Effects of Repeated Inhalation of Sevoflurane During Infancy on Cognitive Function and Hippocampal Volume of SD Rats in Childhood and Adulthood

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Abstract

Background: It has been reported that repeated exposure to sevoflurane, a widely used general anesthesia in pediatric surgeries, may lead to brain defect in infant. However, the long-term effect of repeated exposure to sevoflurane during infancy on the learning behavior and neuro-development is less investigated yet.

Methods: Sixty-four SD (sprague dawley) rats were randomly divided in to two groups, the experimental group (n=32) was exposed to sevoflurane (2.6%, 2 h) and the control group (n=32) was exposed to carrier gas (1 L/min O₂ + 1 L/min Air, 2 h) for three times at infancy (P (postnatal day) 7, P14, and P21). At childhood (P32 to P36), SD rats in each group (n=16) received Morris Water Maze (MWM) test, and then Magnetic Resonance Imaging (MRI) was used to scan their brain and hippocampus at P37. Subsequently, the same NWM test and MRI scanning was conducted for the remaining SD rats in their adulthood (P92 to P97) (n = 16/group).

Results: After being exposed to sevoflurane in infancy, the hippocampal volume of SD rats significantly decreased in their childhood and adulthood, their whole brain volume was also shrunken in adulthood; however, MWM test showed there is no obvious change in their spatial learning and memory recall performance either in childhood or in adulthood.

Conclusions: Although repeated exposure to sevoflurane in infancy did not affect the spatial learning and memory performance of rats, however, it could result in the decease of hippocampal and brain volume in their adulthood. This study suggests that repetitive sevoflurane exposure in infancy may exert long-term risk in brain development.

Background

Sevoflurane is one of the most popular anesthetics, it is widely used in pediatric anesthesia due to low pungency, steady anesthesia, rapid recovery and small respiratory stimulation in recent years. McCann et al. reported that infants expose to anesthetic for once (less than 1 h) won't cause any neuro-developmental outcome when they are 5 years old [1]. However, some children may experience multiple sevoflurane anesthesia due to complex pediatric surgeries that need to be performed several times, such as cleft lip and palate and fracture fixation.

A Mayo Anesthesia Safety in Kids (MASK) study indicated that single anesthesia exposure before age 3-years-old won't lead to deficit in general intelligence, but multiple exposures are associated with some behavioral and learning difficulties [2]. Although clinical studies favored that single brief exposure during infant stage is not connected with impairment of neuro-developmental process [3], experimental researches reported that neonatal anesthesia exposure in rodents and nonhuman primates may impair different parts of brain regions, including prefrontal cortex, hippocampus and amygdala, and cause behavior deficits [4-6]. The outcome of multiple anesthetic exposures and its long-term effect still need to be discussed.

Hippocampus is the most important brain region related to the ability of learning and memory, it is also vital for cognitive development from infancy to adulthood. The volume of Hippocampus is positively related to accurate episodic memory [7], the decrease of hippocampal volume in adults might cause early dementia and cognitive decline [8]. The maturity levels of hippocampus is correlated with age differences in the memory encoding [9]. Previous studies reported that experimental animals' exposure to sevoflurane inhalation in infancy may impair their learning process, reduce neurogenesis and its plasticity, and finally cause neurotoxicity in the hippocampus of rodents [10-12].

In order to investigate the long-term influence of repeated exposures to sevoflurane in infancy, the SD rats were exposed to sevoflurane three times in their infancy in this study (P (postnatal day) 7, P14 and P21). Then their spatial learning and memory performance were evaluated by Morris Water Maze (MWM) test, then their hippocampus and brain volumes were scanned by MRI in their childhood and adulthood, respectively. Our finding may provide further evidence for the potential long-term risk of repeated exposure to sevoflurane for infants in pediatric surgery.

