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Effects of Long-Duration Administration of 1% Isoflurane on Resting Cerebral Blood Flow and Default Mode Network in Macaque Monkeys

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Abstract

Isoflurane is an inhalational anesthetic that is widely used in medical procedures or biomedical research. The duration of anesthesia administration varies from minutes to hours. It is known that isoflurane has dose-dependent effects on brain functionality and physiology, and long-duration anesthesia administration could cause neurocognitive decline in animals and humans. However, the duration effect of isoflurane on the brain physiology and functionality still remains poorly understood. In the present study, cerebral blood flow (CBF) and functional connectivity of adult rhesus monkeys (maintained with 1% isoflurane for 4 h) were examined by using magnetic resonance imaging. The results demonstrate that long-duration isoflurane exposure could result in CBF reduction in most brain areas and functional connectivity decrease in the dominant default-mode network. This study reveals the anesthetic duration effects in the central nervous system of anesthetized subjects and suggests that such duration effects should be considered in examining the brain function of anesthetized animals or humans with contemporary neuroimaging approaches.

Keywords: anesthesia; cerebral blood flow; default-mode network; non-human primate; pseudo continuous arterial spin-labeling

Introduction

Isoflurane is an inhalational anesthetic that is widely used for general anesthesia in medical procedures and biomedical studies. In particular, it is commonly utilized for sedation purpose in various *in vivo* neuroimaging (such as magnetic resonance imaging [MRI] and positron emission tomography [PET]) examinations of animals or uncooperative patients. The duration of anesthesia administration varies from minutes to hours, depending on the requirement of each specific surgical procedure or experimental design. It can last up to 24 h in some clinical cases (Bomberg et al., 2016).

Previous studies have reported that repeated exposure to anesthesia in children is an important factor of learning disabilities (Flick et al., 2011). Also, it has been demonstrated that the duration of anesthesia is one of the risk factors for early postoperative cognitive dysfunction (Moller et al., 1998), and the duration of general anesthesia is associated with the risk of cell death in the developing brains (McCann and Soriano, 2012). Meanwhile, it has been reported that an increased risk for neurobehavioral disturbances correlates positively with the duration of anesthesia exposure (Block et al., 2012; Wilder et al., 2009). Animal studies have further demonstrated that spatial memory was impaired for 2 weeks after long-duration administration of isoflurane in aged rats (Culley et al., 2004) and isoflurane might cause brain cell death, neurocognitive decline in immature rats (Stratmann et al., 2010).

Cerebral blood flow (CBF) quantifies the blood supply to the brain, is highly auto-regulated to maintain normal brain functionality, and closely coupled to brain metabolism. CBF decrease has been seen in normal aging (Chen et al., 2011) and is associated with cognitive decline (Birdsill et al., 2013; Hirsch et al., 1997; Jagust et al., 1992; Poels et al., 2008). CBF and cerebral metabolism can be altered by sedatives, analgesics, and anesthetics dramatically and the effects vary substantially from one drug to another (Van Aken and Van Hemelrijck, 1991; Werner, 1995). Isoflurane has strong dose-dependent effects on CBF as demonstrated in prior studies of anesthetized macaques or baboons (Li et al., 2013, 2014; Van Aken et al., 1986). The duration effects of isoflurane on CBF have also been previously examined in anesthetized dogs (McPherson and Traystman, 1988) and goats (Albrecht et al., 1983), and monkeys (McPherson et al., 1994), but the conclusions remain controversial.

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Resting-state functional MRI (rsfMRI) is a robust tool that is used to examine the intrinsic functional connectivity, including the default mode network (DMN), in the brains of awake human subjects or anesthetized animals (Biswal et al., 1997; Deshpande et al., 2011; Keilholz et al., 2013; Koch et al., 2012; Liu et al., 2011; Meng et al., 2016; Murnane et al., 2015; Santhanam et al., 2011; Vincent et al., 2007; Wu et al., 2015; Zhao et al., 2008), and it is associated with CBF (Jann et al., 2015; Li et al., 2012). Therefore, we hypothesized that the CBF and brain connectivity might be affected by the duration of anesthesia administration in subjects maintained with isoflurane.

