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Regional Cerebral Blood Flow and BOLD Responses in Conscious and Anesthetized Rats Under Basal and Hypercapnic Conditions: Implications for Functional MRI Studies

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Summary: Anesthetics, widely used in magnetic resonance imaging (MRI) studies to avoid movement artifacts, could have profound effects on cerebral blood flow (CBF) and cerebrovascular coupling relative to the awake condition. Quantitative CBF and tissue oxygenation (blood oxygen level–dependent [BOLD]) were measured, using the continuous arterial-spin-labeling technique with echo-planar-imaging acquisition, in awake and anesthetized (2% isoflurane) rats under *basal* and *hypercapnic* conditions. All basal blood gases were within physiologic ranges. Blood pressure, respiration, and heart rates were within physiologic ranges in the awake condition but were depressed under anesthesia ($P < 0.05$). Regional CBF was heterogeneous with whole-brain CBF values of 0.86 ± 0.25 and 1.27 ± 0.29 $mL \cdot g^{-1} \cdot min^{-1}$ under awake and anesthetized conditions, respectively. Surprisingly, CBF was markedly higher (20% to 70% across different brain conditions) under isoflurane-anesthetized condition compared with the awake state ($P < 0.01$). Hypercapnia decreased pH, and increased P_{CO_2} and P_{O_2}. During 5% CO_2 challenge, under awake and anesthetized conditions, respectively, CBF increased 51 ± 11% and 25 ± 4%, and BOLD increased 7.3 ± 0.7% and 5.4 ± 0.4%. During 10% CO_2 challenge, CBF increased 158 ± 28% and 47 ± 11%, and BOLD increased 12.5 ± 0.9% and 7.2 ± 0.5%. Since CBF and BOLD responses were substantially higher under awake condition whereas blood gases were not statistically different, it was concluded that cerebrovascular reactivity was suppressed by anesthetics. This study also shows that perfusion and perfusion-based functional MRI can be performed in awake animals. **Key Words:** CBF—Spin labeling—fMRI—Awake animals—Anesthesia—Carbon dioxide.

Most magnetic resonance imaging (MRI) and spectroscopy studies on animal models involve general anesthesia to eliminate movement artifact. The use of anesthetics, however, could have profound influence on cerebral circulation, metabolism, and neural–vascular coupling. Anesthetics in general are known to suppress neural activity (Ueki et al., 1992), basal cerebral blood flow (CBF) (Ueki et al., 1992), hemodynamic coupling, and functional MRI (fMRI) responses (Lahti et al., 1998; Peeters et al., 2001). Previous studies have shown that basal CBF varies dramatically depending on the anesthetics used. For example, CBF in rats anesthetized with isoflurane is substantially higher than the CBF in rats anesthetized with fentanyl or pentobarbital (Hendrich et al., 2001). Although many studies have compared CBF and metabolism under different anesthetics, little is known regarding how they differ from conscious condition.

Anesthetics also affect the fMRI signal responses. Functional MRI is widely used as a noninvasive tool to image brain functions ranging from perceptions to cognitive processes in both animal models and humans (Bandettini et al., 1992; Kwong et al., 1992; Ogawa et al., 1992). Functional MRI does not measure neural activity directly; rather, it indirectly detects hemodynamic changes associated with increased neural activity. Therefore, detection of the fMRI signal responses relies on intact coupling between hemodynamic and metabolic responses associated with increased neuronal

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activity. Such coupling could be markedly affected by anesthetics.

Cerebrovascular coupling has been widely studied using hypercapnic challenge in which carbon dioxide is added to the breathing gas (Weiss et al., 1983; Bandettini and Wong, 1997; Kim and Ugurbil, 1997; Davis et al., 1998; Hoge et al., 1999; Posse et al., 2001). Carbon dioxide, a potent vasodilator and a key modulator of stimulus-induced blood flow response, evokes large CBF and blood oxygen level–dependent (BOLD) increases without concomitant increase in metabolic rate. There is strong evidence that cerebrovascular reactivity to carbon dioxide is also perturbed by anesthetics (Strebel et al., 1993; Bonvento et al., 1994), therefore further supporting the notion that the neural–vascular coupling associated with fMRI is affected by anesthesia.

Although most fMRI studies on animal models are done with the animals under general anesthesia, there is increasing interest in fMRI studies of awake and restrained monkeys (Logothetis et al., 1999; Zhang et al., 2000; Dubowitz et al., 2001; Ferris et al., 2001; Vanduffel et al., 2001), rabbits (Wyrwicz et al., 2000), and rats (Lahti et al., 1998). Functional MRI of awake animals offers many distinct advantages. First, the confounding effects of anesthesia can be avoided. Second, general neural activity is not suppressed (Ueki et al., 1992), which leads to increased fMRI signal changes (Lahti et al., 1998; Peeters et al., 2001) and, potentially improved detection. Finally, subcortical and higher-order cognitive functions can be studied in the awake model. Such studies would be very difficult if not impossible using the anesthetized preparations. The disadvantages of performing awake MRI studies are potential motion artifact and stress.

Although animal studies under anesthetized conditions remain valuable and unavoidable under many circumstances, developing an awake animal model in which perfusion and perfusion-based fMRI can be studied under the fully conscious condition could have many important applications. As a first step to evaluate such an awake rat model, quantitative perfusion was measured using the continuous arterial spin-labeling technique with echo-planar-imaging acquisition to evaluate CBF in awake and anesthetized rats under basal and hypercapnic challenge. The main goals of this study were (1) to determine whether CBF can be measured under awake and restrained conditions using MRI, (2) to identify major regions of the brain that have substantial perfusion differences between awake and anesthetized conditions, and (3) to evaluate the CBF and BOLD responses to hypercapnia under awake and anesthetized conditions. To the best of our knowledge, this is the first noninvasive measurement of quantitative perfusion and perfusion-based fMRI in fully conscious animals using magnetic resonance imaging.

