Effects of the $\alpha_2$-adrenergic receptor agonist dexmedetomidine on neural, vascular and BOLD fMRI responses in the somatosensory cortex

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Abstract
This article describes the effects of dexmedetomidine (DEX) – the active ingredient of medetomidine, which is the latest popular sedative for functional magnetic resonance imaging (fMRI) in rodents – on multiple unit activity, local field potential (LFP), cerebral blood flow (CBF), pial vessel diameter (indicative of cerebral blood volume [CBV]), and blood oxygenation level-dependent (BOLD) fMRI. These measurements were obtained from the rat somatosensory cortex during 10 s of forepaw stimulation. We found that the continuous intravascular systemic infusion of DEX (50 $\mu$g/kg/h, doses typically used in fMRI studies) caused epileptic activities, and that supplemental isoflurane (ISO) administration of -0.3% helped to suppress the development of epileptic activities and maintained robust neuronal and hemodynamic responses for up to 3 h. Supplemental administration of $N_2O$ in addition to DEX nearly abolished hemodynamic responses even if neuronal activity remained. Under DEX + ISO anesthesia, spike firing rate and the delta power of LFP increased, whereas beta and gamma power decreased, as compared with ISO-only anesthesia. DEX administration caused pial arteries and veins to constrict nearly equally, resulting in decreases in baseline CBF and CBV. Evoked LFP and CBF responses to forepaw stimulation were largest at a frequency of 8–10 Hz, and a non-linear relationship was observed. Similarly, BOLD fMRI responses measured at 9.4 T were largest at a frequency of 10 Hz. Both pial arteries and veins dilated rapidly (artery, 32.2%; vein, 5.8%), and venous diameter returned to baseline slower than arterial diameter. These results will be useful for designing, conducting and interpreting fMRI experiments under DEX sedation.

Introduction
Anesthesia is commonly used in functional brain imaging studies that investigate a wide variety of neuroscience research questions, including neurovascular relationships, pain mechanisms, consciousness, longitudinal functional development, and reorganisation. Imaging studies are often performed in immobilised animals to avoid motion artefacts. As immobilisation without stress is not often possible, particularly for relatively long recording sessions in alert animals, the use of anesthesia is beneficial. Anesthesia, however, can modify neuronal and hemodynamic activity (Berwick et al., 2002; Martin et al., 2006). Thus, it is critical to understand the impact of anesthesia on neuronal and hemodynamic responses.

Two anesthetic agents, isoflurane (ISO) and medetomidine (MED), are often used for functional magnetic resonance imaging (fMRI) experiments, because of their ability to enable longitudinal studies. Our group previously explored the impact of ISO anesthesia on neurovascular responses in the rat forepaw model (Masamoto et al., 2007, 2009; Kim et al., 2010). ISO at approximately one minimum alveolar concentration (1.38%) (White et al., 1974) provides a stable physiological situation for experiments lasting for > 6 h. However, ISO strongly suppresses neuronal activity (Banoub et al., 2003) and dilates blood vessels (Lida et al., 1998), thus reducing evoked hemodynamic responses. Thus, the use of ISO for fMRI studies has been limited. Meanwhile, MED has recently become popular for rodent fMRI studies over various brain regions, including the brain stem, hippocampus, basal ganglia, and neocortex (Van Camp et al., 2006; Weber et al., 2006; Zhao et al., 2008; Angenstein et al., 2010), largely because MED yields robust hemodynamic responses; however, this effect lasts for < 3 h (Pawela et al., 2009). Despite the popularity of MED, its effects on physiological parameters, such as cerebral blood flow (CBF), cerebral blood volume (CBV), and neuronal activity, at the doses typically used in fMRI studies (100 $\mu$g/kg/h) have not been established.

