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ORIGINAL PAPER

Juvenile Rats Show Altered Gut Microbiota After Exposure to Isoflurane as Neonates

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Abstract

Inhaled anesthetic agents may be neurotoxic to the developing brain of a neonatal rodent. Isoflurane is a commonly used volatile anesthetic agent for maintenance of general anesthesia in various types of surgery. Neonatal exposure to isoflurane has been implicated in long-term neurocognitive dysfunction in children. The mechanisms of isoflurane-induced neurotoxicity have not been fully elucidated. Disruption of gut microbiota is currently attracting considerable interest as a vital pathogeny of some neurologic disorders. In the rat model, it is unknown whether neonatal exposure to isoflurane impacts the gut microbiota composition of juvenile animals. In the present study, postnatal 7-day-old male rats were exposed to 1 minimum alveolar concentration isoflurane for 4 h. Non-anesthetized rats served as controls. The fecal microbiomes of rats were observed using 16S RNA sequencing technique on postnatal day 42. Results indicated that composition of gut microbiota of isoflurane-exposed rats was different from controls. Several bacteria taxa in isoflurane-exposed rats were different from those of controls at various taxonomic levels. In particular, the abundance of *Firmicutes, Proteobacteria, Clostridia, Clostridiales*, and *Lachnospiraceae* were significantly increased in exposed rats and the abundance of *Bacteroidetes, Actinobacteria, Bacteroidia* and *Bacteroidaceae* were significantly decreased compared to controls. These results may offer new insights into the pathogenesis of isoflurane-induced neurotoxicity.

Keywords Isoflurane · Anesthetic neurotoxicity · Gut–brain axis · Gut microbiota

Introduction

Millions of babies with different pathophysiologic conditions receive operative procedures requiring general anesthesia. Safety of pediatric anesthesia is a major health and social issue. Findings from animal models indicate that exposure to commonly used anesthetics during neonatal stages produces cognitive decline and neurotoxicity in juvenile animals [1, 2]. Moreover, study in humans suggest an association between impaired neurodevelopment and general anesthesia exposure in early infancy [3]. These findings have aroused concerns about the possible harmful effects of general anesthesia in the pediatric population. In the last decade, despite extensive research exploring the potential pathogenesis of anesthesia-induced neurotoxicity, the mechanisms are still not fully elucidated.

Although anesthetic agent-induced neurologic changes in the neonatal brain have been extensively studied within the central nervous system (CNS), there are few studies that have investigated the peripheral effects of general anesthesia causing neurotoxicity. Emerging evidence from animal and human investigations indicate a close connection between the intestinal microbiota and the brain, which is termed the gut–brain axis [4]. A dynamic and highly complex community of micro-organisms with thousands of individual strains are colonized in the mammalian digestive tract. These gut microbes substantially influence the function and

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microenvironment of the brain [5]. Moreover, the differentiation and function of immune cells in the CNS are controlled by the gut microbiota [6]. Therefore, gut microbiota is also thought to be a key regulator of neuroinflammation [7, 8]. Mounting evidence indicates that gut dysbiosis, rather than a comorbidity, leads to pathological abnormalities and behavioral impairments in various spectrum of neuropsychological disorders [9, 10]. For example, expression of proteins with Alzheimer disease (AD)-related mutations directly interferes with gut microbiota [11]. And antibiotic-induced perturbations in gut microbiota could exacerbate the pathologic changes of AD in mice [12]. Moreover, alteration of the microbiota is considered an important pathogeny of neuropsychiatric symptoms such as depression, anxiety, and cognitive dysfunction [13]. Studies of aging adults with cognitive impairments have reported gut dysbiosis with decreased abundance of anti-inflammatory microbes and increased abundance of pro-inflammatory microbes [14]. Further, people with AD have been found with significant changes in gut microbiota composition and diversity compared with cognitively normal persons [15].

Isoflurane is a commonly used volatile anesthetic agent for maintenance of general anesthesia in various types of surgery. Previous studies showed that neonatal exposure to isoflurane can lead to neurotoxicity and long-term cognitive deficiencies [16]. Based on the evidence that brain function is impacted by gut microbiota, we designed this study to determine whether neonatal exposure of rats to isoflurane could impact the gut microbiota of the juvenile animals in order to improve our understanding on mechanisms of anesthesia-related neurotoxicity.

Materials and Methods

Experimental Animals

All experimental procedures were performed in accordance with animal care guidelines and approved by The Ethics Committee of the Peking University Health Science Center (LA2016320). Specific pathogen free (SPF) Sprague–Dawley rats were obtained from Beijing Vital River Experimental Animals (Beijing, China). Animals were maintained under SPF environmental conditions and housed on a 12:12 light:dark cycle at 20 ± 2 °C, and humidity of 50 ± 10%. Standard chow and water were available ad libitum.

