Effects of alfaxalone on cerebral blood flow and intrinsic neural activity of rhesus monkeys: a comparison study with ketamine

Chun-Xia Li, Doty Kempf, Leonard Howell, Xiaodong Zhang

Abstract

Objective: Alfaxalone has been used increasingly in biomedical research and medication of large animal anesthesia in recent years. However, its effects on the cerebral blood flow (CBF) physiology and intrinsic neuronal activity of anesthetized brains during neuroimaging study remain poorly understood.

Methods: Four healthy adult rhesus monkeys were anesthetized initially with alfaxalone (0.125mg/kg/min) or ketamine (1.6mg/kg/min) for 50 minutes, then administrated with 0.8% isoflurane for 60 minutes. Heart rates, breathing beats, and blood pressures were continuously monitored. CBF data was collected using pseudo-continuous arterial spin-labeling (pCASL) MRI technique and rsfMRI data were collected using single-shot EPI sequence for each anesthetic.

Results—Both the heart rates and mean arterial pressure (MAP) remained more stable during the alfaxalone infusion than those seen during ketamine administration. Alfaxalone reduced CBF substantially compared to ketamine anesthesia (grey matter, 65±22 vs 179±38 ml/100g/min, p<0.001; white matter, 14±7 vs 26±6 ml/100g/min, p<0.05); In addition, general CBF increase was seen in all selected cortical and subcortical regions of alfaxalone-pretreated monkey brains during isoflurane exposure, very different from the findings in isoflurane-exposed monkeys pretreated with ketamine. Also, alfaxalone showed similar suppression effects on functional connectivity of the monkey brain as ketamine.

Conclusion—Alfaxalone showed strong suppression effects on CBF of the monkey brain. The residual effect of alfaxalone on CBF of isoflurane-exposed brains was monotonous when used as induction agent for inhalational anesthesia. In particular, alfaxalone resulted in stable
physiological readings when used alone or as induction agent and showed similar suppression effect on intrinsic neuronal activity of the brain in comparison with ketamine. These findings suggest alfaxalone can be a good alternative to veterinary anesthesia in examination of brain physiology and functionality of large animal models in which CBF and functional connectivity are critical measures to characterize the brain lesion evolution and functional alteration and recovery.

Introduction

Alfaxalone is a synthetic neuroactive steroid anesthetic and has been used as an induction and maintenance agent for anesthesia of large animals [1]. Alfaxalone-2-hydroxpropyl-β-cyclodextrin (Alfaxalone-HPCD) is its newest formulation and was approved by FDA for use in dogs and cats in the United States in 2012[2]. Alfaxalone produces satisfactory induction and maintenance of anesthesia such as onset of anesthesia, fast redistribution, short elimination half-life, short duration of action [3], no cardiovascular depression at clinical doses [4, 5], and a lower frequency of apnea [6]. In recent years, alfaxalone has been increasingly used in horses [7], dogs [1], pigs [8], cats [3, 9], and non-human primates (NHPs) [10–12], suggesting alfaxalone has the potential to become an alternative anesthetic for induction and maintenance anesthesia of large animals. However, alfaxalone’s effects on the cerebral blood flow (CBF) physiology and neural activity of large animals remain poorly understood.

CBF quantifies the blood supply to the brain and is highly coupled to brain metabolism and functionality [13–16]. Several non-invasive perfusion MRI techniques can be used to perform quantitative CBF measurement in large animals like macaque monkeys non-invasively [17], and examine the effects of anesthesia on CBF and autoregulation of large animals [18, 19]. In addition, resting state functional MRI (rsfMRI) can detect the intrinsic neural activity in the brain and has been widely used to examine the functional connectivity in the brain [20, 21] and anesthetized subjects [12, 22–24]. Both CBF and rsfMRI techniques are robust and non-invasive approaches to investigate the effects of anesthesia on the brain physiology and functionality in animals and human subjects [16, 25–29].