Methods

Animals

Sixty-four neonatal healthy SD rats were randomly divided into experimental group (n=32) and control group (n=32) and kept in the experimental animal center of Zunyi medical University. All procedures were performed in accordance with the regulations of the Animal Management Regulations and Administrative Measures on Experimental Animal. Three life stages were adopted in this study: neonatal period (P0-P21), childhood (P22-P60) and adulthood (2-20 month).

Sevoflurane anesthesia treatment

All SD rats were placed in a clear acrylic box connected to the anesthesia machine or the gas monitor. The experimental group (n=32) was exposed to 2 L/min, 2.6% sevoflurane (Southern Shandong Bert Pharmaceutical company, Shandong, China) for 2 h, at P7, P14 and P21; the control group (n=32) was exposed to the carrier gas (1 L/min O₂ + 1 L/min Air, 2 h) at the same periods (Fig. 1).

Morris Water Maze (MWM) test

To investigate the spatial learning and memory recall performance in the development stage of childhood, both experimental group and control group received seven-days MWM test at childhood (P32-37) and adulthood (P92-97), respectively. Each time 16 rats from each group were randomly selected to receive the MWM test in each group, and they only received the MWM test once (Fig. 1).

The Morris water maze consisted of a round pool (120 cm in diameter, 60 cm in height) containing a black platform (20 cm in diameter, 30 cm in height) in the center of the southeast quadrant and an image acquisition system. The pool was divided into four quadrants named I, II, III and IV at equal distances to the edge of the pool. The water was made opaque with 600 g milk powder. The water temperature was maintained at $25 \pm 1^\circ\text{C}$ and the illumination was kept constant. The platform was submerged 1.4 cm below the water surface and could not be seen by the rats. The test was managed by an operator blinded to the group conditions. In each acquisition trial, animals were placed in the pool facing the wall from a selected quadrant (I, II, III and IV). The test included a directional navigation test and a probe test of spatial exploration, performed over 6 days. During the first 5 days of spatial acquisition phase, rats were trained with 4 trials per day with 5 min intervals. Rats were allowed to search for platform for 90 s and to remain on it for 30 s once they found it. If a rat failed to find the platform within 90 s, the rat would be guided gently to the platform by the experimenter and allowed to stay on it for 30 s. The swimming trial was monitored by a video-tracking system (Chengdu Thai Union Technology co., Chengdu, China). After 5 days of training, rats were returned to the pool for a probe trial. The platform was removed from the pool. The rats were placed in any quadrant and allowed to swim freely for 90 s. The previous platform area crossing times and the times the rats stayed in the target quadrant within 90 s were recorded. After the trial, each rat was dried and then returned to the experimental animal center.

Magnetic Resonance Imaging (MRI)

After MWM tests, the brain volumes of SD rats were scanned immediately (at P37 and P97, respectively) by a 3.0-T MRI scanner (GE Aircraft Engines Group, American) with a dedicated coil (4CH/5 cm, Suzhou Zhongzhi Medical Technology, Suzhou, China) at the radiology department of Affiliated Hospital of Zunyi Medical College. Rats received intraperitoneal injections of 30 ml/kg 1% sodium pentobarbital at P37 or P97. The acquisition of the MRI data was identical in transverse, coronal, and sagittal planes for all rats, and the head position was corrected for bilateral symmetry using BRAVO sequences with 3T MR imaging. T2-weighted BRAVO sequence parameters were the following: TR (time of repeat) 12.9 ms, TE (time of echo) 5.1 ms, TI (time of inverse) 450 ms, flip angle 12° , matrix 224×192 pixels, slice thickness 0.3 mm (no interslice gap), voxel size $0.3 \times 0.3 \times 0.3$ mm, FOV (field of view) 25 mm^2 (5×5 mm), bandwidth 19.2 kHz. Following experiment was conducted after the rat naturally wakes up.