Non-human primates (NHPs) resemble most aspects of humans and are widely used in preclinical studies and various neuroscience investigations. Furthermore, previous studies have confirmed the existence of a DMN in monkeys and demonstrated the topological similarities of the monkey's DMN with the human's DMN (Mantini et al., 2011). One to 3% isoflurane (up to 5% for induction) is usually used for general anesthesia of NHPs or other animals. As isoflurane has a strong dose-dependent suppression effect on neural activation in the brain, a lower dosage of isoflurane is usually applied for light anesthesia in various neuroimaging studies of NHPs. In the present study, adult rhesus monkeys were used to examine the long-duration effects of 1% isoflurane on CBF and DMN by using the continuous arterial spin-labeling perfusion MRI and rsfMRI techniques.

Materials and Methods

Animal preparation

Adult female rhesus monkeys ($n = 5$, 7–11 years old) were employed in the present study. The animals were initially anesthetized with Telazol (5 mg/kg, i.m.), and then switched to $\sim$1% isoflurane mixed with 100% oxygen by using an isoflurane vaporizer (Patterson Veterinary, Devens, MA). The anesthetized and spontaneously breathing animals were intubated and immobilized with a homemade head holder and placed in the ''supine'' position during MRI scanning for about 4 h.

Respiration rate, isoflurane concentration (end-tidal), and Et-CO_2 were continuously monitored with a PROCARE Monitor B40 anesthesia machine (GE Healthcare, Milwaukee, WI); heart rate and O_2 saturation with a Nonin pulse oximeter (Nonin medical, Plymouth, MN); blood pressure by a Surgivet V6000 (Smiths Medical PM, Waukesha, WI); and body temperature with a Digi-Sense Temperature controller (Cole-Parmer, IL), respectively. Lactated ringer's solution was administered intravenously to prevent dehydration during scanning. The physiological parameters were recorded and maintained in normal ranges (Li et al., 2013). All procedures followed the protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Emory University in accordance with the NIH Guide for Care and Use of Laboratory Animals.

Data acquisition

MRI data collection was conducted by using a Siemens 3T Trio scanner (Siemens Medical Solutions, Malvern, PA) with an 8-channel phased-array volume coil (Invivo, Inc., FL). rsfMRI was conducted by using a gradient-echo Echo Planar Imaging (EPI) sequence (time of repetition [TR]/time of echo [TE] = 2060/25 ms, 34 contiguous slices to cover the whole brain, 430 volumes, field of view [FOV] = 96 × 96 mm, spatial resolution = 1.5 × 1.5 × 1.5 mm^3). The single-shot EPI was applied for CBF measurement with the pseudo-continuous arterial spin-labeling MRI technique (Li et al., 2014; Wu et al., 2007). The MRI parameters were as follows: TR/TE = 3830/21 ms, FOV = 96 × 96 mm, data matrix = 64 × 64, 16 slices with slice thickness = 1.5 mm, labeling offset = 55 mm, post-labeling delay = 0.8 sec, and labeling duration = 2.0 sec. Eighty pairs of control and labeling images with six repetitions were acquired. CBF and rsfMRI data collection started $\sim$15 min after the animal was moved into the scanner and then repeated $\sim$3.5 h later.

T_2-weighted images were acquired by using fast spin-echo sequences with TR/TE = 5040/125 ms, FOV = 96 × 96 mm, matrix = 128 × 128, slice thickness = 1.5 mm, 16 slices, and 2 averages. High-resolution structural T_1-weighed images were acquired by using a 3D magnetization-prepared rapid gradient echo sequence with the generalized autocalibrating partial parallel acquisition ($R = 2$) (TR/TE = 3000/3.51 ms, FOV = 96 × 96 mm, spatial resolution = 0.5 × 0.5 × 0.5 mm^3). The field map (TR/TE1/TE2 = 1100/5.36/7.82 ms, FOV = 96 × 96 mm) was obtained for each animal. The T_1, T_2-weighted MRI and field map and Diffusion Tensor Imaging (not reported in the present study) scans were conducted after the first set of rsfMRI, and CBF data were collected. Each scanning session lasted about 4 h.

Image data processing and analysis

CBF data analyses were performed by using home-built Matlab scripts (MathWorks, MA) and Stimulate software (www.cmrr.umn.edu/stimulate) (Li et al., 2013). The bilateral caudate, putamen, globus pallidus, anterior cingulated cortex (ACC), posterior cingulate cortex (PCC), thalamus, cerebellum, white matter (WM), grey matter (GM), and cortical and subcortical cortex were selected as regions of interest (ROIs) to acquire averaged CBF values (Fig. 1). Paired *t*-test was performed to analyze the CBF differences statistically.