Large animals (like pigs, dogs, rabbits, cats, and NHPs, et al) are usually required to be sedated for performing invasive procedure or non-invasive imaging scans in biomedical and neuroscience research. As a popular practice in neuroimaging study, the animals are firstly knocked down with an induction agent (like ketamine) and then maintained with inhalational anesthesia (such as isoflurane) for the entire duration of scan session. The anesthesia effects of ketamine and isoflurane on the CBF and neural functionality of the brain have been explored extensively in previous studies [15, 19, 25, 30–35], demonstrating the CBF and neural functionality could be dramatically affected by applied anesthetics in a dose- and duration-related manner. We hypothesized the CBF and neural activity of the brain could be affected by alfaxalone when it is used alone or as an induction agent in veterinary anesthesia of large animals. In the present study, the anesthesia effects of alfaxalone on the CBF and intrinsic neural functionality were investigated using macaque monkeys and compared with the effects of ketamine.
Methods and Materials

Healthy female rhesus monkeys (n=4, 11–15 years old) were employed in the present study. Alfaxalone (Alfaxan, Jurox, MO, USA) was given initially via intramuscular injection (induction, 5 mg/kg) followed by intravenous infusion (0.125 mg/kg/min) for about 50 minutes during MRI scanning (dosage was practically modified to satisfy the clinic anesthesia requirement [36]). Then the anesthetic was switched to isoflurane (~0.8% with 100% Oxygen) (IsoThesia, Henry Schein Animal Health, NY, USA) to keep the animal sedated continuously until the end of each scan session. The anesthesia procedure with the same time frame was repeated on the same animal in another session but Alfaxalone was replaced with ketamine administrated via intramuscular injection (induction, 10–11 mg/kg), then followed by intravenous (IV) infusion (~1.6 mg/kg/min) and intramuscular (IM) injection (2.6–2.8 mg/kg, every ~15 min, as supplement if needed) for about 50 minutes. The alfaxalone and ketamine were administrated with minimum dosage which could practically satisfy the anesthesia requirement in clinic [36, 37]. The monkeys were immobilized using a custom-built head-holder in the supine position and allowing spontaneous breathing during scanning. Two scans for each animal were separated for at least 3 weeks. Also, the monkeys were randomly assigned to receive alfaxalone or ketamine anesthesia in their first scans.

Physiological parameters such as O₂ saturation, blood pressure, heart rate (HR), respiration rate, body temperature, and PaCO₂ were monitored continuously and maintained within normal ranges [19]. When MRI scans were completed, animals were returned to their home cages after fully recovering from anesthesia. No abnormality was observed during anesthesia induction, maintenance and recovery of the animals.

MRI scans started ~15 minutes after animals were moved into a Siemens 3T Trio scanner with an 8-channel Tx/Rx volume coil (Siemens Healthineers USA, Malvern, PA). The CBF measurements were conducted about 35 minutes after ketamine (or alfaxalone) (IV) administration and repeated 40 minutes after isoflurane administration by using the pseudo-Continuous Arterial Spin Labeling (pCASL) MRI technique [15]. The perfusion 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 5 repetitions were acquired. The multiband EPI pulse sequence showed improved detection of intrinsic functional activity in anesthetized monkey brains [38], and was used for resting-state functional MR images (rsfMRI) scan with the parameters: TR=1000ms, TE=25ms, FOV = 96 mm × 96 mm, spatial resolution= 1.5x1.5x1.5mm³, 34 contiguous slices to cover the whole brain, 550 volumes, multiband factor = 2, GRAPPA factor = 2. Corresponding 3D T₁ weighted images and field map images were acquired during the transition period from ketamine (or alfaxalone) to isoflurane exposure. The rsfMRI data collection was conducted 20–25 minutes after animals were moved into the scanner during alfaxalone (or ketamine) administration, and repeated ~30 minutes after isoflurane exposure. B0 field inhomogeneity was optimized with a novel and automatic shimming procedure reported previously [39].
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.

**CBF and rsfMRI data processing and analysis**

CBF maps were obtained using home-built Matlab scripts (Mathworks, MA). Averaged CBF of each ROI (region of interest) was further processed by using ImageJ 1.51j8 (Fig 1). The cortical regions including media prefrontal cortex (MPFC), anterior cingulated cortex (ACC), posterior cingulate cortex (PCC) were selected for ROI analysis as they are associated to multiple psychiatric [40] and developmental diseases [41] and have been explored in previous fMRI studies of default mode network [42]. Also, the subcortical regions (the caudate, putamen, globus pallidus (GP), thalamus and cerebellum) were examined as they are critical and related to neurological diseases such as stroke, seizures [43], Parkinson disease [44, 45], and Huntington disease [46], et al.

