU+/NeuN+ neurons in the SGZ zone or GCL zone to BrdU+ cells in the whole layer on PND14 (C) and PND21 (D) were calculated separately. DCX-positive neurons (Green) on PND14 (E, F) and PND21 (G, H) (values are mean\( \pm \) SD (n = 5). One-way ANOVA with Tukey post hoc multiple comparison tests were used for data analysis. \( * p < 0.05; ** p < 0.01 \) vs Sham group; \( ^* p < 0.05; ** p < 0.01; \) \( ^*** p < 0.001 \) vs HI group. \( ^p < 0.05; ^{**} p < 0.01; ^{***} p < 0.001 \). Scale bar = 50 \( \mu m \).

![Fig. 5](image)

Fig. 5 The protection of sevoflurane postconditioning reorganizing dentate gyrus neuron distribution was blocked by piceatannol. Representative photographs of Nissl stained-Dentate gyrus on PND21 (A). Neuronal density relative ratio (B). Values are mean\( \pm \) SD (n = 5). One-way ANOVA with Tukey post hoc multiple comparison test was used for data analysis. \( \text{****} p < 0.0001 \) vs Sham group; \( \text{^^} p < 0.01 \) vs HI group. \( ^{**} p < 0.05 \) vs HI + Sev group. Scale bar = 50 \( \mu m \).
$p < 0.001$; HI + Sev versus HI, $p < 0.05$), and piceatannol blocked this beneficial effect (Fig. 8C: HI + Sev + G versus HI + Sev, $p < 0.05$). Interestingly, WME, which represents short-term memory, showed no difference among the four groups (Fig. 8D: $p > 0.05$ compared with all groups). After the 8-arm radial maze test, we assessed the quantity of
neurons in hippocampal DG region by immunohistochemistry test (Fig. 9A). The rats in HI group showed a reduced neuron quantity compared with that in Sham group (Fig. 9B, HI versus Sham, \( p < 0.05 \)). However, SPC elevated the numbers of NeuN\(^+\) cells in HI + Sev group compared with that in HI group (HI + Sev versus HI, \( p < 0.05 \)). The relative numbers of NeuN\(^+\) cells in the HI + Sev + G group were significantly decreased compared with that in the HI + Sev group (Figs. 9B: HI + Sev + G versus HI + Sev, \( p < 0.05 \)).

**Discussion**

Our study showed that HIE inhibited neuron migrating outward and disordered DG neuron cytoarchitecture, leading to long-lasting neurological deficits. SPC can abolish these impairments and ultimately facilitate hippocampal-dependent long-term spatial learning and cognition. The specific mechanism of SPC neuroprotection may involve Reelin expression inhibition and Reelin/Dab1 signaling pathway suppression.

HIE is a devastating consequence of perinatal asphyxia that leads to severe brain structure aberration and long-term neurocognitive sequelae such as mental retardation (Barkhuizen et al. 2017; Li et al. 2012; Ma and Zhang 2015; Millar et al. 2017). The lack of an effective therapy for brain injury has therefore aroused intense interest in investigating appropriate drugs for administration.

Whether the effect of anesthetics on the developmental brain is neurotoxic or protective has been a hot issue. Recently, clinical multicenter experiments GAS and PANDA gave the preliminary answers. According to GAS investigation, general anesthesia did not cause abnormal neurodevelopmental outcomes in children compared to spinal anesthesia (36th International Symposium on Intensive Care and Emergency Medicine: Brussels, Belgium. 15–18 March 2016). PANDA studies confirmed inhalation anesthetic exposed children had no differences in cognition and
behavior compared to their unexposed siblings (Huang et al. 2016). In addition, the MASK study concluded that multiple, but not single, exposures before the age of 3-year-old children are associated with the impairment of learning and memory (Warner et al. 2018). All clues indicated that certain dose-and-time exposure of sevoflurane may not exert neurotoxicity on children. Furthermore, to our knowledge, sevoflurane is a popular general inhaled anesthetic which is particularly used in pediatric operation. The safety of the clinical and subclinical dose of sevoflurane has been reported to repair neurological impairments after brain injuries. In addition to the traditionally accepted protective effect of sevoflurane postconditioning, including neural death reduction, overactive autophagy suppression, and long-term memory consolidation (Ren et al. 2014; Wang et al. 2016; Lai et al. 2016), recent reports indicate that the appropriate dose of SPC also facilitates endogenous hippocampal neural network reconstruction through arising neurogenesis and neural migration (Chen et al. 2015; Yu et al. 2019; Chen et al. 2018). In our present study, we investigated abnormal DG neuron migration and cytoarchitecture disorganized after HIE and examined the neurological effect of sevoflurane postconditioning. Consistently with the findings of previous studies, we found that normal DG neuron migration was significantly inhibited, and neural orientation was disordered after HIE (Golan et al. 2009). Interestingly,
sevoflurane postconditioning exerted an obvious protective effect on loosely distributed neuron reorganization and neuronal migratory promotion to some extent.

