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Evaluation of prolonged administration of isoflurane on cerebral blood flow and default mode network in macaque monkeys anesthetized with different maintenance doses

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Abstract

Object—Isoflurane is a commonly used volatile anesthetic agent in clinical anesthesia and biomedical researches. Prior study suggested the cerebral blood flow (CBF) and default mode network (DMN) could be changed after prolonged administration of isoflurane. The normal maintenance doses of isoflurane may vary from light (~0.75%) to deep (~1.5 or 2%) anesthesia. However, it is not clear how the duration effects are affected by the altered doses. The present study is aimed to examine if the duration effects are affected when isoflurane concentration is altered within normal maintenance doses.

Materials and Methods—Adult rhesus monkeys (n=5, 8–12 years old, 8–10 kg) were anesthetized and maintained at isoflurane levels 0.89±0.03%, 1.05±0.12%, or 1.19±0.08 %. CBF and DMN of monkeys were examined using arterial spin-labeling perfusion and resting state functional MRI techniques.

Results—the functional connectivity (FC) in the dominant DMN (posterior cingulate cortex (PCC) to anterior cingulated cortex (ACC) or media prefrontal cortex (MPFC)) decreased substantially and similarly during 4-hour administration of isoflurane at any given maintenance dosage. CBF changes varied with isoflurane dosage. At the low dose (~0.89 %), CBF decreased in most brain regions. In contrast, no obvious changes was seen in those regions (except for the subcortex) when higher doses of isoflurane were applied.

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Conflict of interest statement
The authors have no conflict of interest to claim.

Compliance with Ethical Standards
All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the studies 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.
Conclusion—FC in DMN was reduced substantially during prolonged administration of isoflurane. The FC reduction was not varying significantly within altered maintenance doses of isoflurane but the duration effect on CBF was dose-dependent. Such duration effects of isoflurane administration on DMN and CBF should be considered in the interpretation of the outcome and further studies in related neuroimaging studies of anesthetized subjects.

Keywords
Anesthesia; CBF; non-human primate; pseudo continuous arterial-spin-labeling (pCASL); functional MRI; default-mode network (DMN); dosage effect

Introduction
Isoflurane is an inhalational anesthetic widely used in medical procedures and biomedical researches of human and animals. In particular, it is extensively used in various neuroimaging studies of animal models since it allows for rapid induction and recovery and flexible control of anesthetic doses and duration of administration and is minimally metabolized by the liver and less toxic to the animal’s metabolism compared to injectable anesthetics [1, 2]. Isoflurane is used in young children as well [3]. Isoflurane has showed profound effects on the brain physiology and functionality. Evident dose-dependent suppression on the brain functionality [4, 5] and biphasic responses on cerebral blood flow (CBF) such as vasoconstriction effect on low dose (~0.5% or lower) and vasodilatation on high dose (~1% or higher) have been demonstrated in a previous report [6].

In preclinical and clinical practice, the duration of anesthesia can vary substantially from minutes to hours. The long duration administration of isoflurane for a few hours has been usually seen in preclinical neuroimaging studies using MRI [4, 7, 8]. Sometimes it can last even much longer as reported in intensive care unit (ICU) patients [9]. Prior study indicated that the default mode network (DMN) and CBF could be affected by the duration of 1% isoflurane administration [10]. As CBF is strongly affected by the isoflurane concentration in anesthetized subjects [11–13], we hypothesized the duration effect of isoflurane on CBF and DMN could be dose-dependent.

Non-human primates (NHPs) resemble most aspects of humans in physiology and neural anatomy and are widely used in various neuroscience studies and translational medical researches [14]. In particular, the old world monkeys have much larger brain volumes (~100ml) compared to rodents and allow for more detailed brain structures and neuronal functions being evaluated using high-field MRI techniques. Resting state functional MRI (rsfMRI) has been demonstrated to be a robust approach to evaluate functional connectivity in human [15, 16] and animal models [17–21] and anesthetized macaques of either healthy [4, 22–24] or disease models [25]. The arterial spin labeling (ASL) perfusion MRI allows to examine the dynamic changes of CBF with high spatial and temporal resolution [12, 26, 27]. In the present study, adult rhesus monkeys were used to examine the duration effects of isoflurane on CBF and DMN of macaques under different maintenance doses by using the pCASL perfusion MRI and rsfMRI respectively.
Materials and Methods