Isoflurane Exposure and Fecal Sample Collection

Pups included in our experiments were from three naive mothers. On postnatal day 7 (P7), ten male pups were randomly allocated to one of two groups, control and isoflurane anesthesia, to exclude litter variability. Five male rats were placed in a plexiglass chambers connected to a vaporizer. The rats were exposed to 1 minimum alveolar concentration (MAC) isoflurane plus 50% O_2 for 4 h as described previously [17, 18]. A total gas flow rate of 6 l was used to maintain a steady state of inhaled anesthetic gas and prevent accumulation of expired CO_2 within the chamber. The control group of five P7 male rats was exposed to the same conditions for 4 h but without isoflurane. Oxygen and anesthetic agent fractions were monitored continuously using a gas analyzer (Datex-Ohmeda, Madison, WI). Spontaneous breathing of animals was observed every 3 min. During anesthetic treatment, the environmental temperature was maintained at 37 ± 0.5 °C. After the procedure, all pups in both groups were returned to their dams and weaned after 21 days. At 42 days of age, rats in both groups were euthanized, and fresh fecal samples were collected immediately after euthanasia from the terminal rectum of each animal into individual sterile EP tubes and then stored at − 80 °C. Meanwhile, on P7, fecal samples from the male littermates of the pups that participated in the experiments were collected and the microbiome of those samples were assessed. No significant differences in the diversity and structure of the microbiota was detected between the litters (see Appendix in Supplementary Material).

DNA Extraction

Fecal DNA of rats was extracted using Omega stool DNA Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's protocol. Purity and quality of the genomic DNA were evaluated by electrophoresis on 0.8% agarose gels. All DNA samples were stored at − 20 °C for further analysis.

Polymerase Chain Reaction (PCR) Amplification

Amplification of 16S rRNA gene was performed with the universal primers 338F (*ACTCCTACGGGAGGCAGCAG*) and 806R (*GGACTACHVGGGTWTCTAAT*), which targets V3–V4 hypervariable region. The 5′ end of forward and reverse primers were added with 10-digit barcode sequence. PCRswere performed in a volume of 50 µl containing 5 µl of 10 × Ex Taq Buffer (Mg^{2+} plus), 4 µl of 12.5 mM dNTP Mix (each), 1.25 U of Ex Taq DNA polymerase, 2 µl of template DNA, 200 nM barcoded primers each, and 36.75 µl of ddH_2O. The following parameters were used: 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s with a final extension at 72 °C for 10 min. The PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany), quantified using real time PCR, and sequenced at Beijing Auwigene Tech (Beijing, China).

High Throughput Sequencing and Data Analysis

Deep sequencing was performed on MiSeq platform using Illumina Analysis Pipeline Version 2.6 (Illumina, San Diego, CA, USA). The raw sequencing data were screened and sequences were removed from consideration if they were shorter than 200 bp, had a low-quality score (≤ 20), contained ambiguous bases or did not exactly match primer sequences and barcode tags. Qualified reads were then separated using the sample-specific barcode sequences and trimmed with Illumina Analysis Pipeline Version 2.6. After trimming, high-quality sequences were classified into different taxonomic groups using the Ribosomal Database Project Classifier tool (rdp.cme.msu.edu/classifier; Center for Microbial Ecology, Michigan State University, East Lansing, MI, USA). The datasets were analyzed using QIIME. The sequences at a similarity level of 97% were clustered into operational taxonomic units (OTUs) in order to generate rarefaction curves and to calculate richness and diversity indices. A Venn diagram of the OTUs for each group was generated using the R package. Alpha diversity was applied to analyze the richness and diversity of species present in a sample. To evaluate α diversity of the gut microbiome, Chao1 and PD_whole_tree and Shannon index was calculated based on rarefied OUT tables. Comparisons of index of α diversity were performed by Mann–Whitney U tests using Graphpad Prism software (GraphPad Software, La Jolla, CA, USA). Beta diversity was used to measure the magnitude of differences in community composition between the samples. Clustering analyses and principal coordinate analyses (PCoA) based on the unweighted UniFrac distances was performed for assessment of the beta diversity of samples. Difference of PCoA between groups was determined using PERMANOVA analysis. Linear discriminant analysis (LDA) coupled with effect size (LEfSe) was employed to identify the characterization of microbial features differentiating the fecal microbiota specific to different taxonomic types [19]. LDA scores were then performed to estimate the effect size of each feature. For all statistical tests, $P < .05$ was regarded statistically significant.