The DG of the hippocampus is a special trilaminar, C-shaped structure where neurogenesis occurs postnatally and has a pivotal role in learning and memory (Hevner 2016). Progenitors are generated in the DG and migrate short distances from the inner SGZ to the superficial molecular layer, and this process is mediated by other intercellular factors, Reelin, for example. The newly generated neurons integrate with the pre-existing hippocampal circuit and form functional synaptic attachments within 2–3 weeks (Bruel-Jungerma et al. 2005) (Hack et al. 2002). Here, we chose 7-day-old rats as experimental candidates during which synaptogenesis reaches its peak period and observation migration till postnatal 21 days when newly generated neuron migration almost finished (Ming and Song 2005). This programmed migratory process is, however, vulnerable to hypoxia and changes in the extracellular matrix, which result in hippocampal neuron migration deficits and aberrations in the orchestration of the hippocampus (Ma and Zhang 2015). The effect of essential migratory regulators, Reelin, that modulate the migration of newborn neurons to their destination in cortex, hippocampus, and cerebellum is clearer in recent studies. It exerts predominant effect on monitoring neuronal migration and dendritic and synaptic growth. As a consequence, abnormal Reelin signaling pathway transduction has been reported to result in autism, depression, and mental retardation. For example, abnormal expression of Reelin induced disruptions of neuronal migration of cortex development which led to lissencephaly and seizure (Bock and May 2016; Komitova et al. 2013; Kerjan and Gleeson 2007). Consistently with other studies, we compared normal migratory process with pathological models and investigated Reelin expression differences. Data may prove that the elevated Reelin expression induces many neurons perturbated beneath the basal layer of DG instead of migrating outwards migration. These results are consistent with the view of Chai et al. that Reelin acted as a “Stop” signal for migratory neurons (Chai et al. 2009). Alternatively, sevoflurane postconditioning effectively facilitated this pre-stop migration process, released these perturbated neurons, and inhibited elevated Reelin expression after HIE.