Animal preparation

Adult female rhesus monkeys (n=5, 8–12 years old) were employed in the present study. The animals were initially anesthetized with Telazol (5mg/kg, i.m.) [12] then intubated and switched to isoflurane mixed with 100% oxygen. The animals were spontaneously breathing and immobilized with a home-made head holder and placed in the "supine" position during MRI scanning for about 4 hours [13]. End-tidal isoflurane concentration, respiration rate and Et-CO\textsubscript{2} were continuously monitored with a PROCARE B40 Monitor (GE Healthcare, Milwaukee, WI). Heart rate (HR) and O\textsubscript{2} saturation were monitored with a pulse oximeter (Nonin medical, Plymouth, MN); blood pressure and body temperature were monitored with a capnometer (Surgivet V6000, Smiths Medical PM, Waukesha WI) and a Digi-Sense Temperature controller (Cole-Parmer, IL, USA) respectively. An intravenous line was placed for administrating Lactated ringer’s solution to prevent the animal from dehydration.

Three dosages of isoflurane (0.89±0.03, 1.05±0.12, and 1.19±0.08 %, mean ± standard deviation (SD), end-tidal concentration) were applied. In each scanning session, one dose was randomly selected and maintained to keep the animal under anesthesia. The animal was given at least two weeks for recovery after every 4-hour long MRI scan.

Data Acquisition

MRI data collection started ~15 minutes after animals were moved into the scanner (Siemens 3T TIM Trio, Siemens Healthcare, Malvern PA) with the high-resolution 8-channel phased-array volume coil. Functional MR images of whole-brain volumes were acquired with 34 contiguous slices (TR/TE=2190ms/25ms), 430 volumes per scan, field of view (FOV) = 96 mm × 96 mm, spatial resolution= 1.5×1.5×1.5mm\textsuperscript{3}, scanning duration = 10 minutes. The single-shot Echo Planar Imaging (EPI) was applied for CBF measurement by using the pCASL MRI technique [12, 28]. The MRI parameters were: TR/TE = 3830ms /21 ms, FOV= 96 mm × 96 mm, data matrix = 64 × 64, 16 slices with slice thickness = 1.5 mm, labeling-offset = 55 mm, post-labeling delay = 0.8 s, labeling duration = 2.0 s. 80 pairs of control and labeling images with 6 repetitions were acquired. The rsfMRI and CBF data were collected firstly (the 0.5-hour time point) and then re-collected (with CBF measured first) about 3 hours later (the 3.5-hour time point) in each scan session.

T\textsubscript{2}-weighted images were acquired with the same slice positions as those in CBF measures by using the fast spin-echo sequence with TR/TE= 5900ms/125ms, FOV = 96 mm × 96 mm, matrix = 128 × 128, slice thickness = 1.5mm, 16 slices, 2 averages. High resolution structural T\textsubscript{1}-weighed images were acquired with a 3D magnetization prepared rapid gradient echo (MPRAGE) sequence with GRAPPA (R=2) (TR/TE = 2300ms/4 ms, FOV = 96 mm × 96 mm, spatial resolution = 0.5x0.5x0.5mm\textsuperscript{3}). The field map (MRI parameters: TR/TE1/TE2= 1100ms/5.36ms/7.82ms, FOV = 96 mm × 96 mm) was obtained for each animal. The T\textsubscript{1}, T\textsubscript{2}, and field map scans were conducted immediately after the first sets of CBF and rsfMRI data were collected. Each study session lasted about 4 hours.
Image data processing and analysis

CBF data analyses were performed using home-built Matlab scripts (MathWorks, MA) to get CBF maps. Stimulate software (http://www.cmrr.umn.edu/stimulate) was used to visualize CBF maps and define the regions of interest (ROIs) by referring to T2-weighted images. The CBF maps were shown together with corresponding T2-weighted anatomical images in Fig. 1. The bilateral caudate, putamen, globus pallidus(GP), anterior cingulated cortex (ACC), posterior cingulate cortex (PCC), thalamus, cerebellum, white matter (WM), grey matter (GM), cortical and subcortical cortex were selected as ROIs (Fig 1). CBF at the end time point (~3.5 hours post isoflurane administration) of each scan session was normalized to that at the start time point (~0.5 hours post isoflurane administration). Paired t-test was performed to analyze the CBF differences statistically.