Results

Characteristics of the High-Throughput Sequence Data

After quality screening, a total of 613,783 of V3–V4 16S rRNA sequence reads from the 10 samples were obtained. We finally detected 220 OTUs, with 125–176 OTUs in each sample, using a 97% similarity level. About 83.2% OTUs were shared between the two groups of fecal bacterial community as shown in the Venn diagram (Fig. 1a). Rarefaction curve analysis indicated that all samples reached stable plateau, implying the sampling was sufficient for the majority of the bacterial communities (Fig. 1b, c).

Community Structure and Diversity of Microbiota

Five major bacterial phyla (*Bacteroidetes, Firmicutes, Verrucomicrobia, Proteobacteria*, and *Actinobacteria*) were represented in the fecal microbiota of rats, up to approximately 99% of the total fecal bacterial community. After neonatal exposure with isoflurane, *Firmicutes* increased from 27.2 to 52.9% ($P < .05$), *Bacteroidetes* dropped from 70.4 to 39.6% ($P < .05$), and *Proteobacteria* increased from 0.68 to 2.59% ($P < .01$) (Fig. 2).

Microbial alpha diversity was measured with Chao1 and PD_whole_tree and Shannon index. No statistical difference was observed in the alpha diversity of microbiota between the two groups (Table 1). Thus, isoflurane did not significantly affect the alpha diversity of the gut microbiome.

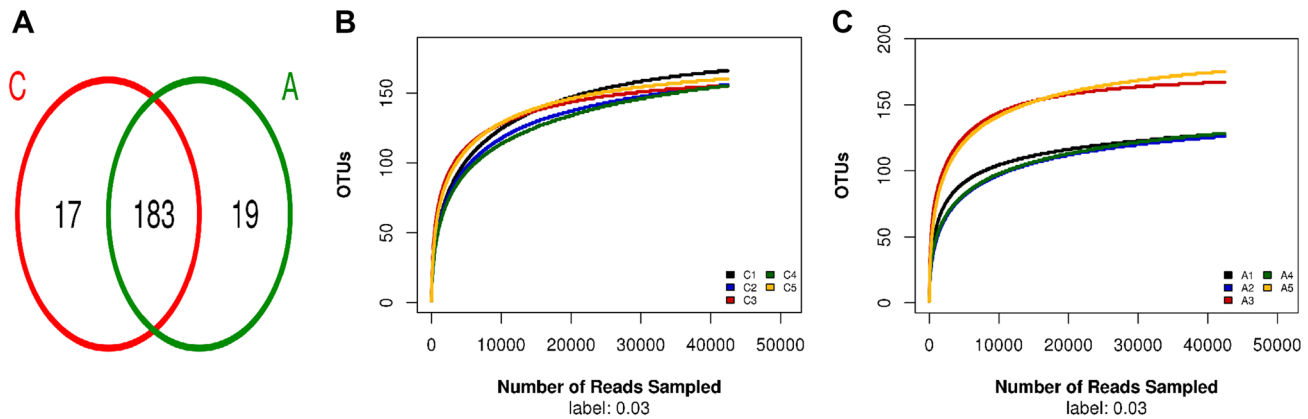


Fig. 1 High-throughput sequence data. **a** Venn diagram of the unique and shared OTUs between two groups. **b** Rarefaction curve for each sample in control group. **c** Rarefaction curve for each sample in anesthesia group. *C* control group, *A* anesthesia group, n = 5 per group