Reelin is a 388-kDa extracellular matrix glycoprotein secreted by GABAergic and plays crucial roles in cortical and hippocampal lamination, synaptogenesis, neurite formation, and neurotransmitter release (Levy et al. 2014; Nichols and Olson 2010). It consists of eight Reelin repeats, and its N-terminal region is required for signal transduction (Kohno et al. 2009; Kubo et al. 2002). Furthermore, proteolytically cleaved by ADAMTS-4, ADAMTS-5 of Reelin full-length (388 kDa) isoform may downregulate...
its activity in downstream signal transduction. Therefore, we used G10 Reelin antibody to detect full-length/N-R2 fragments with N-terminus and piceatannol, which causes Reelin full-length isoform aggregation in vivo, to prove that Reelin acted as a stop signal and prevented neuron of dentate gyrus from migrating outward throughout the whole layer after HIE attack. Consistent with our hypothesis, data showed that sevoflurane functionally decreased the elevated Reelin expression level and its full-length isoform after HIE. In vivo aggregation of Reelin by piceatannol also presented a similar cytoarchitecture phenotype in the HI group. Furthermore, accumulating evidence has shown that disrupted Reelin expression may be a potential reason for various neurodegenerative disorders such as seizure and mental retardation (De Rubeis et al. 2014; Pujadas et al. 2010). A previous study also revealed that Reelin can stabilize the actin cytoskeleton and microtubule cytoskeleton by binding to ApoER2 or VLDLR receptors to promote nuclear translocation acting on these networks (Chai and Frotscher 2016). Evidence based on prenatal hypoxia-induced impaired neural migration emphasized the relationship between Reelin-associated migration deficits and hypoxia stimuli (Frotscher et al. 2003). In addition, data revealed abnormal expression of Reelin during the prenatal period may lead to hippocampal-dependent cognitive impairments in offspring (Pocock and Hobert et al. 2008). It is reasonable to hypothesize that sevoflurane could ameliorate the impairments after HIE by regulating DG neuron migration. Our study data revealed that SPC may markedly disengage perturbed prelabeled BrdU+/NeuN+ cells from the SGZ zone and promote their percentage in the GCL on PND14 and PND21. The application of piceatannol implies the effect of Reelin on regulating DG lamination and the vital role that the Reelin/Dab1 interaction plays in SPC-induced neuroprotection after HIE. The role of the canonical Reelin-mediated signaling pathway in stabilizing the cytoskeleton by binding with receptors to activate the Dab1-associated cascade has been highly debated (Chai et al. 2009). The cytoskeleton rearrangement ability of modifying Tau and MAP1B might involve protein-directed kinase GSk3 and CDK5 (Gonzalez-Billault et al. 2005). A study on the relationship between Reelin-mediated neuron migration and microtubules showed that Reelin dynamically promoted the polymerization of microtubules, leading to inhibited nuclear translocation (He et al. 2010). In the present study, HIE obviously enriched Reelin expression and activated phospho-Dab1 at Tyr198, phosphor-GSK-3β at Ser 9, and phosphorylated Tau at Ser396. SPC can suppress the activated Reelin/Dab1 signaling pathway. Notably, an injection of piceatannol abolished this phenomenon, suggesting that the Reelin/Dab1 pathway, which influences microtubule polymerization, was involved in the protective effect of SPC after HIE. This indicates that HIE may cause the spread of DG neurons without tight distribution, and this impairment may be associated with abnormal Reelin expression and overactivation of the Reelin/Dab1 cascade; however, these injuries may be reduced by SPC to some extent.

To our knowledge, endogenous hippocampal events, including neurogenesis and neuron migration, contribute to hippocampal memory and learning (Abrous and Wojtowicz 2015; Lepousez et al. 2015; Gustavsson et al. 2005). To verify whether SPC promotes long-term hippocampal spatial memory after HIE, the MWM test was conducted. The results demonstrated that in the absence of motor disability and anxiety motion, hippocampal spatial and learning memory were extremely damaged by HIE due to injuries in DG which is consistent with that colchicine-induced DG lesion–damaged DG-dependent learning ability (Gilbert et al. 2001). Moreover, we conducted the 8-arm radial maze test to investigate DG-dependent pattern separation as it incorporates the transformation of similar memories or events into non-overlapping representations (Chen et al. 2015). Our study data showed that RME, which represents long-term memory, was increased in the HI group. The phenomenon that rats in the HI group entered the working arm of the 8-arm maze without food rewards after training was abolished significantly by SPC. Piceatannol injection showed a similar harmful effect as that observed for the HI group which indicated Reelin-regulated DG migration was critical in long-term memory storage. Interestingly, WME, which represents short-term memory retrieval, showed no difference among the four groups. This finding implied that HIE may particularly damage DG-dependent long-term memory storage instead of short-term memory, which may indicate that neuron migration disorder after HIE mainly influenced the integration of short-term memory into long-term memory instead of inhibiting its production. These results are consistent with previous research on traumatic brain injury effect on pattern separation (Sebastian et al. 2013).

Our study had several limitations. Firstly, we mostly focused on clarifying the neuron migration disorder resulting from HIE and the neuroprotective effect of SPC on modulating this impairment, instead of neurogenesis and relative molecular mechanisms. Secondly, we used the BrdU pulse–labeled technique, which may have increased the inaccuracy in absolutely qualifying and tracing single neuron migration destinations. We are currently working on improving this method and will hopefully present the dynamics of the neuron migration pathway in a better way in further investigations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12640-021-00377-3.

Author Contribution YZ and PZ contributed to research design. YZ, QG, and PZ contributed to the planning of the work. YZ performed all the experiments with the help of QG, HX, and ZW. YZ, QG, and XH participated in the analysis or interpretation of data. YZ wrote the manuscript. PZ approved of the submission and final versions.
Consent for Publication All data is transparency and can be obtained from the author.