The rsfMRI data were preprocessed for image distortion correction by using the FSL software (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FUGUE). Slice timing correction, rigid body registration, regressing out of WM and cerebrospinal fluid time series, temporal filtering with 0.009–0.0237 Hz band-pass, and spatial smoothing with 2.5-mm full width at half maximum Gaussian blur were performed by using a script of AFNI (http://afni.nimh.nih.gov) (Li et al., 2008). Anatomical ROI for the whole PCC, ACC, and dorsal/media prefrontal cortex (DMPFC) were selected by using the graphical user interface of AFNI software (https://afni.nimh.nih.gov) with the monkey brain atlas (Saleem and Logothetis, 2007) and anatomical T_1-weighted images as references. PCC was used as the seed region for the seed-based functional connectivity data analysis.

Z transformation was applied to the individual correlation maps to show normalized correlation maps. The averaged *z* values of connectivity between PCC and ACC or DMPFC were examined for statistical differences.

All statistical analyses were conducted in SPSS 21.0. *p* Values less than 0.05 were considered statistically significant.

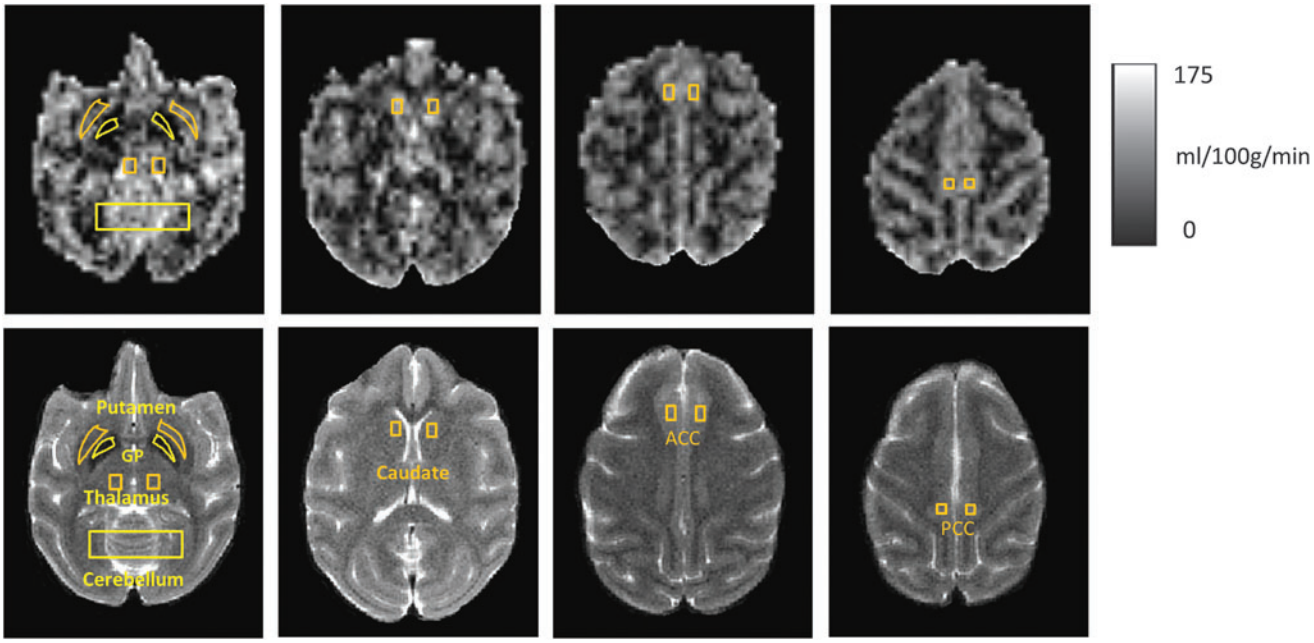

FIG. 1. CBF maps of an adult macaque monkey acquired with pCASL at 3T. ROIs are illustrated on the CBF maps (top) and corresponding T_2-weighted structural images (bottom). ACC, anterior cingulated cortex; CBF, cerebral blood flow; GP, globus pallidus; PCC, posterior cingulate cortex; pCASL, pseudo-continuous arterial spin-labeling; ROIs, regions of interest.