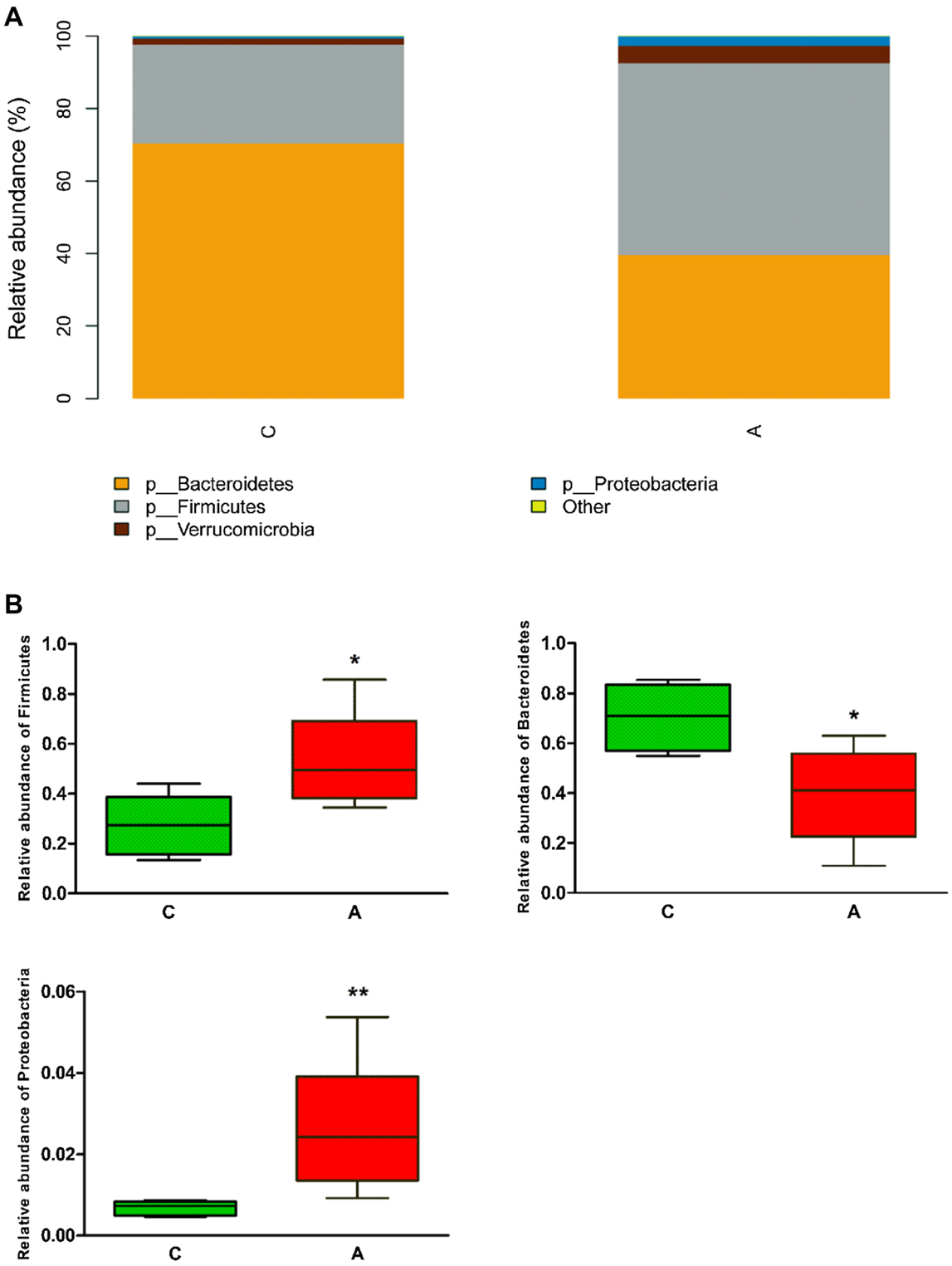


Fig. 2 Fecal microbiota compositions of control and anesthesia groups. **a** Overall microbial composition of the two groups. **b** Differences in relative abundance of *Firmicutes, Bacteroidetes*, and *Proteobacteria* were statistically significant. $^{*}P < .05$, $^{**}P < .01$. *C* control group, *A* anesthesia group, n = 5 per group

Table 1 Alpha diversity indices for gut microbiome in each group

Group	Chao1	PD_whole_tree	Shannon
Control	172.88 [163.97–185.83]	12.01 [11.91–12.41]	3.48 [3.48–3.55]
Anesthesia	139.01 [134.71–180.07]	10.30 [9.9–12.75]	3.60 [3.59–4.34]

No statistical difference was observed in the alpha diversity of microbiota. Values are expressed with median [interquartile range], n = 5 per group

Beta diversity between the two groups was examined by PCoA analysis based on the unweighted UniFrac distances and clustering analysis. OTU community comparisons by hierarchical clustering showed that samples from the two groups clustered, and samples from isoflurane-inhaled rats were separated from of those of the control group (Fig. 3a). Similar results were obtained with PCoA, which revealed that the gut microbiota of juvenile rats exposed neonatally to isoflurane were significantly distinct from controls ($P < .05$) (Fig. 3b).

Variations in Microbiota Composition Related to Isoflurane

We applied the LEfSe algorithm to identity the differences on microbial composition between the anesthesia and control groups (Fig. 4a, b). LDA > threefolds was considered significant. LEfSe analysis showed that a total of 33 features had significantly different abundance between the two groups. At the phylum level, fecal microbiota of neonatal rats exposed to isoflurane were differentially enriched with *Firmicutes* and *Proteobacteria*, whereas control rats were enriched with *Bacteroidetes*. At the class level, the microbiota of isoflurane-inhaled rats was characterized by a preponderance of *Clostridia* and *Gammaproteobacteria*, whereas the microbiota of control rats was characterized by a preponderance of *Bacteroidia* and *Actinobacteria*. At the order level, the abundance of the *Clostridiales* and *Bacillales* in feces of isoflurane-inhaled rats were significantly higher compared to the control group. In addition, at the family level, rats exposed neonatally to isoflurane showed an increased abundance of *Lachnospiraceae, Bacillaceae*, and *Burkholderiaceae* than did control rats.