METHODS

Animal preparation

All animal experiments were approved and monitored by the University of Massachusetts Medical Center, Department of Animal Medicine. These procedures were in accordance with guidelines published by the National Institutes of Health. Two groups of Sprague-Dawley rats (250 to 300 g) were imaged and each rat was studied once. In group 1 ($n = 8$), quantitative basal CBF was measured under awake and anesthetized conditions in the same animals. In group 2 ($n = 7$), CBF and BOLD changes were simultaneously measured in association with hypercapnic (CO_2) challenges under awake and anesthetized conditions in the same animals. The femoral artery was catheterized for monitoring mean arterial blood pressure, heart rate, and for sampling blood gases. Animals were secured in a rat restrainer with custom-designed ear-, nose-, tooth-, and shoulder-bars, a body-restraining tube, and built-in radiofrequency electronics for imaging, similar to the restrainer reported previously (Lahti et al., 1998). All animals respired without mechanical ventilation. Air or premixed CO_2 gas was delivered via a tube to the animal's chamber at a flow rate of 2 L/min. For the anesthetized condition, 2% isoflurane was used. The rectal temperature was monitored and maintained at 37 ± 1°C throughout the study via a feedback-regulated, circulating-water pad.

Physiologic measurements. Mean arterial blood pressure, heart rate, and respiration rate were continuously monitored and recorded. These parameters were quantified under basal and hypercapnic conditions in awake and anesthetized rats during imaging. Blood gases were sampled immediately at the end of MRI measurement of each CO_2 challenge while the animals were in the magnet, to avoid the effect of withdrawing blood on the MR signals.

Hypercapnic challenges (group 2). For each animal, two (5 and 10%) carbon dioxide concentrations with 21% O_2 and balance N_2 were studied under anesthetized and awake conditions. In each MRI experiment (≈200 seconds), the animal was exposed to air (baseline) for 40 seconds, followed by 5% or 10% CO_2 gas for 160 seconds. Typically, at least 15 minutes elapsed between each CO_2 challenge and between awake and anesthetized conditions.

Magnetic resonance experiments. All MR experiments were performed on a 4.7-T/40-cm horizontal magnet (Oxford Instrument, Oxford, U.K.) equipped with a Biospec Bruker console (Bruker, Billerica, MA, U.S.A.) and a 20-G/cm magnetic field gradient insert (ID = 12 cm) capable of 120-μs rise time (Bruker). An actively decoupled surface coil (2.3-cm inner diameter) was used for brain imaging and a neck coil (Silva et al., 1999; Duong et al., 2000*b*) for perfusion labeling. Coil-to-coil electromagnetic interaction was actively decoupled. Anatomic images were acquired using the fast spin-echo (rapid acquisition with relaxation enhancement) pulse sequence with repetition time (TR) = 2 seconds (90° flip angle), 8 echo trains, effective echo time (TE) = 65 milliseconds, matrix = 256 × 256, field of view = 2.5 × 2.5 cm^2, and six 1.5-mm slices. Four transients were acquired for signal averaging.

Cerebral blood flow measurements were made using the continuous arterial spin-labeling technique (Silva et al., 1999; Duong et al., 2000*b*) with single-shot, gradient-echo, echo-planar-imaging acquisition. Paired images were acquired alternately: one with arterial spin labeling and the other without spin labeling (control). The MR parameters were: data matrix = 64 × 64, field of view = 2.5 × 2.5 cm^2, and six 1.5-mm slices, TE = 15 milliseconds, and TR = 2 seconds (90° flip angle).

Continuous arterial spin labeling used a 1.78-second square radiofrequency pulse to the labeling coil in the presence of 1.0 G/cm gradient along the flow direction such that the condition of adiabatic inversion was satisfied (Detre et al., 1992). The sign of the frequency offset was switched for control (nonlabeled) images. For each set of CBF measurement, 51 pairs of images (≈200 seconds) were acquired and the first two pairs were discarded and excluded from analysis.

Blood oxygenation level–dependent images were taken from the nonlabeled (control) images of the CBF measurements, and no additional data acquisition was needed. Note that the optimal gradient-echo BOLD contrast is obtained with echo time set at tissue T_2*, which is ≈20 milliseconds at 4.7 T. In this study, the echo time was 15 milliseconds, which was chosen to compromise between BOLD contrast and CBF signal-to-noise ratio. Thus, larger BOLD percent changes would be expected if the optimal echo time was used. Nevertheless, the overall conclusions in this study were not altered.

For group 1, each CBF measurement set (51 pairs) was typically repeated three times and acquired alternately between awake and anesthetized conditions. For group 2, each CBF measurement set (51 pairs) was typically repeated twice for each gas condition and acquired alternately between awake and anesthetized conditions.

Data analysis. Image analysis used codes written in Matlab (MathWorks Inc., Natick, MA, U.S.A.) and the STIMULATE software (Strupp, 1996). All statistical tests used the Student's paired *t*-test. All reported values and error bars on plots were in mean ± SD.