The pharmacological effects of MED are attributed to its optical isomer, dexmedetomidine (DEX), and thus both MED and DEX exert the same physiological effects (Vickery & Maze, 1989; MacDonald et al., 1991; Savola & Virtanen, 1991; Schmeling et al., 1991). DEX is a highly selective $\alpha_2$-adrenergic receptor agonist that crosses the blood–brain barrier. DEX produces sedation and analgesia dose-dependently, with a minimal effect on the respiratory system, despite the muscular relaxation that it causes (Doze et al., 1989; Kalso et al., 1991; Correa-Sales et al., 1992; Guo et al., 2002; Martin et al., 2006). Thus, it is critical to understand the impact of the physiological effects of MED on neuronal and hemodynamic activities and local field potential (LFP) activity.
1.4% ISO in O2-enriched air (25°C, 35% O2) for at least 30 min before experiments were started. During experimental recordings, the mean arterial blood pressure (MAP), heart rate, respiration rate, end-tidal CO2, O2, N2O and ISO levels were monitored (Capnomac Ultima; Datex Engstrom, Tewksbury, MA, USA) and recorded with a polygraph data acquisition system (MP150 and ACK100W Acknowledge; Biopac Systems, Goleta, CA, USA). Rectal temperature was maintained at 37.0°C with a feedback-controlled heating pad (40-90-8°C; FHC, Bowdoinham, ME, USA).

Experimental recordings of evoked somatosensory activity were performed under 1.3–1.4% ISO. After these experiments, 50 µg/kg DEX (Dexdomitor; Pfizer, NY, USA) was injected intravenously as a bolus, and the ISO level was reduced to 0.5%. Fifteen minutes after the bolus injection, continuous intravenous infusion of DEX at a rate of 50 µg/kg/h was commenced. The infusion liquid also contained pancuronium bromide (1.5 mg/kg/h) and 5% dextrose. It should be noted that a dose rate of 50 µg/kg/h DEX is equivalent to 100 µg/kg/h MED, which is the typical dose rate used in most published fMRI studies (Ramos-Cabrer et al., 2005; Weber et al., 2006, 2008; Pawela et al., 2008, 2009; Zhao et al., 2008; Airaksinen et al., 2010; Angenstein et al., 2010; Seehafer et al., 2010; Williams et al., 2010; Krautwald & Angenstein, 2012; Nasrallah et al., 2012).

For electrical stimulation, two needle electrodes were inserted between digits 2 and 4 under the palmar skin of either the left or right forepaw. Electrical pulses were generated with a waveform generator (Master 8; AMPI, Israel) and delivered with a constant current isolator (Iso-Flex; AMPI) to the forepaw contralateral to the recording site. Electric pulses with a width of 1 ms and a current of <1.5 mA were delivered for 10 s. No MABP change resulting from the stimulation was observed.

Materials and methods
Twenty-five male Sprague-Dawley rats (260–450 g; Charles River Laboratories, Wilmington, MA, USA) were used, following an animal protocol approved by the University of Pittsburgh Institutional Animal Care and Use Committee, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Twenty-one rats were used for non-fMRI studies, and four rats were used for fMRI studies with DEX + ISO anesthesia.

Animal preparation
The rats were initially anaesthetised with 5% ISO in O2-enriched air (30–35% inspired O2) for intubation. Then, lidocaine gel (2%) was applied locally for placement of catheters in a femoral artery and vein under 2–2.5% ISO. Atropine was not used in the present studies, because its anticholinergic activity can cause adverse cardiovascular effects with DEX administration [for a review, see Sinclair (2003)]. The respiration rate and ventilation volume of the ventilator (TOPO; Kent Scientific, Torrington, CT, USA) were adjusted to maintain normal blood gas levels throughout experiments. Approximately 0.1 mL of blood was withdrawn from the femoral artery for measurement of the arterial partial pressure of oxygen (PaO2), arterial partial pressure of carbon dioxide (PaCO2) and pH with a blood gas analyser (Stat Profile, Nova Medical Corp., Waltham, MA, USA). For non-fMRI physiological studies, the rats were placed in a stereotaxic frame (SR-5R; Narishige, Tokyo, Japan). Lidocaine gel (2%) was applied at pressure points of the stereotaxic frame, and 0.3 mL of 2% lidocaine was administered under the skin over the targeted craniotomy location. A custom-made recording chamber (outer diameter, 2.2 cm) was mounted on the exposed skull over the forepaw cortical area with dental acrylic. A 5 × 7-mm portion of the skull, centered 3.5-mm lateral and 1.5-mm rostral from bregma, was thinned with a dental drill, and then removed with forceps. The cerebrospinal fluid was released by performing a dural puncture of the cisterna magna prior to duratomy, to minimise herniation. The dura mater over the forepaw cortical area was resected. Both the chamber and the dural puncture site were then sealed with agarose gel (typically 0.4%) at body temperature.