Discussion

The bidirectional communication between the gut and brain is termed the gut–brain axis. Substantial evidence from animal and human investigations indicate that the gut microbiota is a key player for regulating this axis. Activity of the developing gut microbiota in infants is indispensable for brain development and can affect behavior [20]. The early neonatal period is a pivotal time window for development and maturation of microbiota composition and host–microbe interactions. During the early postnatal period, the microbiota is strongly influenced by environmental factors [21]. Evidence from clinical trial indicated that administration of

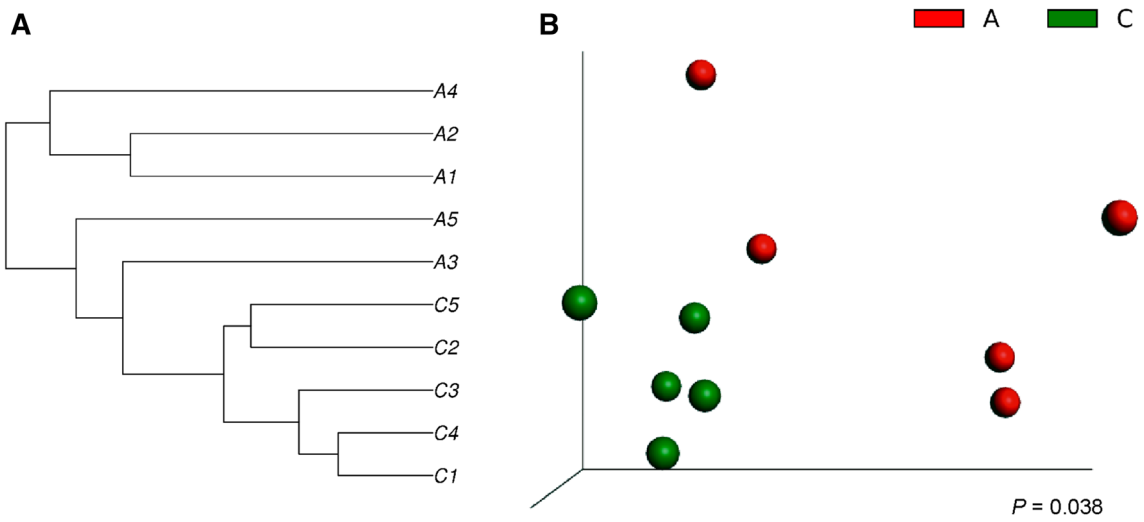
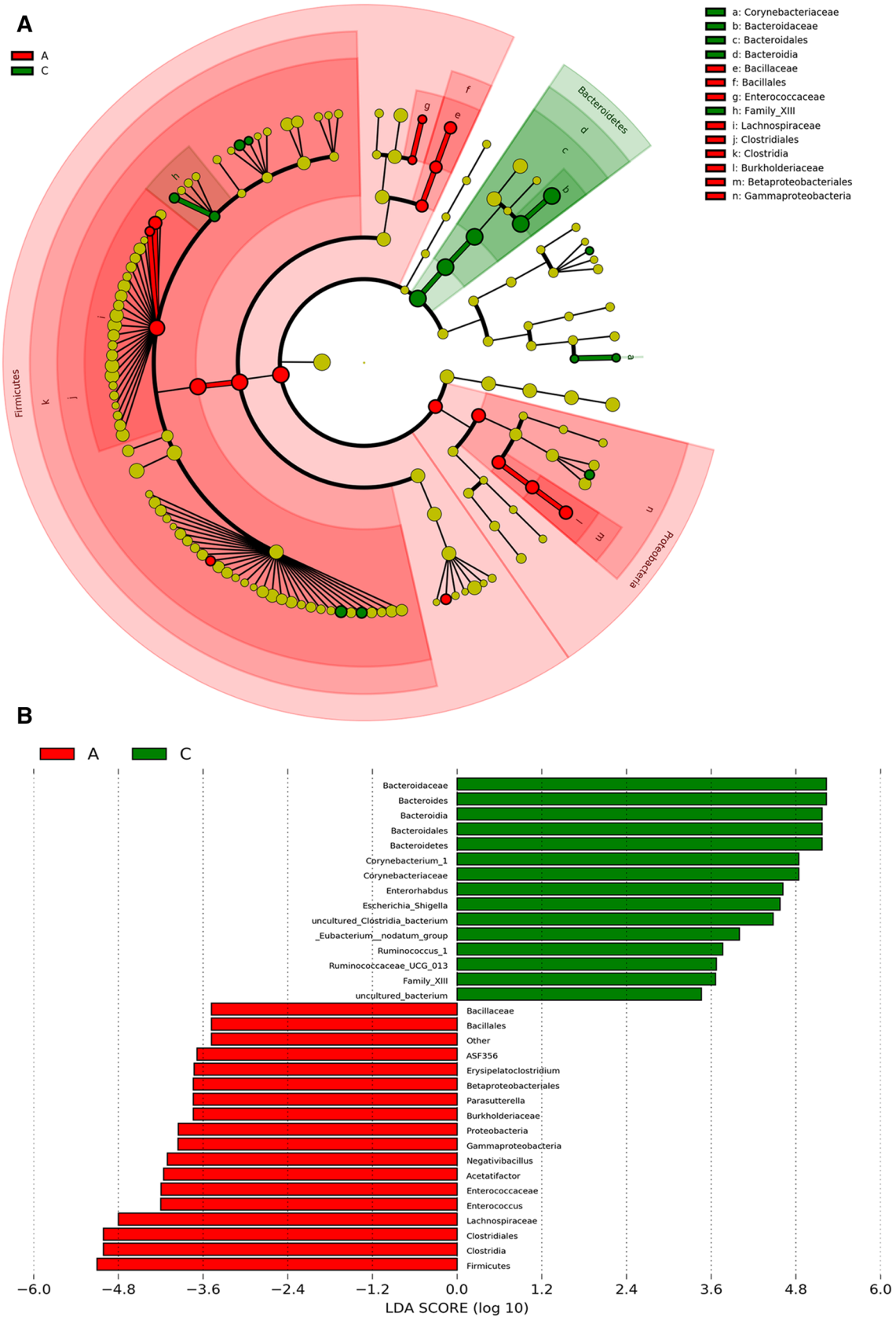