The rsfMRI data were preprocessed firstly by applying field map for image distortion correction with FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FUGUE). Slice timing correction, rigid body registration, regressing out of white matter and cerebrospinal fluid (CSF) time series with a general linear model, temporal filtering with 0.009 Hz – 0.0237 Hz band-pass, spatial smooth with 2.5-mm full width at half maximum Gaussian blur were performed using a script of AFNI (http://afni.nimh.nih.gov) [29]. ROIs including the whole PCC, ACC and MPFC (media prefrontal cortex) were selected using the graphical user interface of AFNI software with anatomical T1-weighted images and the monkey brain MRI atlas as reference [30]. The averaged time courses of rsfMRI signal in the whole PCC were used as the seed for calculating its correlation with the voxels in other brain regions. Z transformation was applied to the individual correlation maps to illustrate normalized correlation maps. The averaged z values of connectivity between PCC and ACC or MPFC were examined for statistical differences.

Repeated ANOVA was also performed to detect the temporal changes in mean arterial pressure (MAP), HR and isoflurane dosages (Fig 2), and the duration effects of isoflurane on CBF and DMN in selected brain regions at different doses (Fig. 3). All statistical analyses were performed with SPSS 22.0. P-values less than 0.05 were considered statistically significant.

Results

Mean arterial pressure (MAP) (Fig. 2 A, D and G or left column) and heart rates (Fig. 2B, E and H or middle column) of the animals were not showing any significant changes during the 4-hour anesthesia administration. Inhaled isoflurane dosage (in %) showed increasing tendency during the 4 hour scan (Fig. 2 C, F and I or right column).

The regional CBF changes after ~3.5 hour administration of isoflurane with different dosages (~0.89, ~1.05, ~1.19 %) are illustrated (Fig. 3). Initial CBF (~0.5 hours post isoflurane administration) from the 3 dosages were normalized as baseline. After the 0.89% isoflurane exposure, CBF decreased significantly in ACC (9.5±7.3%, p<0.05), PCC (12.5±7.7%, p<0.01), thalamus (25.6±8.9%, p<0.01), and cerebellum (16.2±7.5%, p<0.05) (Fig 3 F, G, D, E). In contrast, no obvious CBF change was seen in those regions when 1.05 or 1.19 % isoflurane was applied. However, CBF in caudate significantly increased
22.7±7.7% (p<0.01) with 1.05% of isoflurane and 35.4±18.2% (p<0.01) with 1.19% isoflurane respectively, compared to either the baseline CBF (Fig. 3 B). Also, CBF of putamen increased significantly 23.6±6.0% (p<0.01) with 0.89%, 41.3±24.8% (p<0.01) with 1.05 %, and 35.5±18.3% (p<0.05) with 1.19% isoflurane respectively, compared to the baseline at each scan (Fig. 3 C).

Significant CBF reduction in both cortical (by 10.1±3.2%, p<0.01) and subcortical regions (by 14.2±7.0%, p<0.05) were seen with 0.89% isoflurane (Fig 3 J, K). However, CBF in the subcortical regions increased 16.0±8.7% (p<0.05) with 1.05% isoflurane, 22.3±15.7% (p<0.05) with 1.19% isoflurane, respectively (Fig. 3 K). No significant CBF change was seen in cortical regions although increase trend was showed in animals with 1.05 or 1.19% isoflurane, compared to the baseline (Fig. 3 J).

CBF in grey matter and white matter decreased by 10.8±3.5% (p<0.01) and 16.1±9.7% (p<0.05) at 0.89% isoflurane, respectively (Fig. 3 H and I). No obvious CBF changes in both grey matter and white matter were observed with the 1.05% and 1.19% isoflurane during the 4-hour anesthesia compared to the baseline (Fig. 3 H and I).