For group 1, CBF image (S_{CBF}) with intensity in milliliters per gram tissue per minute was calculated pixel-by-pixel using (Silva et al., 1997*b*)

$$S_{CBF} = \frac{\lambda}{T_1} \frac{S_C - S_L}{S_L + (2\alpha - 1)S_C} \quad (1)$$

where S_C and S_L are signal intensities of the control and labeled images, respectively. λ is the water brain–blood partition coefficient, T_1 is that of tissue, and α is the arterial spin-labeling efficiency (Silva et al., 1997*b*). λ was taken to be 0.9 (Herscovitch and Raichle, 1985). T_1 and α were measured and they were 1.5 seconds and 0.75, respectively.

Multiple CBF measurements from each animal were averaged to obtain a single multislice set of awake CBF images and a single multislice set of anesthetized CBF images. Alignment of CBF images in a study was checked by visually evaluating the time-series "movies" created by appending images acquired at different time points. Regions of interests (ROIs) were analyzed in both hemispheres and were carefully drawn with references to both anatomy and echo-planar images. These ROIs were cerebral cortex, corpus callosum, hippocampus, thalamus, and caudoputamen. The same ROIs were used for the awake and anesthetized conditions in the same animal. Subtraction maps between awake and anesthetized rats were also calculated and color-coded.

For group 2, hypercapnia-induced CBF and BOLD changes were analyzed. Conservative ROIs enclosing the entire brain but avoiding the edge of the brain were used. Percent change maps were obtained by using *t*-test analysis with images 2 to 11 as baseline and images 16 to 51 as stimulation. Images 12 to 15 were not included in the *t*-test because CO_2 and fMRI signals were dynamically changing and not at steady state.

Functional contrast-to-noise ratios were calculated pixel-by-pixel as the hypercapnia-evoked percent changes divided by the standard deviations of the baseline images before CO_2 challenge. Rise times were not calculated because of the relatively long TR used in this study.

RESULTS

Physiologic measurement under awake and anesthetized conditions

The number of struggling movements as well as the number of vocalizations markedly decreased after the first hour of being awake in the restrainer and remained low during the entire MR study, suggesting that the animals had acclimated to the restrainer to some extent. Therefore, all MR and physiologic measurements were made with animals being awake for at least 1 h in the restrainer.

Physiologic measurements under basal and hypercapnic conditions are summarized in Table 1. Comparisons of these physiologic parameters between awake and anesthetized conditions were made in the same animals. Blood pH, P_{CO_2}, P_{O_2} and oxygenation saturation under both awake and anesthetized conditions were within normal physiologic ranges (Flecknell and Waynsorth, 1992; Sharp and LaRegina, 1998) and these parameters were not statistically different between awake and anesthetized rats ($P > 0.05$). The respiration rate, heart rate, and mean arterial blood pressure under the awake condition were consistent with established data in rats (Flecknell and Waynsorth, 1992; Sharp and LaRegina, 1998), where the respiration rate was reported to range from 70 to 115 breaths per minute, heart rate 300 to 450 beats per minute, systolic blood pressure 116 to 180 mm Hg and diastolic blood pressure 60 to 145 mm Hg. The respiration rate, heart rate, and mean arterial blood pressure under isoflurane anesthesia, although slightly depressed ($P < 0.05$), were within normal physiologic ranges.

Under hypercapnic condition (also shown in Table 1), there were no statistical differences in blood-gas values between awake and anesthetized conditions for each CO_2 gas conditions; for example, under 5% CO_2 challenge, blood-gas values were not statistically different between awake and anesthetized conditions. During hypercapnia, pH decreased, and P_{CO_2} and P_{O_2} increased; however, there were no statistical differences in heart rate, mean arterial blood pressure, and respiration rate relative to the basal condition. A general increase in respiration volume was observed during hypercapnia (data not shown).

Basal cerebral blood flow differences between awake and anesthetized rats

Representative anatomic images obtained from an awake rat are shown in Fig. 1A. There were no obvious movement artifacts in the anatomic images. Superimposed on these anatomic images are ROIs of five major brain structures used in our quantitative CBF analysis, namely, the cerebral cortex, caudoputamen, thalamus, corpus callosum, and hippocampus. Representative single-shot, echo-planar images obtained from an awake

TABLE 1. *Physiologic measurements of basal condition and hypercapnic challenges under awake and anesthetized conditions*

Group	Gas condition	MABP	HR	RR	pH	P_{CO_2}	P_{O_2}	O_2 Sat
Anesthetized	Air	110 ± 17	353 ± 25	54 ± 8	7.44 ± 0.04	36 ± 2	86 ± 4	95 ± 4
	5% CO_2	110 ± 17	356 ± 15	58 ± 6	7.38 ± 0.03	50 ± 5	97 ± 7	97 ± 1
	10% CO_2	109 ± 9	355 ± 23	59 ± 4	7.24 ± 0.02	69 ± 2	108 ± 6	97 ± 2
Awake	Air	137 ± 5	427 ± 06	106 ± 1	7.43 ± 0.03	36 ± 3	92 ± 3	98 ± 1
	5% CO_2	140 ± 15	406 ± 19	105 ± 2	7.39 ± 0.02	47 ± 4	118 ± 5	98 ± 1
	10% CO_2	146 ± 17	416 ± 34	116 ± 3	7.27 ± 0.07	65 ± 5	120 ± 8	98 ± 1

Mean arterial blood pressure (MABP), heart rate (HR), respiration rate (RR), pH, P_{CO_2}, P_{O_2}, and oxygen saturation (O_2 Sat) were measured on the same group of animals (n = 7). During hypercapnia, pH decreased and P_{CO_2} and P_{O_2} increased, as expected; however, there were no statistical differences in HR, MABP and RR. MABP, HR and RR under the awake condition were statistically different from those under anesthetized ($P < 0.05$). However, there were no statistical differences in blood-gas values between awake and anesthetized conditions for each gas condition (air, 5% or 10% CO_2).

rat are shown in Fig. 1B. No gross misalignment was observed in the time-series "movies" generated from multiple repeated measurements.