The anesthesia and breathing gas mixture were adjusted to 1.3–1.4% ISO in O2-enriched air (25–30% inspired O2) for at least 30 min before experiments were started. During experimental recordings, the mean arterial blood pressure (MAPB), heart rate, respiration rate, end-tidal CO2, O2, N2O and ISO levels were monitored (Capnomac Ultima; Datex Engstrom, Tewksbury, MA, USA) and recorded with a polygraph data acquisition system (MP150 and ACK100W Acknowledge; Biopac Systems, Goleta, CA, USA). Rectal temperature was maintained at 37.0°C with a feedback-controlled heating pad (40-90-8°C; FHC, Bowdoinham, ME, USA).

Experimental designs
Three major experiments were performed: (i) evoked neural activity and hemodynamic responses were recorded under DEX only vs. DEX + ISO vs. DEX + N2O; (ii) spontaneous neural activity and baseline hemodynamics were recorded under ISO only vs. DEX + ISO; and (iii) evoked neural activity and hemodynamic responses under DEX + ISO were characterised. All functional studies under DEX were performed at least 30 min after the bolus injection of DEX. Neural activities [i.e. local field potentials (LFPs) and multiple unit activity (MUA)] were measured with a metal microelectrode; CBF was measured with laser Doppler flowmetry (LDF); vessel diameters were measured with optical imaging; and blood oxygenation level-dependent (BOLD) fMRI was performed with a 9.4-T magnetic resonance imaging scanner. Specific experiments were as follows (for a summary, see Table 1).

Experiment 1.1 – Evoked LFP and CBF responses under DEX only
To examine whether robust and stable evoked hemodynamic responses can be obtained under DEX-only anesthesia, electrical stimulation at a frequency of 3, 4, 6, 8, 9, 10 or 12 Hz for 10 s with a 70-s interstimulus interval (ISI) was intermittently delivered to the forepaw to evoke LFP and CBF responses in the somatosensory cortex. These experiments were conducted in five rats, and experimental recording lasted for >120 min, during which DEX alone was continuously infused (50 µg/kg/h). To attempt to extend the effects of DEX demonstrated by Pawela et al. (2009), the infusion dose rate was increased from 50 µg/kg/h to 150 µg/kg/h (equivalent to 300 µg/kg/h MED) 120–156 min after the initial DEX bolus administration in three rats, and cortical evoked responses to forepaw stimulation (4, 6, 8, 10 or 12 Hz for 10 s with a 70-s ISI) were recorded. The stimulation frequency over all experiments was randomised between trials. At least two trials were recorded for each frequency in all experiments.
TABLE 1. Summary of experiments

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**Experiment 1.2 – Comparison between DEX + ISO and DEX + N2O conditions**

To examine the effect of inspired N2O on evoked responses in DEX-sedated rats, LFP and CBF responses to forepaw stimulation at four different frequencies (3, 6, 9 or 12 Hz for 10 s with a 70-s ISI) were recorded under DEX + N2O (30% O2/70% N2O) and DEX + ISO (< 0.5% ISO in 30% O2/70% N2) in seven rats. It should be noted that ISO did not decrease to zero for a long time after the anesthetic vaporiser was completely turned off (> 1 h). As 0.1% ISO has almost no synergistic effect in combination with N2O (Sloan et al., 2010), experiments under DEX + N2O were initiated when the ISO level was < 0.1%.