The duration effect of isoflurane administration on the DMN of monkeys look similar with all given doses. The representative changes of the DMN of the monkeys under 1.05% isoflurane is illustrated in Fig 4. The z score changes in PCC-ACC and PCC-MPFC of macaque brains during 4-hour isoflurane exposure at 0.89, 1.05, and 1.19 % are tabulated in Table 1. It is seen that the normalized correlation degree (z score) of PCC with either MPFC or ACC was obviously decreased after ~3.5 hours isoflurane administration, and the decrease of the PCC-ACC connectivity was substantial and significant in all animals with 0.89, 1.05, or 1.19 % isoflurane (p < 0.05) (see Table 1).

**Discussion**

Animal neuroimaging studies are widely conducted under isoflurane anesthesia with the maintenance dose varying from %0.75 (light anesthesia) to %1.5 or more (deep anesthesia). The experiments can last for hours. However it remains poorly understood how the neurophysiology could be affected by the duration of anesthesia. Our results demonstrated significant decrease of PCC-ACC connectivity during the 4-hour duration of isoflurane administration with any given doses. Meanwhile, the effects of prolonged isoflurane administration on CBF were dose- and region-dependent, suggesting the neurophysiology and functional connectivity would be affected by both the anesthetic dosage and duration of administration in anesthetized subjects.

As isoflurane allows for rapid induction and recovery, less toxic to the animal’s metabolism, and flexible control of the length and dose of administration, it is a very popular inhalation anesthetic used in neuroimaging studies of animals or uncooperative subjects like young children and critical patients. Like any other anesthetics, isoflurane has comprehensive effects on the brain physiology [6]. CBF increase has been seen generally in animals and humans due to the isoflurane vasodilation effect. In particular, such effect is dose-dependent and regional specific in the brain [11, 13]. The duration effects of isoflurane exposure on
CBF have been reported previously. By using transcranial Doppler (TCD) ultrasonography to measure time-averaged mean velocity in the middle cerebral artery (Vmca) of patients, Kuroda et al reported that CBF equivalent (CBF divided by cerebral metabolic rate for oxygen (CMRO2)) was maintained with minimal fluctuation during prolonged (3 hours) inhalation of 1.5 MAC isoflurane [31, 32] and 1.0 MAC for 4 hours [33] in humans.

Our present finding demonstrated no significant CBF changes in cortical areas when ~1.0–1.2% isoflurane was applied, in good agreement with the Kuroda et al’s reports in human. An early study by McPherson and colleagues reported increased CBF in forebrain and hindbrain regions of NHPs with 1 MAC isoflurane administration for 4 hours (measured with radiolabeled microsphere techniques)[11]. To some extent, our current result are consistent with the NHP study [11] because increased CBF was shown in subcortical region in our animals with 1.05 % or higher isoflurane (Fig. 3K) and increasing CBF tendency also was seen in cortical regions (Fig. 3J).

Prior macaque studies indicate CBF in subcortical are more susceptible to the dosage effect of isoflurane [12, 13]. The present study suggests the duration effect of isoflurane exposure on CBF in the subcortical region is also sensitive to the isoflurane dosages. CBF reduction was seen in most cortical and subcortical ROIs during the 4-hour administration of 0.89% isoflurane. In contrast, stable or mild increase CBF was observed in cortical ROIs (Fig. 3 F, G and J) under the 4-hour administration of ~1.05 or 1.19 % isoflurane. In particular, Significant CBF increase was seen in subcortical areas, especially in putamen and caudate (Fig. 3 B, C and K). Therefore, it can be inferred that the duration effects of isoflurane on CBF are pronounced in subcortical regions (Fig. 3K).

Vincent et al [22] have demonstrated that default-mode network (DMN) in human exists in macaques under isoflurane (0.8%–1.5%) in which PCC shows as a dominant region in DMN. Therefore PCC was chosen to examine the duration and dose dependent effect of isoflurane on DMN in the present report. The result showed that ACC-PCC correlation substantially and significantly decreased during the 4-hour administration of isoflurane (0.89 – 1.19%) (Table 1). Anesthesia suppresses the brain neuron activity substantially and functional connectivity patterns can be affected differently with different anesthetics. The suppression effect of isoflurane is probably because the anesthetic results in breakdown of large-scale synchronization between brain regions [4, 34] and a global loss of functional segregation/specialization, the regional specificity is decreased or becoming more homogeneously connected to each other [4] and increased randomness of spontaneous brain activity in global level[35, 36]. In consideration of the BOLD signal mechanism of FC and the progressive increase of isoflurane concentration in the lipid tissue of the nervous system during the prolonged experiment, longer silent periods or decreased neuronal activity could be resulted as seen in the suppress-burst neuronal activity pattern of isoflurane [37]. Consequently the low frequency fluctuation (the basis of connectivity analysis) activities are decreased, resulting in reduced FC after prolonged administration of isoflurane.