Fig. 3 Beta diversity of microbiota showed significant difference between the control and anesthesia groups. **a** Hierarchical clustering analysis of bacterial community of feces. **b** PCoA of bacterial community of feces (green = control group, red = anesthesia group). *C* = control group, A = anesthesia group, n = 5 per group. (Color figure online)


A
A
C
a: Corynebacteriaceae
b: Bacteroidaceae
c: Bacteroidales
d: Bacteroidia
e: Bacillaceae
f: Bacillales
g: Enterococcaceae
h: Family_XIII
i: Lachnospiraceae
j: Clostridiales
k: Clostridia
l: Burkholderiaceae
m: Betaproteobacteriales
n: Gammaproteobacteria
Bacteroidetes
Firmicutes
Proteobacteria
B
A
C
Bacteroidaceae
Bacteroides
Bacteroidia
Bacteroidales
Bacteroidetes
Corynebacterium_1
Corynebacteriaceae
Enterorhabdus
Escherichia_Shigella
uncultured_Clostridia_bacterium
_Eubacterium__nodatum_group
Ruminococcus_1
Ruminococcaceae_UCG_013
Family_XIII
uncultured_bacterium
Bacillaceae
Bacillales
Other
ASF356
Erysipelatoclostridium
Betaproteobacteriales
Parasutterella
Burkholderiaceae
Proteobacteria
Gammaproteobacteria
Negativibacillus
Acetatifactor
Enterococcaceae
Enterococcus
Lachnospiraceae
Clostridiales
Clostridia
Firmicutes
−6.0
−4.8
−3.6
−2.4
−1.2
0.0
1.2
2.4
3.6
4.8
6.0
LDA SCORE (log 10)

◂**Fig. 4** Significant difference in gut microbiome of rats, as revealed by LEfSe and LDA analysis based on OTU-characterized microbiomes. **a** Cladogram using LEfSe, indicating the phylogenetic distribution of fecal microbes of rats. **b** LDA scores of significant differences in bacteria between the two groups. *C* control group, *A* anesthesia group, n = 5 per group

antibiotics during the first year of life has been associated with poorer emotional, cognitive and behavioral endpoints throughout childhood [22]. The effects of antibiotics on the gut microbiota and neurotoxicity during the growth period have also been modelled in rodents. Desbonnet et al. [23] found that antibiotic-induced gut dysbiosis in early juvenile mice could cause a long-term cognitive decline. Moreover, Fröhlich et al. [24] revealed that the neurotoxic effects of antibiotics were due to antimicrobial agents inducing gut dysbiosis rather than from a systemic antibiotic response. These findings suggest that disturbances in microbial organization in early growth could have long-term impact on the brain and behavior, resulting in brain dysfunction.