The rsfMRI data were preprocessed for image distortion correction by applying a field map with FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FUGUE). Additional pre-processing included 1) slice timing correction, 2) rigid body registration, 3) regressing out signal in white matter and cerebrospinal fluid time series with a general linear model, 4) temporal filtering with 0.009 Hz ~0.0237 Hz band-pass (this filter allowed to provide similar topology with the monkey’s DMN reported previously) [25, 47, 48], 5) spatial smooth by a Gaussian blur with 2.5-mm full width at half maximum and normalizing individual brains to a template brain [49] using the AFNI software (http://afni.nimh.nih.gov) [50]. Selected ROIs (regions of interest) (including PCC (the seed), ACC and MPFC) were illustrated in a monkey brain template (Fig 1 (bottom row)). The averaged time courses of rsfMRI signal in PCC were used for seed-based correlation analysis separately. 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 MPFC were examined for statistical differences.

**Statistical Analysis**

Statistical analyses were performed with SPSS-26 (Statistical Package for the Social Sciences, version 24). Statistical significance was set at p < 0.05, two-tailed for all reported results. In case of significance, post hoc tests with ANOVA were performed with Least Significant Difference (LSD) correction for multiple comparisons. Repeated ANOVA was used to evaluate the temporal changes in HR and MAP (Fig 2). Paired t-test was used to analyze the CBF and FC differences statistically between alfaxalone or ketamine administration and following isoflurane exposure.

**Results**

The temporal changes of mean arterial pressure (MAP) and HR of monkeys during the entire scanning session were examined and illustrated in Fig 2. No significant MAP change was seen during the entire period of alfaxalone infusion. Continuous reduction of MAP was observed during ketamine and following isoflurane administration. The MAP was always reduced after the anesthetic (alfaxalone or ketamine) was switched to isoflurane. No
significant changes of breathing rates were seen in any scenario (data not shown). HR was stable during alfaxalone administration and showed nearly significant decrease during the successive isoflurane administration. In contrast, HR was gradually increasing during the entire session of ketamine and successive isoflurane administration.

Dramatic CBF changes in selected cortical and subcortical regions during administration of alfaxalone (or ketamine) and successive isoflurane exposure were observed (Fig 3). Obviously, CBF was affected by alfaxalone very differently from ketamine administration. The mean CBF during alfaxalone administration was substantially lower than that with ketamine in cortical regions (ACC, PCC, MPFC) (38.7±14 vs. 155±22, unit: ml/100g/min) (Fig 3A), and in the subcortical regions (caudate, putamen, GP, thalamus, cerebellum) (32±9 vs.107±18, unit: ml/100g/min) (Fig 3C). In particular, alfaxalone resulted in substantial reduction of CBF in grey matter (65±22 vs. 179±38) and white matter regions (14±7 vs. 26±6) (Alfaxalone vs. ketamine, unit: ml/100g/min).

In order to examine the adverse impact of alfaxalone on CBF during successive inhalation anesthesia, the CBF was examined after isoflurane was given for over 30 minutes. When alfaxalone was induced initially, the mean CBF during isoflurane exposure was increased by 92% (38±14 vs. 74±20, unit: ml/100g/min) in cortical regions, 55% (32±9 vs. 49±14) in subcortical regions, 20% (65±22 vs. 78±20) in grey matter, and 43% (14±7 vs. 20±9) in white matter. In contrast, in the monkeys pretreated with ketamine, the mean CBF during isoflurane exposure decreased 22% (155±22 vs. 120±10) in cortical regions, 16% (107±18 vs. 90±11) in subcortical regions, 33% (179±38 vs. 119±5) in grey matter, but increased 15% (26±6 vs. 30±4) in white matter. The adverse effects of each induction agent on CBF during isoflurane exposure in the ROIs (MPFC, ACC, PCC, GP, caudate, putamen, thalamus, and cerebellum) were illustrated in Fig 3B and 3D. Obviously, when the anesthetic was changed from alfaxalone to isoflurane, CBF was monotonously increased in all selected cortical and subcortical regions. In contrast, both CBF increase and decrease were seen in different regions of the ketamine-pretreated monkey brains during isoflurane administration (Table 1).