Isoflurane is known for vasodilation effects and strong neural suppression. Isoflurane dilates the arterioles in a dose-dependent manner as seen in prior dog study [38]. The suppressing effect on resting-state BOLD signal was reported in a prior rat study [39] and monkey study.
The suppression effect is dose dependent, as observed in the isoflurane-induced burst suppression pattern [37]. Also, the absolute strength of stationary FC significantly decreased in a monotonic manner when the isoflurane concentration increased [36].

Prior baboon study has demonstrated that the CBF and brain metabolite coupling was intact under low level of isoflurane. Brain metabolite decreased but CBF increased at 0.95% and 1.4% isoflurane, indicating impaired CBF and brain metabolism coupling at mean or deep level of isoflurane anesthesia [6]. Similar finding was also seen in a rat study in which the local coupling of CBF to cerebral glucose utilization was compromised with the increased concentration of isoflurane or desflurane [41]. In addition, region-dependent effects of isoflurane on CBF and brain metabolism were reported in a rat study with 2% isoflurane [42]. Higher CBF was reported in thalamus and basal ganglia (10–15%), and pons (7–10%) (as compared to cortex) in previous SPECT study of human with 1 MAC isoflurane [43], and subcortical regions are more vulnerable to high concentration of isoflurane in monkeys [12].

Regional increase of BOLD signal is normally associated with increased CBF due to the brain metabolite coupling mechanism. FC reflects the degree of correlation of spontaneous fluctuations in the BOLD signal across brain networks. Prior human study has demonstrated FC strength (or degree of correlation) is correlated with the regional increase in CBF-BOLD coupling strength [44]. The relationship of FC and CBF is spatially varying as seen in previous human studies [45, 46]. Obviously the relationship between CBF and FC (or BOLD) can be compromised by the dose-dependent effects of isoflurane on brain vascular function and neuronal activation, as demonstrated by previous findings of isoflurane on cerebral metabolites and CBF in human and animals.

As reported in the present study, subjects were anesthetized with light, mean, or deep level of isoflurane during each scan session. Consistent FC reduction was seen in any given dose of isoflurane. Such FC reduction at light level of isoflurane was in agreement with the corresponding CBF decrease caused by isoflurane induced reduction of neuronal activation in the brain, as expected. In contrast, abnormal CBF changes in subcortical regions were observed with higher isoflurane levels, indicating the impaired CBF-brain metabolite coupling under high-dose isoflurane administration, in agreement with prior findings in rats and baboons.

**Limitations**

Isoflurane maintenance doses can vary substantially in each study of preclinical or clinical practices. 1–2% isoflurane is usually used to sedate the animals (like rodents or NHPs) without combining with other anesthetics during neuroimaging procedure. Isoflurane concentration can be reduced by combining with other drugs like dexmedetomine to improve the fMRI sensitivity [20]. Deep anesthesia with 2–3% isoflurane is usually used in surgical procedures. In the present study, the effects of isoflurane within the normal maintenance doses were investigated. Although the given doses did not cover large ranges of isoflurane concentration, this study still provides information of neurophysiology of anesthetized subjects as these doses are most commonly utilized in various neuroimaging studies.
Also, the anesthesia levels were measured with end-tidal concentrations in the present study. Even though the isoflurane dosage of each animal was carefully administrated in order to maintain the animal in light, mean, or deep anesthesia levels during each scan session, the end-tidal concentration for each anesthesia level (light, mean, or deep) varied from animal to animal due to the individual response difference. As a result, the range for three levels of isoflurane (0.89±0.03, 1.05±0.12, and 1.19±0.08%) was overlapped slightly within the standard deviations. In practice, the depth of real anesthetic to each animal could be different due to individual response difference. 1.05% of isoflurane in one animal may have lower anesthetic effect than 0.89% of another animal. Therefore, the results of the isoflurane dosage effect might be biased when small sample size was used. A comprehensive study can be performed by using a larger sample size and EEG-based monitors to detect the actual depth of anesthesia in future studies [47].