**Experiment 1.3 – Effective duration of DEX + ISO for functional studies**

To examine the stability of evoked responses over time, LFP and CBF responses to forepaw stimulation (8 Hz for 10 s with a 70-s ISI) were monitored for > 180 min during continuous infusion of DEX (50 μg/kg/h) with ISO (0.1–0.5%) in six rats. Two to five 8-Hz stimulation runs were recorded and averaged. To compare the effects of DEX + ISO and those of ISO only, LFP and CBF responses evoked by 10 s of 8-Hz stimulation (ISI of 70 s) were also recorded under ISO only. Five runs were averaged.

**Experiment 2.1 – Baseline MUA and LFP measurements before and after DEX administration**

To examine the effects of DEX on spontaneous neural activities, MUA and LFP were recorded for 5 min under ISO only and DEX + ISO in the absence of evoked stimulation in nine rats.

**Experiment 2.2 – Baseline CBF and vessel diameter measurements before and after DEX administration**

To examine the effects of DEX on baseline CBF and pial vessel diameters, LDF was performed simultaneously with pial vessel imaging under ISO only and DEX + ISO in 15 rats. The baseline CBF level and pial vessel diameters were compared between these two conditions.

**Experiment 3.1 – Frequency-dependent evoked LFP and CBF measurements under DEX + ISO**

LFP and CBF responses to forepaw stimulation at five different frequencies (4, 6, 8, 10 and 12 Hz for 10 s with a 70-s ISI) were recorded under DEX + ISO in seven rats.

**Experiment 3.2 – Frequency-dependent BOLD fMRI measurements under DEX + ISO**

BOLD fMRI responses to forepaw stimulation at five different stimulation frequencies (4, 6, 8, 10 and 12 Hz for 10 s with an 80-s ISI) were recorded under DEX + ISO in four rats. A total of 20–46 runs were repeated for each frequency.

**Experiment 3.3 – Evoked arterial and venous vessel diameter measurements under DEX + ISO**

Pial vessel diameters were measured during 10 s of forepaw stimulation at a frequency of 8 Hz with an ISI of 70 s in 11 rats. Five runs were performed.

**Data acquisition**

**Optical imaging**

Prior to the physiological experiments described above, the forepaw area was mapped with flavoprotein autofluorescence imaging over the primary somatosensory cortex under 1.3–1.4% ISO (Vazquez et al., 2010b, 2012). Flavoprotein autofluorescence images were acquired using an imaging software (MetaMorph, Molecular Devices, CA, USA) on an epi-fluorescence microscope (MVX-10; Olympus, Tokyo, Japan) equipped with a ×1 (0.25 NA) objective (Olympus) and a digital cooled-CCD camera (1392 × 1040 imaging pixels, 6.45 × 6.45 μm²/pixel, CoolSnap HQ2; Photometrics, Princeton, NJ, USA). A mercury lamp light source coupled to a low-noise power supply (100 W; Opti Quip, Highland Mills, NY, USA) was used. The transmitted light was filtered between 450 and 490 nm while the camera recorded the fluorescence emission between 500 and 550 nm. Images were captured at 10 frames/s with a field of view of 5.7 × 4.3–8.9 × 6.7 mm², depending on the microscope magnification. The camera’s exposure time was set to...
100 ms for an effective pixel resolution between 15.4 and 25.6 μm. Forepaw stimulation was performed at the optimal frequency of 12 Hz for 2 s with an ISI of 16 s. Ten runs were repeated and averaged to improve the signal-to-noise ratio.

Images of the cortical surface were captured to measure the diameter of pial vessels (Experiments 2.2 and 3.3). For this purpose, oblique light guides transmitting filtered yellow–green light (570 ± 10 nm) connected to a halogen light source (250 W: Thermo-Oriel, Stratford, CT, USA) were used for illumination. At this wavelength, the light absorption of oxyhemoglobin and that of deoxyhemoglobin are nearly equal, and thus both arteries and veins are equally visible. To prevent artefacts stemming from the LDF probe (780-nm laser light), a low-pass interference filter (<700 nm) was placed in front of the camera. Images were captured over a field of view of 1.7 × 1.2–8.9 × 6.6 mm² with an effective pixel resolution between 2.56 and 6.40 μm for the baseline study (Experiment 2.2), and over a field of view of 1.9 × 1.6–3.3 × 2.5 mm² at 10 frames/s with an effective pixel resolution between 2.6 and 5.1 μm for the functional study (Experiment 3.3).