Code Availability SPSS22.0, Prism 7.0 and Image J.

Declarations

Ethics Approval All animal experimental procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, approved by the Laboratory Animal Care Committee of China Medical University (Shenyang, China; Approval No.: 2016PS337K).

Consent to Participate Our research did not involve human subjects, and no organs/tissues were obtained from prisoners. All the animal treatments were according to the ethics guidelines.

Consent for Publication I understand that the text and any pictures or videos published in the article will be used only in educational publications intended for professionals. If the publication or product is published on an open access basis, I understand that it will be freely available on the internet and may be seen by the general public.

References

36th International Symposium on Intensive Care and Emergency Medicine (2016) Brussels, Belgium. 15–18 March 2016 Critical care (London, England) 20:94. https://doi.org/10.1186/13054-016-1208-6
Abrous DN, Wojtowicz JM (2015) Interaction between neurogenesis and hippocampal memory system: New Vistas Cold Spring Harbor perspectives in biology 7. https://doi.org/10.1101/cshperspect.a018952
Barkhuizen M, van den Hove DL, Vles JS, Steinbusch HW, Kramer BW, Gavilanes AW (2017) 25 years of research on global asphyxia hypoxic-ischemic brain injury. Pediatr Res 81:624–631. https://doi.org/10.1007/s12633-017-1146-2
Bartley J, Soltau T, Wimborne H, Kim S, Martin-Studdard A et al (2005) BrdU-positive cells in the neonatal mouse hippocampus following hypoxic-ischemic brain injury. BMC Neurosci 6:15. https://doi.org/10.1186/1471-2202-6-15
Bingham B, Liu D, Wood A, Cho S (2005) Ischemia-stimulated neurogenesis is regulated by proliferation, migration, differentiation and caspase activation of hippocampal precursor cells. Brain Res 1058:167–177. https://doi.org/10.1016/j.brainres.2005.07.075
Bock HH, May P (2016) Canonical and Non-Canonical Reelin Signaling Front Cell Neurosci 10:166. https://doi.org/10.3389/fncel.2016.00166
Bruel-Jungermann E, Laroche S, Rampon C (2005) New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. Eur J Neurosci 21:513–521. https://doi.org/10.1111/j.1460-9568.2005.04375.x
Caffrey JR, Hughes BD, Britto JM, Landman KA (2014) An in Silico Agent-Based Model Demonstrates Reelin Function in Directing Lamination of Neurons during Cortical Development PloS One 9:e110415. https://doi.org/10.1371/journal.pone.0110415
Chai X, Frotscher M (2016) How does Reelin signaling regulate the neuronal cytoskeleton during migration? Neurogenesis (austin, Tex) 3:e1242455. https://doi.org/10.1080/23262133.2016.1242455
Chai X, Forster E, Zhao S, Bock HH, Frotscher M (2009) Reelin Acts as a Stop Signal for Radially Migrating Neurons by Inducing Phosphorylation of n-Cofilin at the Leading Edge Commun Integr Biol 2:375–377
Chen C, Shen FY, Zhao X, Zhou T, Xu DJ, Wang ZR, Wang YW (2015) Low-dose sevoflurane promotes hippocampal neurogenesis and facilitates the development of dentate gyrus-dependent learning in neonatal rats ASN Neuro 7. https://doi.org/10.1177/1759091415575845
Chen X, Zhou X, Yang L, Miao X, Lu DH et al (2018) Neonatal exposure to low-dose (1.2%) sevoflurane increases rats’ hippocampal neurogenesis and synaptic plasticity in later life. Neurotox Res 34:188–197. https://doi.org/10.1007/s12640-018-9877-3
D’Arcangelo G (2005) The reeler mouse: anatomy of a mutant. Int Rev Neurobiol 71:383–417. https://doi.org/10.1016/S0021-9247(05)71016-3
De Rubeis S, He X, Goldberg AP, Poulton CS, Samocha K et al (2014) Synaptic, transcriptional and chromatin genes disrupted in autism. Nature 515:209–215. https://doi.org/10.1038/nature13772