The effects of alfaxalone and ketamine on functional connectivity (FC) of DMN (MPFC-PCC, ACC-PCC, PCC-PCC) were examined and illustrated in Fig 4 and Table 2. Obviously, no significant difference of functional connectivity was observed in the interested default mode networks between any anesthetic administrations.

**Discussion**

Alfaxalone has been increasingly used as induction agent or used alone for general anesthesia of large animals in recent years. Our results indicated that alfaxalone had evident suppression effect on CBF compared to ketamine (and isoflurane) and showed comparable suppressive effects on the intrinsic neural activity with ketamine. In particular, alfaxalone resulted in more stable physiology when used alone or as induction agent. Also, alfaxalone’s residual effect on CBF of the monkey brain is monotonous when used as induction agent for inhalational anesthesia. These findings suggest alfaxalone is a good alternative to veterinary anesthesia as either an induction or maintenance anesthetic agent in biomedical research or
neuroimaging studies of large animal models. However, the alfaxalone’s effects on CBF should be considered in experimental design and data interpretation.

**The effects of alfaxalone on physiological readings**

Ketamine is a popular induction and maintenance agent and has been used in veterinary anesthesia for decades. However, it could cause HR increase and MAP decrease (Fig 2). The animal may require additional doses to maintain adequate sedation during prolonged scanning duration. In comparison, the animals showed stable MAP and HR readings during alfaxalone administration. The physiological findings of alfaxalone anesthesia are consistent with that seen in previous reports on dogs and cats [51–53] and a recent BOLD fMRI study with cat [54]. In addition, alfaxalone had shorter induction time [55] and showed better quality of induction than ketamine [56] in large animals. The physiological evidences suggest that alfaxalone could be an effective and good alternative for induction and maintenance anesthesia of large animals. The slight MAP reduction after alfaxalone was changed to isoflurane during scanning is most likely caused by the vasodilation and blood pressure reduction effects of isoflurane [15, 25].

**The effects of alfaxalone on CBF when used alone**

It was predicted that alfaxalone might decrease CBF due to its depressant effect in intracellular neuronal metabolism [57]. Our CBF results directly support this assumption, and are in good agreement with the early reports of dogs [58], cats [59], and humans [60] administrated with althesin (the older alfaxalone-alfadolone co-formulation).

In particular, our perfusion results also demonstrated that alfaxalone resulted in CBF decrease substantially in the whole brain compared to ketamine (and isoflurane). Such suppression effect on CBF is probably related to widespread distribution of GABA_A receptor in central nervous system[61] since alfaxalone acts on GABA_A receptor to produce depressant effect on intracellular neuronal metabolism [62, 63]. In contrast, ketamine has vasodilation effect and would increase CBF substantially in spontaneously-breathing goats [35] and rabbits [34] and humans [33, 64, 65], in agreement with our present findings. Isoflurane is also well-known for its vasodilation effect [66–68]. Accordingly, alfaxalone and ketamine showed contrary effects on CBF of the monkey brain.

**The residual effects of alfaxalone on CBF during inhalational anesthesia**

It is a typical procedure to use ketamine as induction agent prior to inhalation anesthesia in large animals. Ketamine is primarily eliminated by the kidneys with the mean terminal half-life of ~155 min in human (in a dose of 0.5mg/kg) [69]. In contrast, alfaxalone can be cleared quickly by the liver and has much shorter terminal half-life time (~45 minutes in cats in a dose of 5 mg/kg) [70].

As alfaxalone has shorter terminal half-life than ketamine, it takes less time to be washed out and the effect of the residual alfaxalone on CBF of animals during isoflurane administration is less compared to ketamine. Therefore, the CBF changes of the brain between alfaxalone and following isoflurane administration are larger than those between ketamine and isoflurane administration (as seen in Fig 3). In addition, the CBF was increased in all
interested regions in alfaxalone-induced monkeys under isoflurane exposure, suggesting alfaxalone’s effect on CBF of the monkey is monotonous in the whole brain. In contrast, the increasing and decreasing effects of ketamine on CBF during isoflurane exposure were region-dependent in ketamine-pretreated monkeys (Table 1).