Results

Mean arterial pressure (MAP) and heart rates were not showing significant changes during the 4-h anesthesia, even though decreasing trends were seen at 3 h post isoflurane administration (Fig. 2a, b). Isoflurane dosage was kept stable during each 4 h scanning session, and no significant changes of end-tidal concentration were seen (Fig. 2c).

The CBF changes in different brain regions during the 4-h isoflurane administration are illustrated in Figure 3. CBF of each ROI at the end time point was normalized to that at the starting time point in each scan to minimize the inter-subject variation. CBF decreased significantly in thalamus (25.6% ± 8.9%, $p < 0.01$), ACC (9.5% ± 7.3%, $p < 0.05$), PCC (12.5% ± 7.7%, $p < 0.01$), and cerebellum (16.2% ± 7.5%, $p < 0.05$) (Fig. 3). In contrast, CBF significantly increased in putamen (15.1% ± 25.9%, $p < 0.05$) (Fig. 3). A significant CBF decrease was seen in both cortical (10.1% ± 3.2%, $p < 0.01$) and subcortical regions (14.2% ± 6.9%, $p < 0.05$) (Fig. 4 left). Also, CBF in both GM (10.8% ± 3.5%, $p < 0.01$) and WM (16.1% ± 9.7%, $p < 0.05$) (calculated with five consecutive slices) reduced significantly (Fig. 4 right).

The duration effect of isoflurane on the default mode network of an adult monkey is illustrated in Figure 5. The rsfMRI results demonstrate that the correlation degree (z score) of PCC with either DMPFC or ACC obviously decreased after 3.5-h isoflurane administration. The decrease of the PCC-DMPFC connectivity was nearly significant statistically ($p = 0.08$), and that in PCC-ACC was significant ($p < 0.01$) (Table 1).

Discussion

This study examined the changes of CBF and DMN in the adult macaque brain during long-duration administration of isoflurane under 1% maintenance dosage. The results demonstrate

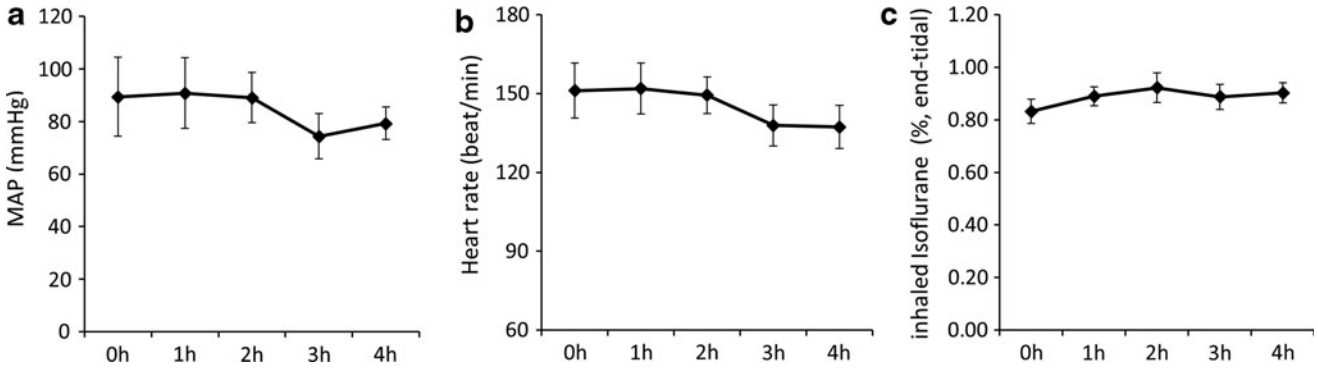

FIG. 2. MAP **(a)**, heart rate **(b)** and end-tidal concentration changes of isoflurane **(c)** of rhesus monkeys during 4-h 1% isoflurane administration. Data are reported as mean ± SEM, *$p < 0.05$ versus 0.5 h isoflurane (baseline). MAP, mean arterial pressure.