The effect of anesthetics on bacteria has been investigated since the last century [25]. Isoflurane was shown in vitro to have activity against growth of both gram-positive and gram-negative bacteria and even multidrug-resistant pathogens [26–28]. Inhaled anesthetics disperse rapidly from the alveoli into the microcirculation and organs, including the gastrointestinal tract. In both humans and animals, the vast majority of the microbial population reside in the gastrointestinal tract. Schoster et al. [29] demonstrated that the fecal microbial structure and some specific bacterial taxa were significantly changed after isoflurane anesthesia was administered in healthy horses. Their findings revealed the short-term antibacterial effects of isoflurane on intestinal microbiota. However, there is a paucity of literature that focus on the long-term effects of isoflurane on gut microbiota, especially during growth and development. Thus, we designed the current study to clarify whether exposure to isoflurane in early growth could cause long-term gut dysbiosis in rats so as to improve our understanding of the systemic mechanisms of anesthesia as related to neurotoxicity. Although there are differences in the overall complexity and timing of development between rodents and humans, the isoflurane exposure in rats on postnatal day 7 coincides with peak neurodevelopment, which corresponds with development in humans aged 0 through the first several months [30]. In our study, we observed the gut microbiome of juvenile rats. This time point overlaps with the corresponding stages of human trials that assessed cognitive deficit in children after anesthesia [31, 32]. In our investigation, we found the effect of isoflurane on microbial alpha diversity was not apparent in juvenile rats. Alpha diversity measures the richness and diversity of unique microbial taxa within a sample. Although maintaining the richness and diversity of gut microbiota and abundance of dominant bacteria is crucial for the health [33], it should be emphasized that alpha diversity or richness of gut microbiota alone may be a poor marker of dysbiosis [34], as some of dysbiosis may exhibit no changes in alpha diversity [35]. In our study, beta diversity as measured by clustering analysis and PCoA analysis was significantly different between the anesthesia and control group rats. These results revealed that after neonatal rats were exposed to isoflurane, the overall composition of gut microbiota in the juvenile animals was significantly altered. Moreover, LEfSe analysis showed that a total of 33 features had significantly different abundance between the two groups. Therefore, our study demonstrated that neonatal exposure to isoflurane can lead to long-term gut dysbiosis in juvenile rats.

We analyzed the variant taxa of the rat gut microbiome at different taxonomic levels. Our results suggest that in future studies attention should be paid to applying non-dietary or dietary interventions to manipulate the microbiota to mitigate the disturbances of specific taxa so as to ameliorate isoflurane-induced developmental neurotoxicity through targeting of the gut–brain axis. *Firmicutes* and *Bacteroidetes* are the most dominant phyla which provide various benefits to the host including protection against pathogenic microbes and instructing of the innate immune system [36]. In this study, for rats exposed to isoflurane anesthesia, the abundance of *Firmicutes* was significantly higher than that of the control animals and the abundance of *Bacteroidetes* was decreased in the isoflurane-exposed rats compared with the controls. Increased abundance of *Firmicutes* paralleled by decreased *Bacteroidetes* is a hallmark of dysbiosis in obesity and metabolic syndrome [37, 38], both of which are associated with a high risk of dementia [39, 40]. Increased abundance of *Firmicutes* accompanied by reduced *Bacteroidetes* have also been reported in AD and autism spectrum disorder in both humans and animal models [12, 41–43]. Thus, overgrowth of *Firmicutes* paralleled by undergrowth of *Bacteroidetes* might be harmful microbial disturbances that are injurious and detrimental to neurologic outcomes. We also found a significantly increased abundance of *Clostridia*, a genus of the *Firmicutes* phylum, in juvenile rats exposed neonatally to isoflurane. *Clostridia* secrete the highest toxin levels of any type of bacteria and are responsible for several diseases in humans and animals [44]. Some toxins can pass through the intestinal barrier and cross the blood–brain barrier, interacting with neurons and thus causing neurotoxicity [44].