**The effects of alfaxalone on intrinsic neural activity in the brain**

The rsfMRI is a robust tool to examine the intrinsic neural activity by measuring the functional connectivity (FC) in human and anesthetized animal brains [25, 29, 71]. The default mode network (DMN) is most commonly defined with rsfMRI for examining the correlation of posterior cingulate cortex (PCC) with other brain areas [72]. PCC is involved in self-related cognitive functions and mainly comprised of the regions of PCC, precuneus and the MPFC [73]. Previous studies have confirmed the existence of the DMN in monkeys under isoflurane and demonstrated the topological similarities of the DMN between macaque monkey and human brains [47, 48].

The functional connectivity of the brain was suppressed by isoflurane [48] and ketamine [27] administration as reported previously. Our results demonstrated that alfaxalone showed similar effect of neural activity suppression as Ketamine when used alone (Table 2). In addition, alfaxalone resulted in no statistically significant changes (but tendency of stronger suppression) of functional connectivity in DMN compared to isoflurane (Table 1). Alfaxalone’s suppression effects on FC become significant when compared to the DMN results acquired at 60 minutes as reported previously [74]. Most likely such significance was caused by the larger sample size (n=5) and longer wash-out time of residual alfaxalone (30 vs. 60 minutes) than those in the present study. Interestingly, the standard deviation of z-scores with the alfaxalone administration (IV) is comparable to that with ketamine, but it is much less than that with isoflurane exposure (Table 1), suggesting the inter-subject variation in the intrinsic neural activity with alfaxalone is much less than that with isoflurane, in agreement with those seen previously in previous BOLD fMRI study in the cat [54].

**Conclusion**

The present study revealed alfaxalone’s effects on CBF physiology and intrinsic neuronal activities of monkeys when used alone or as induction agent for inhalational anesthesia. The findings suggest alfaxalone can be a good alternative to veterinary anesthesia in biomedical research and neuroimaging study of brain physiology and functionality. As large animals (such as pigs and non-human primates) are increasingly used for study of neurodegenerative diseases (like stroke, traumatic brain injury (TBI)) in recent years [43, 75–77], the present findings may facilitate alfaxalane’s application in the neuroimaging studies of large animal models in which CBF and functionality are critical measures to characterize the brain lesion evolution and functional alteration and recovery.

**Acknowledgements**

The authors are grateful to Sudeep Patel and Ruth Connelly for assistance in data acquisition and animal handling, and the Center for Magnetic Resonance Research (CMRR) of University of Minnesota for sharing the multiband EPI pulse sequence with us. The project is supported by the Office of Research Infrastructure Programs (OD P51OD011132).
References


Magn Reson Imaging. Author manuscript; available in PMC 2022 January 01.


Alfaxalone reduced CBF in the whole brain substantially

The residual effects of alfaxalone on CBF were monotonous when used for induction

Alfaxalone resulted in similar suppression effect on intrinsic neural activity of the monkey brain as ketamine
Figure 1.
Demonstration of representative cerebral blood flow (CBF) map (top row), axial T₂ weighted image (T2W, middle row) with selected ROIs (regions of interest) of a monkey brain. MPFC, medial frontal cortex; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; GP, Globus pallidum; GM, grey matter; WM, white matter.
Figure 2.
Demonstration of temporal changes of mean arterial pressure (MAP, left) and heart rate (HR, right) of the monkeys during administration of anesthetic (alfaxalone/Ketamine) and 0.8% isoflurane. Alfaxalone: 0.125mg/kg/min; Ketamine: 1.6 mg/kg/min. Data are reported as mean ± standard deviation error. *, p<0.05 compared with the measure at drug start; #, p<0.05; @, p<0.07 compared with the measure at isoflurane. Repeated AVOVA was applied.
Figure 3.
The CBF changes in selected cortical (top, A and B) and subcortical regions (bottom, C and D) of macaque brains with alfaxalone, ketamine at pre isoflurane (A and C) and post isoflurane (B and D) exposure. Data are reported as mean ± standard deviation, **, p<0.01; *, p<=0.05 vs ketamine. MPFC, media prefrontal cortex; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; GP, Globus pallidum; GM, grey matter; WM, white matter; Cereb, cerebellum. Alfaxalone: 0.125mg/kg/min; Ketamine: 1.6 mg/kg/min.
Figure 4.
Demonstration of seed (PCC) based averaged z maps of the default mode network (DMN) in the monkey brains (n=4) after administration of ketamine (or alfaxalone) and 0.8% isoflurane. The color bar: the magnitude of the regression coefficient (z-score, corrected joint threshold $p<0.001$ plus 0.63mm$^3$). L, left hemisphere; R, right hemisphere. Alfaxalone: 0.125mg/kg/min; Ketamine: 1.6 mg/kg/min. MPFC, medial frontal cortex; ACC, anterior cingulated cortex; PCC, posterior cingulated cortex.
The CBF changes in selected cortical (top) and subcortical regions (bottom) of macaque brains with ketamine, alfaxalone (pre-isoflurane) and isoflurane exposure.