The volumes of brain and hippocampus

The visual assessment method is an anatomy-based volumetric method used to measure the brain volume (BV), left hippocampal volumes (LHV), right hippocampal volumes (RHV), brain length (BL), left hippocampal length (LHL), right hippocampal length (RHL), brain width (BW), left hippocampal width (LHW) and right hippocampal width (RHW). The ratios of the bilateral hippocampal volume and the BV (LHV/BV and RHV/BV) were also calculated. Processing the scanned images at the post processing workstation at the GE ADW4.6 and then the scanned images were analyzed by MIP system, which could

simultaneously display three different planes (sagittal plane, coronal plane and cross-section) of the same structure, depict the border of brain and hippocampus on coronal plane layer by layer and finally automatically calculate and display indicators such as the volume of the brain or hippocampus. All volumes were measured three times and averaged to increase its reliability.

Statistical analysis

Statistical analyses were performed by GraphPad Prism 5 (GraphPad Software, San Diego, CA). All data was given as means $\pm$ SE. Student's T-test was used for single comparisons. Two-way ANOVA was used for analyzing statistic difference of learning curves (based on escape latency) among rats in different groups in the MWM. $P < 0.05$ was considered as statistically significant.

Results

The results of Morris Water Maze (MWM) test in the rats' childhood and adulthood

In their childhood (P32 to P36), the escape latencies of rats from both experiment and control groups gradually decreased, and there was no statistical difference between groups (Fig. 2A). At P37, after removing the platform, the traces (Fig. 2B-C), the total moving distance (Fig. 2D), the percentage of the time spent in the target quadrant (Fig. 2E), the total time spent in the target quadrant (Fig. 2F) and the number of crossings over the previous platform area (Fig. 2G) were also not difference between the sevoflurane-inhaled rats and control rats.

Similarly, there was no significant difference in the rats' escape latencies between groups in adulthood (P92-P96) (Fig. 3A). After removing the platform (P97), each index in experimental group had no significant difference from that in control group (Fig. 3B-G). Repeated sevoflurane exposures in infancy did not affect the spatial learning and memory recall performance in childhood and adulthood.

The size change of hippocampus in the rats' childhood

We quantified the brain and hippocampal volume of experimental group (n=16) and control group (n=16) at P37 with high-resolution MRI (Fig. 4). Student's t-test showed there were no significant difference in the brain volume, length and width between experimental rats and control rats (Fig. 5A, D and G). The left hippocampal volume of experimental rats was smaller than that of controls (Fig. 5B-C), the length of bilateral hippocampus in experimental rats were shorter than that of control (Fig. 5E-F) and the width of bilateral hippocampus were no significant difference between groups (Fig. 5H-I). The ratio between the bilateral hippocampal volume and the brain volume showed no significant difference between experimental and control rats.

The size change of brain and hippocampus in the rats' adulthood

Similarly, at P97, we quantified the volume ratio between hippocampus and brain in the experimental group (n=16) and control group (n=16). The brain volume of experimental rats was significantly smaller than that of the control group. Both volume and length of brain in the experimental rats were reduced (Fig. 6A and D), but there was no significant difference in the width of brain (Fig. 6G). The bilateral hippocampal volume of experimental rats was smaller than that of controls (Fig. 6B-C), however, there were no significant differences in the length and width of bilateral hippocampal volume (Fig. 6H-I). It is worth noting that there was no observed significant difference in the ratio of left hippocampal volume to brain volume between groups, but the ratio of right hippocampal volume to brain volume of experimental rats was higher than that of controls, suggesting that atrophy of the whole brain in adulthood may be more significant than that in hippocampus after multiple exposures to sevoflurane in infant.

Discussion

The present study demonstrated that after SD rats was exposed to 2.6% sevoflurane for three times in their infancy, their hippocampal volume significantly decreased in childhood; in their adulthood, the brain and hippocampal volumes both decreased. However, MWM tests showed no significant changes in their spatial learning and memory performance.