**MUA, LFP and CBF measurements (all experiments except for 3.2)**

On the basis on the functional map generated, a microelectrode with a tip diameter of 5 μm (Carbostar-1; Kation Scientific, Minneapolis, MN, USA) was placed at a depth of ~0.3 mm below the cortical surface of the forepaw area (Fig. 1) to record neural activity extracellularly. Neural activity was recorded with an electrophysiological data acquisition system (MAP; Plexon, Dallas, TX, USA) at a 1-kHz sampling rate. Baseline neural activity (Experiment 2.1) was acquired for a 5-min period. MUA data were bandpass filtered between 400 Hz and 1 kHz, and LFP activity was bandpass filtered between 3.3 and 88 Hz.

A needle-type LDF probe with tip diameter of 450 μm (PeriFlux 4001 Master System; Perimed, Sweden) was placed on the cortical surface over the forepaw area, avoiding large pial vessels, to measure parenchymal CBF (Fig. 1). Relative changes in CBF were acquired by the LDF system with a time constant of 0.03 s, and recorded with the polygraph data acquisition software at a frequency of 100 Hz.

**BOLD fMRI (Experiment 3.2)**

BOLD fMRI experiments were performed on a 9.4-T MRI system with a Unity INOVA console (Varian, Palo Alto, CA, USA). The gradient coil used is actively shielded, and has an inner diameter of 12 cm, a maximum gradient strength of 40 G/cm, and a rise time of 0.12 ms (Magnex, Abington, UK). A surface coil (diameter, 2.3 cm) was positioned on top of the rat’s head for imaging. The magnetic field homogeneity was optimised by localised shimming to yield a typical water spectrum line-width of ~20 Hz. All fMRI images were acquired with a gradient echo echo-planar imaging sequence with an echo time of 20 ms, a repetition time of 1000 ms, an in-plane matrix of 64 × 64, a field of view of 2.3 × 2.0 cm, and a slice thickness of 2 mm, over four contiguous slices. All data were collected within 2 h after the bolus DEX administration.

**Data analysis**

All of the data were analysed with MATLAB (Mathworks, Natick, MA, USA). Optical images, LFPs, CBF data and BOLD fMRI data were averaged over multiple runs from identical stimulus frequency conditions. Analyses were performed on each rat separately before group averaging. All graphs, including scatter plots, box plots, and power law curve fitting, were produced with ORIGIN 7 (OriginLab Corp., MA, USA).

**Flavoprotein autofluorescence imaging map**

To determine forepaw area, activation maps were generated from flavoprotein autofluorescence images by calculating the relative increase in fluorescence (∆F/F). The differential image (∆F) was obtained by subtracting the average image over 1 s prior to stimulation (F) from the average image over the initial 1 s of stimulation. The maps were smoothed by use of a Gaussian kernel with a width of 5 × 5 pixels, and pixels over a threshold of >67% of the largest increase in fluorescence (∆F/F) were considered to be part of the active area (Fig. 1).

**Vessel diameter measurement (Experiments 2.2 and 3.3)**

Pial arteries and veins were visually distinguished on the basis of the differences in their color under the microscope with white light illumination (arteries are light red, and veins are dark red). The intraluminal vessel diameter was calculated from 570-nm optical images by placing a region of interest (ROI) with a four-pixel width perpendicular to the vessel direction. The image within the ROI was linearly interpolated, and the intensity along the four-pixel direction was measured. Assuming that the vessel is cylindrical, the actual diameter corresponds to 15.5% over the full-width-at-half-minimum value (Vazquez et al., 2010a).
For the study of evoked vessel diameter changes, feeding arteries and draining veins of the active area were chosen. To evaluate whether vessel diameter changed during stimulation, vessel diameters sampled from 5 s prior to stimulation and the 10-s stimulation period were compared by use of a Kruskal–Wallis test (one-way analysis of variance by ranks). If a significant difference was found in the median ($P < 0.05$), the vessel diameter was considered to significantly change with stimulation. To calculate evoked vessel diameter changes as a function of time (Experiment 3.3), the vessel diameters were normalised by their baseline value (5 s prior to stimulation onset). The maximal diameter change during the 10-s stimulation period was then extracted after low-pass filtering the time course with a cut-off frequency of 2 Hz. To examine the dynamic properties of the changes in vessel diameter, normalised time courses were averaged across all active arteries and veins.