Neuroinflammation is receiving attention as a mechanism of inhaled anesthetic-induced neurotoxicity and cognitive impairment in developing animals [36, 45]. Mounting evidence indicate that gut microbiota is a key regulator of neuroinflammatory responses. Microglia, which are a specialized macrophage population within the brain, are the key cells that induce the neuroinflammatory response

[46]. Broad et al. [47] demonstrated that isoflurane evokes significant overactivation of microglia in the neonatal brain and is involved in the mechanism of isoflurane-induced developmental neurotoxicity. Gut microbiota is crucial for activation and function of microglia [6]. Chunchai et al. [48] indicated that obese-insulin resistant rats show disturbance of the gut–brain axis as characterized by gut dysbiosis and increased activation of microglia in brain. Interestingly, in the present study, rats exposed to isoflurane showed a similar phenotype with increased abundance of *Firmicutes* and decreased abundance of *Bacteroidetes* to rats with dysbiosis in the Chunchai et al. study. Thus, to verify our results and those of others, further investigations are needed that focus on the link between gut microbiota and isoflurane-induced microglial activation and neuroinflammation.

Attention should also be paid to the systemic effects of general anesthesia that produce inflammation and contribute to neurotoxicity. Systemic inflammation during the developmental period can negatively affect brain development [49, 50]. Elevated systemic inflammation causes neurons to be more prone to a pro-inflammatory response in the presence of tissue damage [51]. Whitaker et al. [52] found that children who received inhaled isoflurane anesthesia without surgery showed a significantly increased plasma level of IL-1β. This is thought to be a mechanism of isoflurane-induced developmental neurotoxicity [52]. The gastrointestinal system is thought to be the largest organ of the immune system. Microbes residing in the gastrointestinal tract are a pivotal source of inflammatory innate immune signals [53]. Shifts in the microbiota composition are of importance as triggers and regulators of systemic inflammation. In the present study, we found perturbations in several taxa related to systemic inflammation. *Proteobacteria*, one of the main phyla of rat gut microbiota, are endotoxin producers and associated with inflammatory diseases [54, 55] and are the most unstable constituents among the main gut microbiota phyla [56]. Our results revealed that rats exposed neonatally to isoflurane led to a significantly increased abundance of *Proteobacteria* in the juvenile animals. Previous evidence indicated that the majority of taxa in the intestine that showed a positive association with pro-inflammatory cytokines belongs to the phylum *Proteobacteria* [57]. Otherwise, in our study, LEfSe analysis also indicated a significant reduction in *Bacteroides* in isoflurane-exposed rats. Some *Bacteroides* sp. participate in regulating the intestinal immunity [58], and depletion of *Bacteroides* in early life potentially contributes to development of inflammation and immunological diseases [59]. Thus, the aforementioned changes in intestinal flora of isoflurane-exposed rats may reflect an unstable structure of gut microbiota, which is associated with a state of inflammation and are linked to negative effects on the developing brain. Another consideration is increased intestinal permeability, which was found in dogs that were exposed to inhaled isoflurane anesthesia [60]. Gut dysbiosis combined with increased intestinal permeability could deteriorate systemic and neural inflammation [61]. Therefore, the "leaky gut" effects of isoflurane may cause an increased entrance into the circulation of inflammatory cytokines derived from gut dysbiosis, thus triggering the neuroinflammatory response.

There are several limitations of the present study. We only included male rats to eliminate the interference of sex. We adopted the protocol of Lee et al. [18], who found that male rats are more vulnerable to long-term cognitive effects after neonatal exposure to isoflurane anesthesia. Thus, we are unable to draw same conclusions regarding the effects of neonatal isoflurane exposure on gut microbiota of female rats. Secondly, we observed the changes in gut microbiota of rats that received only a single exposure to isoflurane at neonatal period. The anesthesia protocol we used has been shown in previous experiments to lead to long-term cognitive dysfunction and neuropathologic impairment [17, 18]. However, repeated neonatal exposure to isoflurane might be associated with more serious long-term neurotoxicity than a single exposure [2, 62]. Hence, it is necessary to perform more well-designed experiments to determine the gut microbial changes in neonatal rats after multiple exposures of isoflurane. Thirdly, previous studies indicated different neurotoxic effects of neonatal exposure to different inhaled anesthetics in rodent models. For instance, in neonatal mice, isoflurane caused greater neurotoxicity in juvenile animals than sevoflurane [2]. In addition, desflurane was found to be more neurotoxic than sevoflurane or isoflurane in the developing brains of mice [63]. Thus, comparative effects of neonatal exposure to other volatile anesthetics on gut microbiota are largely unknown.

In conclusion, findings in the present study indicate that neonatal rats exposed to isoflurane had their gut microbiota altered by the time they were juveniles. These results suggest that anesthesia-induced neurotoxicity might not be confined to the brain itself, and that brain homeostasis is closely associated with that of the whole body. More important, our study presents a potential new approach for microbial manipulation to prevent and treat anesthesia-induced cognitive decline in the pediatric population.

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Compliance with ethical standards

Conflict of interest None of the authors has a conflict of interest to declare.

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