Although it remains controversial whether there are some correlation between the exposure to general anesthesia in infancy and the impairment of neuronal development, some clinical evidences showed repeated exposure to an aesthetic during infancy may have some negative influences [2, 3, 5, 13]. In addition, animal researches provided evidence that exposure to general anesthetics, e.g. sevoflurane, is linked with morphological and functional changes in the central nervous system, which is involved in the impairment of neuro-cognitive performance. Satomoto et al. [4] found that neonatal exposure to sevoflurane (3%, 6 h) in infant mice at P6 significantly induced the caspase-3-related apoptosis in several brain regions, including hippocampus, cortex and thalamus. Additionally, the single sevoflurane exposure could cause persistent learning deficits and impairment of social behavior in adulthood (>8 weeks old). Similarly, rats exposed to sevoflurane at P3 exhibited worse spatial memory performance in the adulthood when compared with which suffered from the same anesthetic at later stage (P7, P14 and P49). These factors were dose- and duration-dependent, which means higher concentrations (4% vs 2% or 1%), longer times (3 h vs 2 h, 1 h or 0.5 h) and multiple exposures (3 vs 2 or 1 exposures in the same day) at the same concentrations may cause stronger and longer adverse effects. In addition, when the interval between exposures was shorten, the negative effects of repeated exposures could be more serious [11].

In the present study, we adopted a moderate sevoflurane concentration (2.6%), inhalation time (2 h) and with a relative longer interval (7 days), this scheme was closer to the clinical situation. Our outcomes showed that although repeated exposures to sevoflurane that did not change learning performance, it may still affect neuro-development and the brain structure during the later developmental process.

Additionally, although the MWM tests showed unaffected performance, it didn't mean that repeated exposures to anesthetic has no influences on the learning/memory ability or other neurocognitive functions. More behavior tests are still needed in the further study to clarify this point.

The development and mature of hippocampus are critical for learning and memory of children. A recent research showed that hippocampal maturity as expressed in the multivariate pattern of age-related differences in hippocampal subregions is specifically related to the ability to lay down highly specific memories [9]. Decreased hippocampal volume is tightly correlated with memory and cognition decline in adult; small hippocampal volume could be a biomarker of neurodegeneration, and the shrinkage of hippocampus leads to memory impairment and increased risk of dementia [8, 14, 15]. In addition, smaller hippocampal is also related to chronic stress, it may be a susceptible factor for stress perceiving [16-18]. The finding is in line with preclinical evidence that multiple sevoflurane exposures in infant monkeys within P28 alter their emotional reactions to an acute stressor [19]. Therefore, our results indicate that repeated exposure to general anesthetics in infancy may increase the risk of cognitive impairment and stress-related neuropsychological disorders in adulthood, such as depression, anxiety or posttraumatic stress disorder (PTSD).

The mechanism of the long-lasting influence of multiple anaesthetic exposures to the brain development is still less understood. It has been generally accepted that postnatal neurogenesis, experience-dependent synaptic remodeling and functional synaptic plasticity (e.g. long-term potentiation) in the hippocampus play important roles in the developmental plasticity. Previous studies show that general anesthetics have cytotoxicity on the hippocampal neurons of new-born animals [5, 20]. Nevertheless, Kato, R., et al. have found that when newborns were exposed to sevoflurane, their long-term hippocampal synaptic plasticity can be suppressed, implicating sevoflurane maybe the impact factor of hippocampus for learning new memory formation [12]. In addition, animal experiments showed that exposure to anesthesia in infancy can induce neuronal death in several brain regions, including hippocampus, thalamus and cortex [10, 21-23]. Therefore, it is possible that multiple exposures to sevoflurane in the infant stages could cause neuronal loss, reduce neurogenesis and impede the neuro-development, then result in the shrinkage of hippocampus in the childhood and the loss of brain volume in the adulthood.

However, the present study still has some limitations, which need to be further investigated in the future. First, we only examined the spatial learning and memory performance by MWM tests, the other types of memory and cognitive behavior still need to be tested. Second, we only measured the whole brain volume and hippocampal volume. Further investigation in other brain regions, such as cortex and amygdala could provide more valuable information.