**LFP, MUA and CBF data**

The evoked LFP data were first fully rectified and summed over the 10-s stimulation period. The summed LFP data were then multiplied by the sampling (0.001 s). For the baseline neural activity data (Experiment 2.1), the mean spike firing rate and LFP power spectral bands were determined for the 5-min recording in each rat. MUA and LFP signals were separated by bandpass filtering between 400 Hz and 1 kHz and between 3.3 and 88 Hz, respectively. To determine the mean spike firing rate (spikes/s), a one standard deviation (SD) threshold was applied for the ISO-only and DEX + ISO conditions (the SDs from both anesthesia conditions were averaged). A spike was considered to be present for each 1-ms time point where the MUA intensity exceeded the SD threshold (i.e. 1 spike/ms). The spike firing rate was obtained for each 1-s time bin, and the mean spike firing rate for 5 min was calculated. To determine the LFP power over specified spectral bands, the 5-min LFP data were Fourier-transformed. Then, the spectrum was divided into delta (1 to < 4 Hz), theta (4 to < 8 Hz), alpha (8 to < 13 Hz), beta (13 to < 30 Hz), and gamma (30 to < 50 Hz).

The CBF data were low-pass filtered with a 5-Hz cut-off frequency. The filtered CBF data were then normalised by their baseline value (5-s period prior to stimulation onset). The normalised CBF responses were summed over the 10-s stimulation period, and multiplied by the sample rate of 0.01 s. To examine the dynamic properties of the changes in CBF, each time course was normalised by its peak intensity and averaged across all rats.

**BOLD fMRI**

Correlation coefficients between the voxel-wise time courses and a canonical reference function were calculated. The fMRI data recorded for 8-Hz stimulation were used to determine the ROI for analyzing frequency-dependent studies. The ROI was defined as the 20 voxels with the highest correlation coefficients in the forepaw cortical area. Then, time courses were obtained from the ROI, and percentage signal changes were calculated from the baseline, the average value over the 5-s period prior to stimulation onset. A positive BOLD response was integrated over the 10-s stimulation period, and a post-stimulus undershoot was integrated over 5–25 s after stimulation offset. In each rat, the integral BOLD responses were determined for the five stimulation frequencies, and these were normalised by their maximum to reduce inter-animal variation.

**Statistical analysis**

Non-parametric statistical tests were used to compare medians. $P$-values of $<0.05$ were considered to be statistically significant. All data are expressed as median and interquartile range (IQR), unless otherwise specified.

**Results**

Blood gas measures of all experiments and their statistical results (Mann–Whitney $U$-test, Bonferroni-corrected) are reported in Table 2. For non-fMRI experiments, DEX administration significantly reduced $P_{O_2}$ ($P = 0.0002$) and $S_O_2$ ($P = 0.0054$), and increased hematocrit ($P = 0.0048$) and the hemoglobin ($P = 0.0051$) concentration, although they remained within normal physiological ranges (see ISO only vs. DEX50 + ISO for non-fMRI in Table 2). No significant difference was found in blood gas measurements between DEX + ISO and DEX-only conditions. For fMRI experiments, no significant difference was found in blood gas measurements between ISO-only and DEX + ISO conditions. However, $P_{CO_2}$ was significantly lower in non-fMRI experiments ($P = 0.0046$; see DEX + ISO (non-fMRI) vs. DEX + ISO (fMRI) in Table 2), possibly owing to hypocapnic conditions in fMRI rats.