<table>
<thead>
<tr>
<th>CBF Changes in the Cortical Regions</th>
<th>Pre-isoflurane</th>
<th>Induction Agent</th>
<th>Isoflurane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alfaxalone</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>44 ± 12</td>
<td>42 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 ± 15</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 ± 22</td>
<td>14 ± 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65 ± 22</td>
<td>18 ** (+91%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>74 ± 65 *</td>
<td>15 ** (+76%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65 ± 14 *</td>
<td>20 ± 9 * (+42%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84 ± 26 * ** (+116%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>78 ± 12 ** (+20%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>116%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>116%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>116%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>116%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>42%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CBF Changes in the Subcortical Regions</th>
<th>Pre-isoflurane</th>
<th>Induction Agent</th>
<th>Isoflurane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alfaxalone</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>42 ± 9</td>
<td>6 ± 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39 ± 13</td>
<td>29 ± 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 ± 7</td>
<td>44 ± 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29 ± 6</td>
<td>63 ± 18 (+50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 ± 17 ** (+38%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>17 ± 15 (+180%)</td>
<td>12 ** (+63%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48 ± 66 ** (+64%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>66 ± 15 (+43%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 ± 4</td>
<td>119 ± 16 ** (+33%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 ± 14</td>
<td>113 ± 13 ** (+24%)</td>
</tr>
</tbody>
</table>

Data are reported as mean ± standard deviation
** p < 0.01
*p ≤0.05, isoflurane vs. alfaxalone/ketamine.

## p < 0.004

# p < 0.05, ketamine vs. alfaxalone. MPFC, medial prefrontal cortex; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; GP, Globus pallidum; GM, grey matter; WM, white matter. Alfaxalone: 0.125 mg/kg/min; Ketamine: 1.6 mg/kg/min. The percentage changes of CBF at 30 min post isoflurane are derived from comparison with CBF during alfaxalone (or ketamine) administration. CBF unit: ml/100 g/min.
Table 2

The z score changes of MPFC-PCC, ACC-PCC and PCC-PCC of macaque brains with alfaxalone (Alf), ketamine (k) (pre-isoflurane) and isoflurane exposure. Data are reported as mean ± standard deviation; MPFC, media prefrontal cortex; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex. Alfaxalone: 0.125 mg/kg/min, Ketamine: 1.6 mg/kg/min. P_{Alf:k}, the p-values for the t-test between ketamine and alfaxalone in pre and post isoflurane.

<table>
<thead>
<tr>
<th></th>
<th>Pre-isoflurane</th>
<th>Isoflurane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPFC-PCC</td>
<td>ACC-PCC</td>
</tr>
<tr>
<td>Alfaxalone</td>
<td>0.08 ± 0.04</td>
<td>0.10 ± 0.06</td>
</tr>
<tr>
<td>Ketamine</td>
<td>0.10 ± 0.04</td>
<td>0.09 ± 0.08</td>
</tr>
<tr>
<td>P_{Alf:k}</td>
<td>0.56</td>
<td>0.56</td>
</tr>
</tbody>
</table>

*p < 0.05.