Representative quantitative CBF images from a rat under awake and anesthetized conditions are shown in Fig. 1C and 1D, respectively. Under both awake and anesthetized conditions, CBF was heterogeneously distributed, likely attributed to regional differences in metabolic rate (Sokoloff et al., 1977) and/or vascular density (Zeman and Innes, 1963; Miyawaki et al., 1998). Surprisingly, global CBF values were significantly lower in the awake condition relative to the isoflurane-anesthetized condition in the same rats. Cerebral blood flow values in different brain regions ranged from 0.9 to 1.44 $mL \cdot g^{-1} \cdot min^{-1}$ (whole-brain average of 1.27 ± 0.29 $mL \cdot g^{-1} \cdot min^{-1}$) under isoflurane-anesthetized condition, and ranged from 0.6 to 1.2 $mL \cdot g^{-1} \cdot min^{-1}$ (whole-brain average of 0.86 ± 0.25 $mL \cdot g^{-1} \cdot min^{-1}$) in the awake state. Furthermore, the CBF subtraction maps (Fig. 1E) of the anesthetized condition from awake condition shows that there were heterogeneous differences between awake and anesthetized CBF. Regions that were less negative (smaller CBF difference [i.e., blue pixels]) in the CBF subtraction maps were indicative of areas with differentially higher basal CBF in the awake condition.

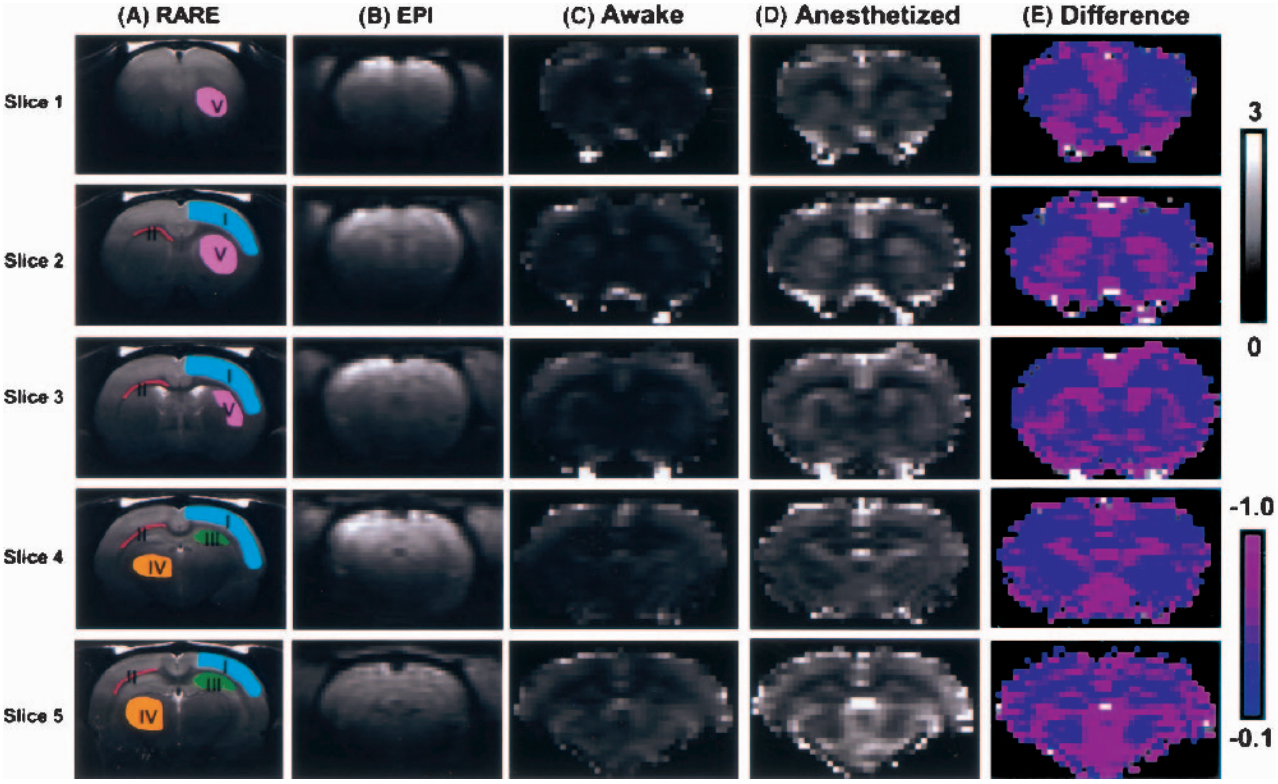
A typical block of CBF measurement over ≈3.3 minutes is shown in Fig. 1F. The CBF-weighted intensities, alternating between nonlabeled and labeled images, are indicative of the presence of labeled blood flow. The CBF contrast, derived from subtracting labeled from nonlabeled image, was ≈8% of the original signal, ranging from 4% to 10% for all animals. These data further showed that there were no obvious movement artifacts in individual CBF measurements under the awake condition.