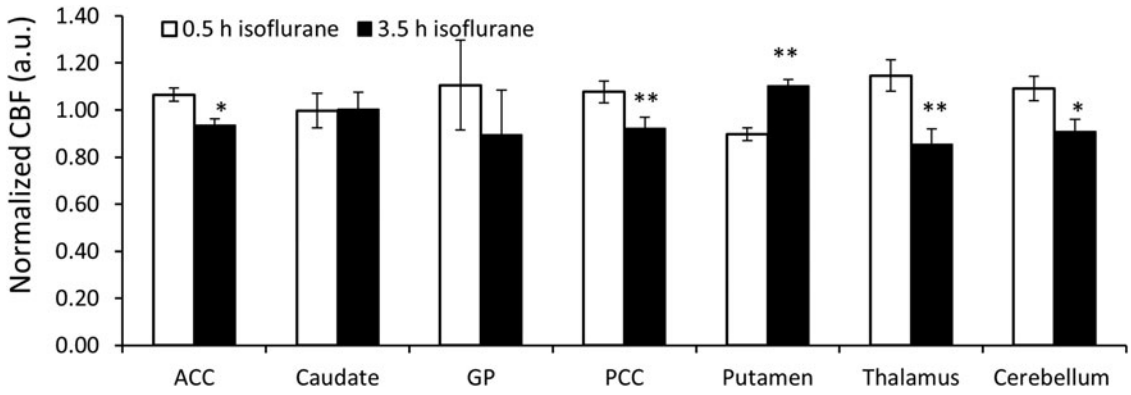

FIG. 3. CBF changes in selected ROIs of normal macaque monkeys maintained with 1% isoflurane administration ($n = 5$); error bar indicate standard deviation. $^{**}p < 0.01$; $^{*}p < 0.05$ versus 0.5 h. h, hour.

significant CBF reduction in most brain regions after 3-h isoflurane exposure. In addition, evident deactivation in the PCC dominant DMN was observed. These findings reveal the evident duration effect of isoflurane administration on the brain functionality of anesthetized macaques.

The administration duration of anesthetics in general anesthesia varies from minutes to hours, and long duration is usually applied in invasive surgical operation or non-surgical procedure in patients and animals by using intravenous sedatives or inhaled anesthetics such as isoflurane. Also, it is a popular procedure in neuroimaging studies of animals or children, uncooperative patients to minimize the motion artifacts. In fact, 1.0% isoflurane is usually applied for sedation purpose in non-surgical procedures such as neuroimaging examinations of animals or patients for a few hours and beyond (Satoh et al., 2002; Stevens et al., 1993; Zhang et al., 2015).

It has been demonstrated that isoflurane has dose-dependent effects on physiology (such as CBF, blood pressure, heart rate, cerebral metabolism, systemic vascular resistance) of monkeys and humans (Kato et al., 1992; Li et al., 2013, 2014; Lorenz et al., 2001; Reinstrup et al., 1995). In addition, neurobehavioral disturbances and cognitive decline have been reported in patients post-anesthesia (Block et al., 2012; Flick et al., 2011; Sprung et al., 2012; Wilder et al., 2009). As the cerebral physiology closely correlates with the neural activation and cognitive performance in the brain, it is important to examine the duration effects of isoflurane on CBF and brain functionality when subjects are anesthetized.

Long-duration effect of isoflurane on CBF

Isoflurane increases CBF in a dose-dependent manner by producing vasodilation through the ATP-sensitive K^+ channel activation (Fujita et al., 2006; Iida et al., 1998). The administration of isoflurane can last for hours in animals and patients. However, the effect of long-duration administration of isoflurane on CBF remains controversial. Our current CBF results are in agreement with prior preclinical studies in anesthetized dogs (McPherson and Traystman, 1988) and goats (Albrecht et al., 1983). Also, the MAP and heart rates of monkeys in the present study were maintained in the normal ranges during the entire study of each session (although a decreasing tendency in MAP or heart rate was seen after 2 h of isoflurane exposure) (Fig. 2), in agreement with those seen in a previous study in rats under 1 minimum alveolar concentration (MAC) isoflurane for 4 h (Stratmann et al., 2010) and dogs (1.4% isoflurane from 2 to 6 h) (Brian et al., 1990).