Another limitation of the present study is the use of a single seed for rsfMRI data analysis. One of the main purposes of the present study is aimed to investigate the duration effects on the brain connectivity. The ACC-PCC network is well-known and selected as a representative to examine the anesthesia effects in the present study. Such effects may be different in different regions and more comprehensive default mode network analysis should be conducted in the future as well.

Neuroimaging has been increasingly and extensively used in preclinical and clinical studies for various neurological diseases [48–53]. In particular, almost all neuroimaging studies of animal models are conducted under anesthesia with the length of anesthesia lasted up to a few hours as seen in stroke studies [7, 54–56]. As functional neuroimaging approaches like fMRI and hemodynamic measurement are robust and sensitive tools to evaluate the development and progress of stroke injury [57–60], the duration related effects of isoflurane (or other anesthetics) on CBF and FC may compromise those measures and furthermore cause bias with the outcome of studies. Meanwhile, such duration effects may exist in other volatile agent like sevoflurane [61] and can be explored as well.

Conclusion

The present results demonstrate the effects of prolonged isoflurane administration on neurophysiology in macaque brains and suggest that CBF and functional connectivity could be altered with the prolonged administration of anesthesia even under normal maintenance doses. In addition, many neuroimaging studies of animal models could last for hours when using multiparameter MRI approach or examining progressive changes of the brain with neurologic diseases, such effects should be considered in the experimental design of functional neuroimaging and data interpretation of animals and humans maintained with isoflurane or other anesthetics.

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References


Highlights

- Prolonged isoflurane administration affects neurophysiology of anesthetized subjects
- The functional connectivity in default mode network reduced during prolonged isoflurane administration
- The duration effect of isoflurane on CBF is dose-dependent
Figure 1.
CBF maps of an adult macaque monkey acquired with the pseudo continuous ASL (pCASL) technique at 3T. Regions of Interest (ROIs) for data analysis are illustrated on the CBF maps (top) and corresponding T2-weighted structural images (bottom). GP: globus pallidus, ACC: anterior cingulated cortex, PCC: posterior cingulate cortex.
Figure 2.
MAP (A, D and G), heart rate (B, E and H) and isoflurane changes (C, F and I) during 4 hours 0.89 (top row), 1.05 (middle row), and 1.19 % (bottom row) isoflurane. Data are reported as means±SEM, *p<0.05 vs 0 h (baseline) isoflurane, MAP: mean arterial pressure, h: hour.
Figure 3.
CBF (normalized to baseline CBF at 0.5 hour) changes in selected regions during 4-hour administration of 0.89 %, 1.05 % and 1.19 % isoflurane in GP, caudate, ACC, PCC, thalamus, cerebellum, putamen, grey matter (GM), white matter (WM), cortical and subcortical areas in adult monkeys (n=5), error bar is indicated standard deviation, *, p<0.05 vs 0.5h (baseline) isoflurane; # p<0.05 vs 0.89 % isoflurane. h, hour.
Figure 4.
The representative changes of default mode network (DMN) in adult rhesus monkeys during 4-hour 1.05% isoflurane (A and B) exposure. The correlation axial maps were generated with PCC as seed. C) The slice locations shown on sagittal image. D), PCC as seed is illustrated on sagittal image. The color bar represents the magnitude of the regression coefficient ($z$-score threshold $p<3\times10^{-18}$ in A) and B), cluster threshold = 376 mm$^3$/overall). ROIs: 1): MPFC, medial prefrontal cortex; 2): ACC, anterior cingulate cortex; 3): PCC, posterior cingulate cortex.
Table 1
The z score changes in PCC-ACC and PCC-MPFC of macaque brains during 4-hour isoflurane exposure at 0.89 %, 1.05 %, and 1.19 %.

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<td>3.5h isoflurane</td>
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Data are reported as mean±SEM, ** vs baseline.