Folsom TD, Fatemi SH (2013) The involvement of Reelin in neurodevelopmental disorders. Neuropharmacology 68:122–135. https://doi.org/10.1016/j.neuropharm.2012.08.015
Frotscher M, Haas CA, Forster E (2003) Reelin controls granule cell migration in the dentate gyrus by acting on the radial glial scaffold. Cereb Cortex 13:634–640
Gilbert PE, Kesner RP, Lee I (2001) Dissociating hippocampal subregions: double dissociation between dentate gyrus and CA1 Hippocampus 11:626–636. https://doi.org/10.1002/hipo.1077
Golan MH, Mane R, Molczadzki G, Zuckerman M, Kaplan-Lousson V, Huleiel M, Perez-Polo JR (2009) Impaired migration signaling in the hippocampus following prenatal hypoxia. Neuropharmacology 57:511–522. https://doi.org/10.1016/j.neuropharm.2009.07.028
Gonzalez-Billault C, Del Rio JA, Urena JM, Jimenez-Mateos EM, Barallobre MJ et al (2005) A role of MAP1B in Reelin-dependent neuronal migration Cereb Cortex 15:1134–1145. https://doi.org/10.1093/cercor/bhh213
Gustavsson M, Anderson MF, Mallard C, Hagberg H (2005) Hypoxic preconditioning confers long-term reduction of brain injury and improvement of neurological ability in immature rats. Pediatr Res 57:305–309. https://doi.org/10.1203/01.pdr.000015122.58665.70
Hack I, Bancila M, Loulier K, Carroll P, Cremer H (2002) Reelin is a detachment signal in tangential chain-migration during postnatal neurogenesis. Nat Neurosci 5:939–945. https://doi.org/10.1038/nn923
He M, Zhang ZH, Guan CB, Xia D, Yuan XB (2010) Leading tip drives soma translocation via forward F-actin flow during neuronal migration. J Neurosci 30:10885–10898. https://doi.org/10.1523/JNEUROSCI.0240-10.2010
Hevner RF (2016) Evolution of the mammalian dentate gyrus. J Comp Neurol 542:578–594. https://doi.org/10.1002/cne.23851
Huang YY, Li L, Monteleone M, Ferrari L, States LJ et al (2016) Use of anesthesia for imaging studies and interventional procedures in children. J Neurosurg Anesthesiol 28:400–404. https://doi.org/10.1097/anr.0000000000000355
Kerjan G, Gleenon JG (2007) Genetic mechanisms underlying abnormal neuronal migration in classical lissencephaly Trends in genetics. TIG 23:623–630. https://doi.org/10.1016/j.tig.2007.09.003
Knuesel I (2010) Reelin-mediated signaling in neuropsychiatric and neurodegenerative diseases. Prog Neurobiol 91:257–274. https://doi.org/10.1016/j.pneurobio.2010.04.002
Kohno S, Kohno T, Nakano Y, Suzuki K, Ishii M et al (2009) Mechanism and significance of specific proteolytic cleavage of Reelin. Biochem Biophys Res Commun 380:93–97. https://doi.org/10.1016/j.bbrc.2009.01.039
Kohno T (2017) Regulatory mechanisms and physiological significance of Reelin function Yakugaku zasshi. J Pharm Soc Jpn 137:1233–1240. https://doi.org/10.1248/yakushi.17-00127
Komitova M, Xenos D, Salmaso N, Tran KM, Brand T et al (2013) Hypoxia-induced developmental delays of inhibitory interneurons are reversed by environmental enrichment in the postnatal
Zhao P, Ji G, Xue H, Yu W, Zhao X et al (2014) Isoflurane postconditioning improved long-term neurological outcome possibly via inhibiting the mitochondrial permeability transition pore in neonatal rats after brain hypoxia-ischemia. Neuroscience 280:193–203. https://doi.org/10.1016/j.neuroscience.2014.09.006

Zhao S, Chai X, Förster E, Frotscher M (2004) Reelin is a positional signal for the lamination of dentate granule cells Development (Cambridge, England) 131:5117–5125. https://doi.org/10.1242/dev.01387

Zhang Y, Zhang LH, Chen X, Zhang N, Li G (2018) Piceatannol attenuates behavioral disorder and neurological deficits in aging mice via activating the Nrf2 pathway. Food Funct 9:371–378. https://doi.org/10.1039/c7fo01511a

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