Isoflurane duration-induced CBF decrease was not observed in prior studies of adults with 1.5 MAC (Kuroda et al., 1996, 1997), children with 1 MAC (Bisonnette and Leon, 1992) during prolonged administration of isoflurane

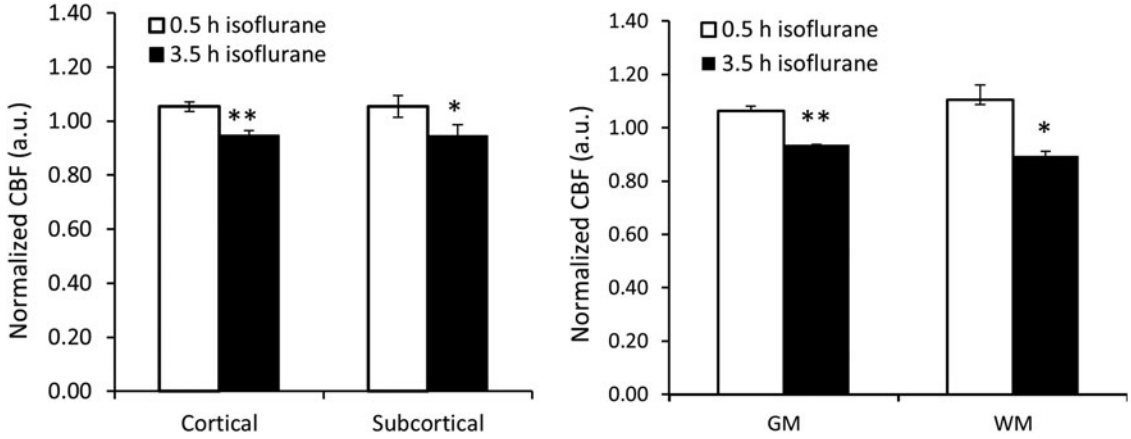

FIG. 4. CBF changes in selected cortical and subcortical regions of macaque monkeys ($n = 5$) maintained with 1% isoflurane administration; error bar indicates standard deviation. $^{**}p < 0.01$; $^{*}p < 0.05$ versus 0.5 h.

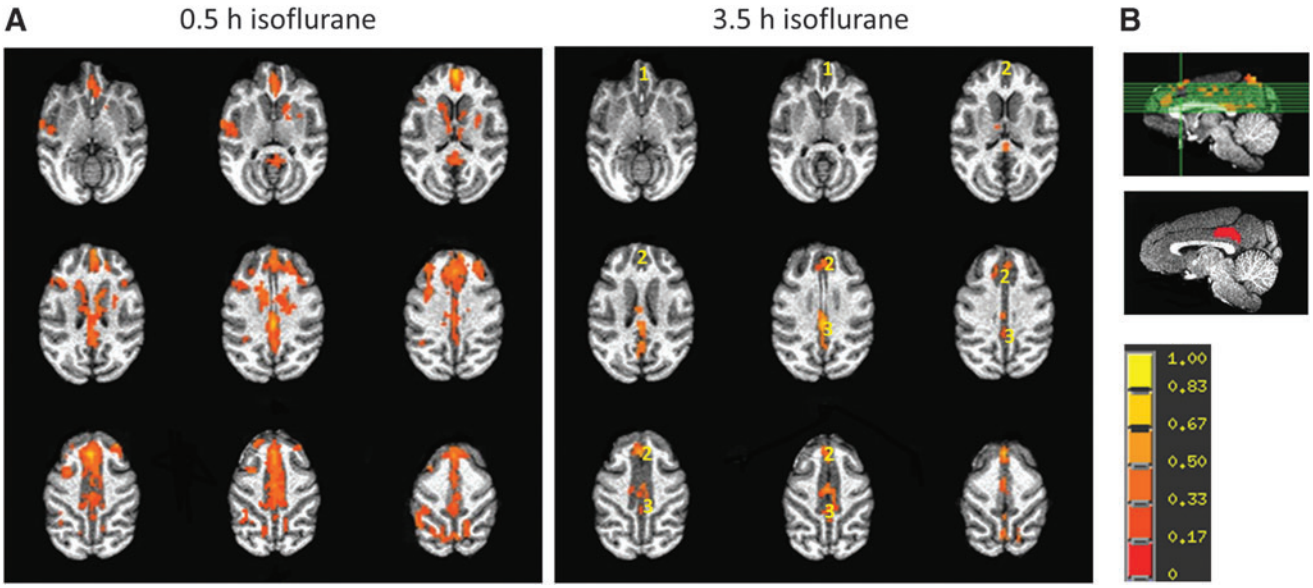

FIG. 5. The representative changes of DMN in an adult rhesus monkey maintained with 1% isoflurane exposure. **(A)** The activation axial maps were generated with PCC as a seed. The color bar represents the magnitude of the regression coefficient (z score threshold $p < 1 \times 10^{-33}$, cluster threshold = 376 mm^3/overall). **(B)** The slice locations are shown on a sagittal view of the monkey brain. The seed with PCC is highlighted as red. ROIs: 1, mPFC; 2, ACC; 3, PCC. DMN, default mode network; mPFC, medial prefrontal cortex.