Although there was some partial volume effect due to limited spatial resolution, many major brain structures were clearly identified. Quantitative CBF values for five major brain substructures from all eight rats were quantified and are summarized in Fig. 2. Among these five structures, the largest differences in CBF between awake and anesthetized rats were in the hippocampus and caudoputamen (≈70%) and the smallest difference in CBF between awake and anesthetized rats was in the cerebral cortex (≈20%). The corpus callosum and thalamic nuclei displayed intermediate difference of the former and latter.

Hypercapnia-induced cerebral blood flow changes in awake and anesthetized rats

Cerebral blood flow and BOLD responses to hypercapnia were evaluated under awake and anesthetized conditions in the same animals and are summarized in Fig. 3. Hypercapnia-evoked CBF and BOLD responses were readily detected without movement artifacts. During 5% CO_2 challenge, under awake and anesthetized conditions, respectively, CBF increased 51 ± 11% and 25 ± 4%, and BOLD increased 7.3 ± 0.7% and 5.4 ± 0.4%. During 10% CO_2 challenge, CBF increased 158 ± 28% and 47 ± 11%, and BOLD increased 12.5 ± 0.9% and 7.2 ± 0.5%. Hypercapnia-induced CBF and BOLD changes were markedly higher under awake condition ($P < 0.05$), whereas blood gases were not statistically different, suggesting that cerebrovascular reactivity to CO_2 was markedly attenuated by anesthesia. It is noteworthy that CBF and BOLD percent changes during 5% CO_2 challenge under the awake state were comparable to those with the 10% CO_2 challenge under the anesthetized conditions. Hypercapnia-induced CBF changes under the awake condition were 2.0 to 3.3 times greater than that observed in the anesthetized condition, whereas hypercapnia-induced BOLD changes under the awake condition were only 1.3 to 1.7 times larger. The differential responses in CBF and BOLD could be indicative of the underlying metabolic differences between the awake and anesthetized conditions.

Basal MRI signal fluctuation under the awake condition was substantially higher than that under the anesthetized condition. Higher basal signal fluctuations under the awake condition, however, did not necessarily arise



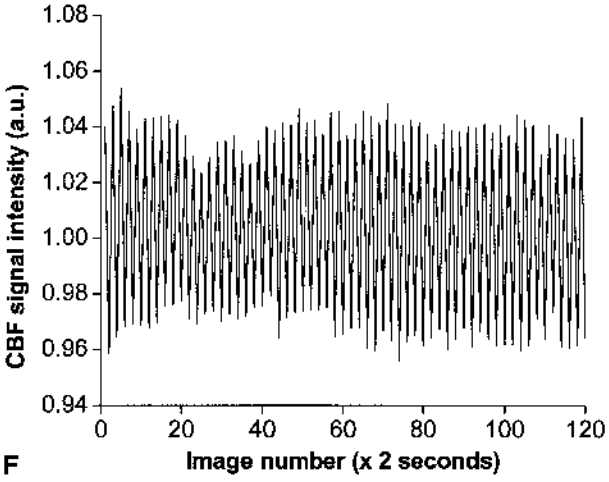


FIG. 1. (A) Anatomic images of an awake rat obtained using fast spin-echo (rapid acquisition with relaxation enhancement [RARE] pulse sequence. Overlaid on the anatomy are representative regions of interest (ROIs) of five major structures used in quantitative cerebral blood flow (CBF) analysis, namely: I. Cerebral cortex—blue; II. corpus callosum—red; III. hippocampus—green; IV. thalamus—yellow; V. caudoputamen—pink. These ROIs, displayed on only one hemisphere for clarity, were conservatively drawn to minimize partial volume effects. **(B)** Representative echo-planar imaging (EPI) images of an awake rat obtained from the continuous arterial spin-labeling measurement. There are no obvious movement artifacts. Representative perfusion images of the awake **(C)** and anesthetized **(D)** conditions in the same rat. The overall cerebral blood flow (CBF) is substantially lower in the awake than in the isoflurane-anesthetized rat. Cerebral blood flow under awake and anesthetized states is heterogeneously distributed. **(E)** The difference by subtracting anesthetized from awake CBF is also heterogeneous. Note that the two gradient-echo EPI (but not the spin-echo RARE anatomy) images show signal drop around the ear canals near the bottom of the brain. Gray scale bar indicates the CBF images ranging from 0 to 3 mL · g^{-1} · min^{-1}. The color bar indicates the difference in CBF values ranging from –0.1 to 1.0 mL · g^{-1} · min^{-1}. **(F)** Representative raw perfusion data obtained from the cerebral cortex and the caudoputamen over the ≈3.3-minute measurement. The oscillations were ≈8% of the original signal (from 0.96 to 1.04), indicative of the labeled and nonlabeled images acquired in an alternate fashion. No obvious movement artifacts in the time course were observed.

from increased movement; they were likely a result of increased basal neural activity and/or increased physiologic "processes" associated with being awake. Functional contrast-to-noise ratios (CNRs) were compared. For the BOLD signals, the CNRs were 20 ± 5 for the 10% CO_2 condition and 10 ± 7 for the 5% CO_2 conditions under the awake state; 16 ± 5 for the 10% CO_2 conditions and 10 ± 6 for the 5% CO_2 conditions under the anesthetized condition. For the CBF signals, CNRs were 14 ± 8 for the 10% CO_2 condition and 8 ± 4 for the 5% CO_2 conditions under the awake state; 6 ± 3 for the 10% CO_2 conditions and 4 ± 2 for the 5% CO_2 conditions under the anesthetized condition. Contrast-to-noise ratios were statistically different between 5% and 10% CO_2 challenges ($P < 0.05$) but not statistically different between awake and anesthetized conditions with the same CO_2 challenge ($P > 0.05$). Although the BOLD CNRs were higher overall, there were no statistical differences between BOLD and CBF contrasts. We concluded that the CNRs were comparable between awake