Conclusions

In this study, the aesthetic models of SD rats showed that although repeated exposure to sevoflurane in infancy seems not to affect their spatial learning and memory performance as they grow, however, their hippocampal volume in early childhood, and both hippocampal and whole-brain volume in the adulthood

significantly shrunk. Our findings suggest the repeated exposure to sevoflurane may have potential long-term risk in brain developmental process.

Abbreviations

BL: brain length; BV: brain volume; BW: brain width; FOV: field of view; LHL: left hippocampal length; LHV: left hippocampal volumes; LHW: left hippocampal width; MRI: Magnetic Resonance Imaging; MWM: Morris Water Maze; P: Postnatal day; RHL: right hippocampal length; RHV: right hippocampal volumes; RHW: right hippocampal width; SD: Sprague Dawley; SEV: Sevoflurane; TE: time of echo; TI: time of inverse; TR: time of repeat

Declarations

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Authors' contributions

YHZ, JJR and ZQZ designed the study, analyzed the experiments, and wrote the paper. CZ, TWG, PCZ, XFL, YY and DXL carried out the data collection and data analysis and revised the paper. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The datasets generated and analyzed in this work are available for anyone who wishes to access the data by contacting the corresponding author.

Ethics approval and consent to participate

This project was approved by the medical ethics committee of Zunyi Medical University (Approval No. 2018. 246).

Consent for publication

Not applicable.

Competing interests

All authors declare that there are no conflicts of interest.

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Figures

Figure 1

The flow chart of the experiment. After 2 h exposed to 2.6% sevoflurane for 3 days, SD rats were used for MWM, brain MRI analysis in the rats' childhood and adulthood.

Figure 2

The results of Morris Water Maze (MWM) test in the rats' childhood. (A) Variation trend of escape latency in control and experimental group; (B, C) Representative swimming traces of control group and experimental group in the retention session (P37). The small circle is the location of the platform, located in the southeast quadrant. Right scales indicate the maximum swimming speed; (D) The total moving

distance; (E) The percentage of the time spent in the target quadrant; (F) The total time spend in the target quadrant; (G) The number of crossings over the previous platform area.

Figure 3

The results of Morris Water Maze (MWM) test in the rats' adulthood. (A) Variation trend of escape latency in control and experimental group; (B, C) Representative swimming traces of control group and experimental group in the retention session (P97), right scales indicate the maximum swimming speed; (D) The total moving distance; (E) The percentage of the time spent in the target quadrant; (F) The total time spend in the target quadrant; (G) The number of crossings over the previous platform area.

Figure 4

Schematic diagram of automatic measurement of hippocampal volume by MIP system. Figures A-J are the MRI images of a SD rat in the experimental group and manually depict the border of hippocampus layer by layer. Figure I showed that the MIP system automatically calculate and display the hippocampus volume, length and width. In particular, the corresponding indicators of the brain are calculated in the same way as above.

Figure 5

The size change of brain and hippocampus in the rats' childhood. (A) The brain volume, BV; (B) The left hippocampal volume, LHV; (C) The right hippocampal volume, RHV; (D) The brain length, BL; (E) The left hippocampal length, LHL; (F) The right hippocampal length, RHL; (G) The brain width, BW; (H) The left hippocampal width, LHW; (I) The right hippocampal width, RHW; (J) The ratio of LHV to BV; (K) The ratio of RHV to BV. *p < 0.05, **p < 0.01, ***p < 0.001 vs controls (n = 8 rats/group).

Figure 6

The size change of brain and hippocampi in the rats' adulthood. (A) The brain volume, BV; (B) The left hippocampal volume, LHV; (C) The right hippocampal volume, RHV; (D) The brain length, BL; (E) The left hippocampal length, LHL; (F) The right hippocampal length, RHL; (G) The brain width, BW; (H) The left hippocampal width, LHW; (I) The right hippocampal width, RHW; (J) The ratio of LHV to BV; (K) The ratio of RHV to BV. *p < 0.05, **p < 0.01, ***p < 0.001 vs controls (n = 8 rats/group).

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