(over 3 h), and dogs with 1% isoflurane for 3–4 h (Roald et al., 1989). In particular, increased CBF was seen in the forebrain and hindbrain of cynomolgus monkeys with 1 MAC isoflurane for 4 h reported by McPherson et al. (1994), different from the present findings. By comparing the experimental methods and materials between these primate and adult studies, such differences in the CBF findings are very likely due to the different experimental setting and procedures. (1) Mechanical ventilation (with pancuronium bromide administered) was applied in McPherson et al.'s (1994) primate study and Kuroda et al.'s (1996, 1997) studies in surgical patients. In addition, Phenylephrine was used in those patients to maintain cerebral perfusion pressure.

Previous reports also have indicated that mechanical ventilation might alter CBF (Baenziger et al., 1994; Raichle et al., 1970). In contrast, our monkeys were breathing spontaneously during each scanning session. (2) Initial CBF baseline might be different due to the usage of different induction drugs. Thiopental could result in lower CBF in comparison with Telazol (Joshi et al., 2005). Therefore, the baseline CBF measured in the present study could be different from Mcpherson et al.'s study due to the difference of the induction drugs. (3) The isoflurane dosage in McPherson's study was 1 MAC, slightly higher than that used in the present study. The dose-dependent effects of isoflurane on regional CBF have been previously reported (Bisonnette and Leon, 1992; Li et al., 2013, 2014; Van Aken et al., 1986). Therefore, the duration effect of isoflurane on CBF may be dose dependent as well.

In consideration of all the approach differences in the two experiments, it is not unexpected to have different findings between the two studies. In particular, these inconsistent findings suggest CBF's sensitivity to the experimental setting and procedures, including induction drugs, anesthetic dosages and durations, ventilation status, and maintenance of physiology of the subjects.

Interestingly, CBF in putamen was found to be significantly increased in our study, different from those in cortical and other subcortical structures such as thalamus (Fig. 3). The opposite changes in fMRI and perfusion measures in putamen were also previously reported in a rat study (Mishra et al., 2011). More investigation is needed to address such neuronal and vascular activity alteration in putamen after long-duration exposure of isoflurane.

The suppression effect of isoflurane on neuronal activation might be a major contribution to the CBF reduction caused by the long-duration administration. It is known that isoflurane induces suppression effect to neuronal activities via exerting antagonistic actions on N-methyl-d-aspartate receptors and enhancing $GABA_A$ receptor-mediated functions (Brosnan, 2011; Dong et al., 2013; Harrison et al., 1993; Shelton and Nicholson, 2010). The suppression effect is dose dependent, as seen in the isoflurane-induced burst suppression pattern (Ferron et al., 2009). The anesthetic potency of isoflurane is highly correlated with the lipid bilayer partition (Smith et al., 1981). As a longer duration of anesthesia allows a

TABLE 1. THE z SCORE CHANGES IN THE NETWORK PCC-ACC AND PCC-DMPFC OF ADULT MONKEYS MAINTAINED WITH 1% ISOFLURANE

	PCC-ACC	*PCC-DMPFC*
0.5 h isoflurane (baseline)	0.67 ± 0.11	0.66 ± 0.10
3.5 h isoflurane	0.36 ± 0.13[a]	0.33 ± 0.15[b]

Data are reported as means ± SEM.
[a] $p < 0.01$.
[b] $p = 0.08$ versus baseline isoflurane.
ACC, anterior cingulated cortex; DMPFC, dorsal/medial prefrontal cortex; PCC, posterior cingulate cortex.

higher concentration of isoflurane in the lipid tissue of the nervous system, the neuronal activity might be decreasing progressively over the duration of anesthesia. Accordingly, CBF is reduced because of its close coupling with the brain metabolism in a normally functioning brain.