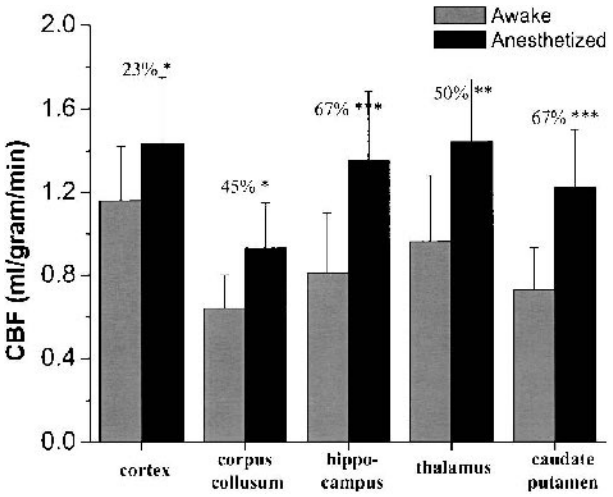


FIG. 2. Quantitative cerebral blood flow (CBF) in awake and anesthetized rats in five major brain structures (n = 8). The percent differences above the bar graphs are the CBF increases by isoflurane anesthesia relative to the awake state. $^{*}P \leq 0.005$, $^{**}P \leq 10^{-4}$; $^{***}P \leq 10^{-7}$. Error bars are standard deviations.

and anesthetized conditions, at least under these experimental conditions.

DISCUSSION

Quantitative perfusion in conscious animals

Despite the widespread use of anesthetics in experimental animal models and in human studies, the effect of anesthetics on CBF remains poorly understood. Although all anesthetics affect basal CBF, metabolism, neural activity, and neural–vascular coupling, little is known regarding the extent to which CBF is modulated globally and in different regions of the brain under anesthesia relative to the awake condition.

Magnetic resonance imaging offers a noninvasive tool to image quantitative CBF at reasonably high spatial and temporal resolution at the level of the capillary and tissue (Kwong et al., 1992; Kim, 1995; Wong et al., 1998; Duong et al., 2001*c*). Although consistent with many established invasive CBF techniques, the accuracy of the continuous arterial spin-labeling CBF technique could be subjected to errors arising from magnetization transfer, transit time, and water-exchange effect. With the use of the actively decoupled two-coil system for CBF measurements, the magnetization transfer effect on quantitative CBF values was not an issue (Silva et al., 1999; Duong et al., 2000*b*). Transit time (Calamante et al., 1999; Zhou and van Zijl, 1999) and water-exchange effect (Silva et al., 1997a; Zhou et al., 2001; Parkes and Tofts, 2002), however, could potentially cause errors in the quantitative blood flow values. These errors, nevertheless, are likely to be small (Silva et al., 1997*a*; Zhou et al., 2001; Parkes and Tofts, 2002) and are not expected to alter the overall conclusions of this study. The continuous arterial spin-labeling technique used herein has also been validated using iodoantipyrine autoradiography (Tsekos et al., 1998).

Most magnetic resonance imaging and spectroscopy studies on animal models involve general anesthesia to

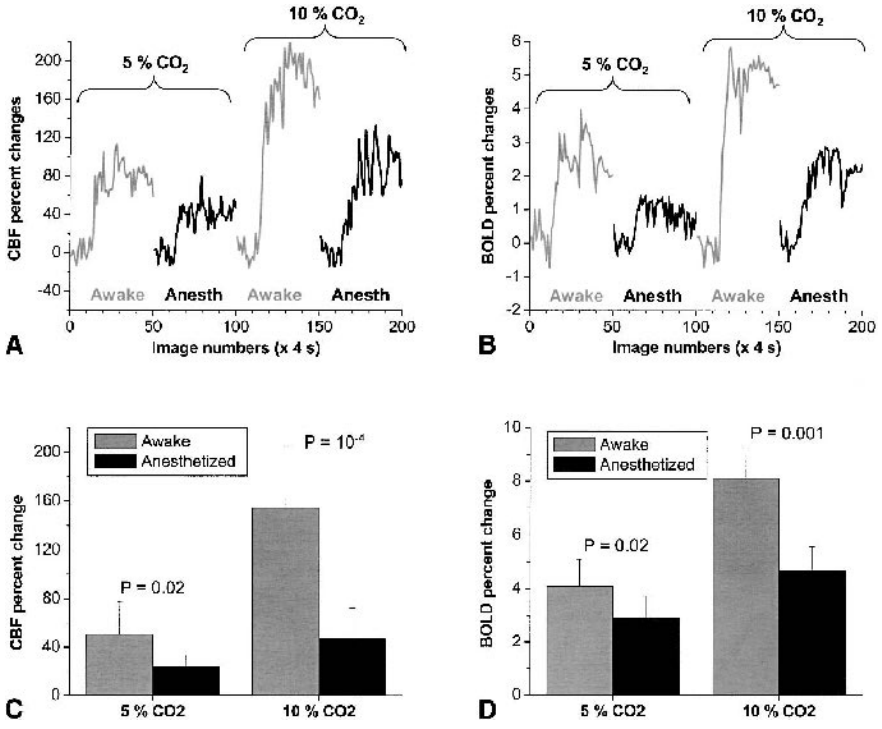


FIG. 3. Representative cerebral blood flow (CBF) **(A)** and blood oxygen level-dependent (BOLD) **(B)** time courses from an animal without signal averaging. Four time courses, namely, 5% CO_2 under awake (gray) and anesthetized (black) conditions and 10% CO_2 under awake (gray) and anesthetized (black) conditions were concatenated. Averaged CBF **(C)** and BOLD **(D)** percent changes during hypercapnic (5% and 10% CO_2) challenge in awake and anesthetized conditions from 7 animals. Error bars are standard deviations. The *P* values are comparison between awake and anesthetized conditions.