Long-duration effect of isoflurane on DMN of the brain

Previous studies have shown that the deactivation of DMN is related with cognitive decline (Hansen et al., 2014; Nelson et al., 2016; Onoda et al., 2012; Vidal-Pineiro et al., 2014). Vincent et al. (2010) demonstrated that DMN in human also exists in macaques under isoflurane (0.8–1.5%) in which PCC is shown as a dominant region in DMN. The resemblance of PCC correlation maps between the monkey and human suggests that many elements of the DMN system may be conserved across primate species.

In the present study, the network of PCC to its association areas was examined to investigate how DMN in monkeys would be affected during long-term isoflurane exposure. ACC is often considered a sub-section of the medial PFC (mPFC) in monkeys (Bissonette et al., 2013; Gabbott and Bacon, 1996). Dorsal PFC in rhesus macaques is comparable to the area nine in Buckner's report and a part of mPFC (Buckner et al., 2008). Therefore, both ACC and DMPFC were selected as hubs of PCC-dominated DMN in data analysis of the present study. Our result indicated that PCC-ACC associations decreased significantly ($p < 0.01$), and PCC-DMPFC associations decreased nearly significantly ($p = 0.08$, most likely due to the sample size) after 3.5 h of isoflurane exposure (Table 1 and Fig. 5). Probably, this is because anesthetic results in breakdown of large-scale synchronization between brain regions (Lu et al., 2007; Vincent et al., 2007).

Also, due to the fact that anesthetic causes a global loss of functional segregation/specialization, regional specificity is decreased or becoming more homogeneously connected to each other (Vincent et al., 2007). Meanwhile, because of the progressive increase of isoflurane concentration in the lipid tissue of the nervous system, longer silent periods or decreased neuronal activity could result in the suppress-burst neuronal activity pattern of isoflurane (Ferron et al., 2009). Consequently, the low-frequency fluctuation (the basis of connectivity analysis) can decrease, resulting in reduced functional connectivity after prolonged administration of isoflurane.

CBF is tightly coupled with neuronal activity and brain metabolism, and resting-state neuronal activity and energetics uses about 80% of brain energy (Shulman et al., 2004). Positive correlation between resting CBF (or brain metabolism) and brain connectivity in several regions (including PCC) was previously reported in arterial spin-labeling-perfusion (Zou et al., 2009) and fluorodeoxyglucose positron emission tomography studies (Di and Biswal, 2012). In addition, a previous study (Li et al., 2012) showed that the resting spontaneous brain activity varied with regional CBF in different structures and positive correlations were demonstrated between the findings with CBF and PCC functional connectivity in DMN.

A similar finding was also reported in another study of humans (Tak et al., 2015). Our finding of decrease in both DMN and CBF of PCC is consistent with that reported in prior studies. In addition, CBF and brain connectivity decrease is usually seen in subjects with cognitive decline (Onoda et al., 2012; Alosco et al., 2013). Our results suggest that the duration effects of isoflurane administration might be associated with the post-anesthesia cognitive decline seen in preclinical and clinical studies. Further behavior studies are needed to validate the relationship.

Isoflurane is a commonly used anesthetic in neuroimaging studies of animals such as rodents and NHPs, serving to eliminate motion artifacts, physiological stress, and training requirements (Hutchison et al., 2014). The animals can be maintained under anesthesia for 3–7 h or even longer. Our results suggest that the acquired neural activation information from anesthetized animals can be biased due to the duration effect of anesthesia. Therefore, CBF or functional MRI measures should be acquired sooner for better sensitivity in anesthetized subjects or the duration-dependent bias should be considered in data collection to avoid or minimize the long-duration effects. In addition, the duration effect of isoflurane on spontaneous functional connectivity can be varying substantially in different networks comprising PCC, ACC, and motor/visual cortex (Supplementary Data; Supplementary Data are available online at www.liebertpub.com/brain), suggesting that such an effect is heterogeneous across different brain networks.

In conclusion, the duration-dependent effect of isoflurane on CBF and brain functional connectivity can cause an unneglectable impact on the brain functionality. In addition, the findings suggest that such an effect can exist in other anesthetics as well and should be considered in various neuroimaging studies of anesthetized subjects.

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Author Disclosure Statement

No competing financial interests exist.

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