avoid movement artifacts. Perfusion imaging is particularly sensitive to movement artifacts because it has low signal-to-noise ratio and it involves paired images acquired at two different time points during which movement could occur. The use of a proper restrainer has provided quantitative perfusion images free of movement artifacts. In addition, data acquisition using the echo-planar-imaging technique was helpful in minimizing the effects of movement artifacts in MR images because this imaging method samples the entire k-space in a single-shot acquisition within ≈20 milliseconds. Therefore, such an approach offers a means to quantitatively evaluate regional CBF in fully conscious rats, allowing direct comparison of quantitative CBF in different regions of the brain under awake and anesthetized conditions in the same animals.

Restraint could have a dramatic effect on animal physiology. However, blood gases, heart rate, respiration rate, and mean arterial blood pressure under the awake preparations were within normal physiologic ranges, and the number of struggling movements markedly decreased after the first hour of being awake in the restrainer. Taken together, these observations suggest that the animals have substantially acclimated to the restrainer during the imaging session. Detailed investigation of the acclimation and restraint effect is currently under investigation. Nevertheless, it is important to keep in mind that the regional difference in CBF values could be influenced by restraint stress.

Awake fMRI studies have used the BOLD technique to detect oxygenation changes (Lahti et al., 1998; Logothetis et al., 1999; Wyrwicz et al., 2000; Ferris et al., 2001) and the monocrystalline iron oxide nanoparticles technique to detect blood-volume changes (Dubowitz et al., 2001; Vanduffel et al., 2001) associated with increased neural activity. This study further showed that perfusion and perfusion-based functional imaging could be performed in fully conscious and restrained animals. Although awake perfusion imaging is without movement artifacts and there are indications that rats had acclimated to the restrainer to some extent, the issue of stress nevertheless needs to be fully addressed.

Effect of anesthesia on cerebral blood flow

Quantitative perfusion in different regions of the brain could be reliably measured within and across different animals under both anesthetized and conscious conditions. The average perfusion value across the entire brain was 1.27 $mL \cdot g^{-1} \cdot min^{-1}$ under the isoflurane-anesthetized condition. These results are consistent with a rat study under 2% isoflurane (mechanically ventilated) (Duong et al., 2001*b*) and the Hendrich et al. (2001) study in rats under 1% isoflurane (mechanically ventilated in the presence of pancuronium paralytics). Cerebral blood flow values in the awake condition were similar to those under chloral hydrate (≈1.14 ± 0.11 $mL \cdot g^{-1} \cdot min^{-1}$, n = 6, unpublished data). By contrast, CBF in the rat brain under α-chloralose was substantially lower (≈0.64 ± 0.17 $mL \cdot g^{-1} \cdot min^{-1}$ using MRI (Duong et al., 2000*b*) and 0.72 ± 0.19 $mL \cdot g^{-1} \cdot min^{-1}$ using iodoantipyrine autoradiography (Tsekos et al., 1998). Cerebral blood flow under pentobarbital is generally even lower (Hendrich et al., 2001). It should be noted that CBF are critically dependent on anesthetic doses. Although there are cross-laboratory differences in animal preparations, blood-gas condition, and anesthetic dose (among others), these comparisons clearly showed that anesthetics have powerful influences on basal CBF.

Literature on CBF comparison between awake and anesthetized conditions is sparse. Cerebral blood flow under awake and restrained conditions in animal models had been measured using autoradiographic techniques (Dahlgren et al., 1981; Young et al., 1991), xenon washout technique (Reinstrup et al., 1995), microsphere technique (Boarini et al., 1984), hydrogen clearance technique (Heiss and Traupe, 1981), and single-photon emission computer-aided tomography (Reinstrup et al., 1995). Most of the aforementioned techniques, however, are highly invasive and yield poor spatial localization, which makes it difficult to quantify and compare CBF in various brain structures. Furthermore, no systematic comparison of CBF under awake and anesthetized conditions was made using the existing invasive techniques in the same animals and in the same regions of interest.

One important and surprising observation is that CBF across the entire brain under isoflurane-anesthetized condition was consistently higher (20% to 70% across different brain regions) than that measured in the awake state. Isoflurane is known to suppress overall electroencephalographic activity (Maekawa et al., 1986) and cerebral metabolism (Sokoloff et al., 1977; Stullken et al., 1977; Wei et al., 1993) relative to the awake condition, and yet surprisingly it yields higher CBF. Higher CBF under isoflurane relative to the awake condition has been previously reported. In a study in which radionuclide-labeled microspheres were used to measure CBF in pigs, CBF under 1.45% isoflurane was reported to be 120% to 145% of that under the awake condition (Manohar and Parks, 1984). Similarly, Hoffman et al. (1991) reported comparable CBF increases in rats under isoflurane anesthesia relative to the awake condition. These observations suggest that the intricate neural–vascular coupling is perturbed by isoflurane (i.e., CBF is not commensurate with neural activity). The most likely explanation is that isoflurane is a potent cerebro-vasodilator, which leads to a global increase in perfusion. Isoflurane's being a strong cerebro-vasodilator had been demonstrated (Hensen et al., 1988; Matta et al., 1999). In addition to a global CBF difference between awake and anesthetized conditions, there are *regional* differences in CBF between awake

and anesthetized conditions, suggesting that there are intrinsic regional differences in metabolism and/or neural activity between the awake and anesthetized conditions, as expected. Regions that have higher metabolism and neural activity (pixels with smaller negative differences in Fig. 1E) under the awake condition relative to the anesthetized condition were the cortex, the corpus callosum, and the thalamus. Preferential higher basal CBF in these three structures in the awake condition are not unexpected. Cortex generally has higher CBF compared with most other brain structures, probably because of the high basal metabolic activity for processing sensory information. Evidently, there is increased sensory processing in the awake condition. The thalamus, whose primary role is to integrate signals from various brain regions and is the gatekeeper of the cortical input and output, is also expected to increase activity under the awake condition. Finally, increased activity associated with being awake also requires increased communication between the two cerebral hemispheres, thus leading to increased CBF in the corpus callosum under awake condition.

Implications in stroke and functional magnetic resonance imaging studies

Anesthetics also affect stroke outcome (Jacewicz and Pulsinelli, 1986; Molinari, 1986; Warner et al., 1995). Both halothane and sevoflurane reduce ischemic damage relative to that incurred in awake rats (Warner et al., 1993). Similarly, isoflurane has been shown to attenuate delayed development of selective neuronal necrosis in periinfarct areas (Kawaguchi et al., 2000). A recent study showed that concurrent or delayed administration of propofol is neuroprotective relative to the awake preparations (Gelb et al., 2002). Furthermore, although numerous therapeutic drugs showed positive results in experimental stroke, none has proven to be effective clinically, especially in terms of neuroprotection (DeGraba and Pettigrew, 2000; Liebeskind and Kasner, 2001). Although there are many possible reasons why this may be so, one crucial factor is that the models to date have been done in anesthetized animals and, thus, anesthesia could be a confounding factor.

Anesthetics could affect fMRI signals by suppressing neural activity, perturbing the neural–vascular coupling associated with increased neural activity and/or modulating basal blood flow. Numerous studies have shown that anesthetics in general, and isoflurane in particular, decrease glucose and oxygen metabolism (Stullken et al., 1977) and EEG activity (Maekawa et al., 1986). Our results showed that the cerebrovascular reactivity was markedly attenuated by isoflurane relative to the awake condition. This observation is consistent with previous studies in which blood flow on the cortical surface was measured using laser Doppler flowmetry. In those studies, it was reported that the vascular reactivity to carbon dioxide under halothane (Bonvento et al., 1994) and isoflurane (Strebel et al., 1993) was markedly reduced relative to the awake condition. Similarly, in another study in which blood flow in the middle cerebral artery was measured using the transcranial Doppler method, ketamine was found to attenuate cerebrovascular response to carbon dioxide in humans (Nagase et al., 2001).

Anesthetics also have an effect on the fMRI signals *per se* because basal blood flow modulates the magnitude and the dynamics of the BOLD responses (i.e., time to peak and full-width at half maximum of the fMRI response function). It has been shown that a higher basal CBF (induced by hypercapnia) yields a smaller stimulus-evoked BOLD percent change and a slower, broader hemodynamic response associated with visual stimulation (Kemna and Posse, 2001; Cohen et al., 2002). Therefore, the BOLD and CBF responses are expected to be dependent on the anesthetics used.

Anesthetics also have important implications in using the early negative BOLD (the initial dip) to map brain functions. Although many laboratories have shown the existence of the initial dip in humans (Menon et al., 1995; Hu et al., 1997) and in animal models (Duong et al., 2000*a*; Kim et al., 2000), and high-resolution mapping of columnar structures has been reported using the initial dip (Duong et al., 2000*a*; Kim et al., 2000), the initial dip remains controversial (Buxton, 2001). The inconsistency across laboratories regarding the existence of the early negative BOLD could arise from many factors, including nature of the stimuli, stimulation duration, areas of the brain stimulated, animal models, data analysis, and anesthetics. For example, in humans, the early negative BOLD can only be detected using a "model function" (Yacoub et al., 1999), and the early dip is completely averaged out if all pixels that show a positive response are considered (Menon et al., 1995; Hu et al., 1997). In marked contrast, the negative BOLD response can be readily detected with an ROI over the entire visual cortex in an anesthetized cat model (Duong et al., 2000*a*; Kim et al., 2000). It has been further shown that the CBF response to stimuli is markedly delayed and sluggish under isoflurane in the cat model, likely to accentuate the initial dip (Duong et al., 2001*a*; Kim and Duong, 2002). This comparison suggests that anesthetics could be a major factor, namely, isoflurane anesthesia could cause a delayed CBF increase (i.e., sluggish CBF responses), resulting in the initial dip. Further investigation is warranted.

CONCLUSIONS

Surprisingly, basal CBF under the awake condition was substantially lower than that under isoflurane anesthesia. Regional CBF differences between awake and anesthetized rats were rather heterogeneous. The hypercapnia-evoked CBF and BOLD percent changes were

substantially higher in the awake than in the anesthetized condition, suggesting that anesthesia markedly suppressed cerebrovascular reactivity to carbon dioxide and thus neural–vascular coupling. This study showed that quantitative perfusion imaging has the potential to be used to investigate brain functions and cerebral ischemia in fully conscious animals.

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