### Table 2. Blood gas tests

<table>
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<tr>
<th>Blood gas parameters</th>
<th>Non-fMRI</th>
<th>fMRI</th>
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<tr>
<td></td>
<td>ISO only ($N = 21$)</td>
<td>DEX50 + ISO ($N = 16$)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.4 (0.2)</td>
<td>37.3 (0.3)</td>
</tr>
<tr>
<td>pH</td>
<td>7.511 (0.049)</td>
<td>7.482 (0.049)</td>
</tr>
<tr>
<td>$P_{O_2}$ (mmHg)</td>
<td>36.1 (5.6)</td>
<td>38.2 (4.7)</td>
</tr>
<tr>
<td>$P_{CO_2}$ (mmHg)</td>
<td>125.1 (16.0)</td>
<td>108.2 (8.0)$^*$</td>
</tr>
<tr>
<td>$S_O_2$ (%)</td>
<td>98.6 (1.2)</td>
<td>97.7 (0.9)$^{****}$</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>37.0 (2.3)</td>
<td>40.0 (4.0)$^{***}$</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>12.4 (0.6)</td>
<td>13.2 (1.3)$^{****}$</td>
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</table>

DEX50, DEX 50 μg/kg/h, intravenous; DEX150, DEX150 μg/kg/h, intravenous; Hb, hemoglobin; Hct, hematocrit; ISO, 1.3–1.4% for ISO only, 0.1–0.5% with DEX; $N$, numbers of rats used. All values are presented as medians (IQRs). Multiple comparisons were performed for four conditions from non-fMRI experiments. Similarly, multiple comparisons were performed for conditions from both non-fMRI and fMRI experiments (ISO only and DEX50 + ISO). Thus, the significant $P$-value of 0.05 is actually 0.0083 after Bonferroni correction. $^*P < 0.05$ vs. ISO only in non-fMRI group by Mann–Whitney $U$-test with Bonferroni correction. $^{**}P < 0.05$ vs. ISO in non-fMRI group by Mann–Whitney $U$-test with Bonferroni correction. $^{****}P < 0.05$ vs. ISO only in non-fMRI group by Mann–Whitney $U$-test with Bonferroni correction. $^{*}n = 3$. $^{**}n = 1.$
**DEX and ISO combination for functional studies**

**DEX only (Experiment 1.1)**

In almost all MED-sedated rat fMRI studies, recording was performed under MED only (typically 100 µg/kg/h, equivalent to 50 µg/kg/h DEX). Therefore, somatosensory evoked LFP and CBF responses from forepaw cortical area in five rats were first recorded under intravenous DEX only (50 µg/kg/h) (Fig. 2A, left column). Although robust, stimulation frequency-dependent CBF responses were observed, consistent with earlier fMRI studies (Zhao et al., 2008; Pawela et al., 2009), periods of epileptic neural discharges followed by electrical silence were also observed, resulting in abnormally prolonged CBF responses (see open arrowheads above the LFP and CBF traces in Fig. 2A, left column). Epileptic responses to 10 s of stimulation were observed in runs at 119–177 min after the initial DEX administration in four of the five rats tested (note – the DEX dose rate was modified for the remaining rat prior to the time period where epileptic activity was observed; Fig. 2B). These results suggest that evoked responses are prone to become epileptic when DEX is infused at a rate of 50 µg/kg/h for longer than 120 min.

To attempt to avoid this epileptic effect, the DEX dose was increased from 50 to 150 µg/kg/h after 120 min from the initial dose in three rats (see two rats’ data in Fig. 2). However, epileptic LFP and CBF responses were elicited as soon as stimulation studies were started (Fig. 2A, right column; Fig. 2B) in all three rats tested. Changes in MABP did not correlate with, or precede, the initiation of the epileptic discharges, suggesting that a systemic physiological perturbation did not trigger the epileptic-like incident at these dose rates (compare MABP and LFP traces in Fig. 2A and B). However, supplementing the intravenous DEX administration at 50 µg/kg/h with inspired ISO at 0.1–0.5% successfully mitigated the stimulation-evoked epileptic response in 15 of 16 rats over a median recording time of 188 min (IQR, 79.3 min; minimum, 123 min; maximum, 306 min). Only one rat showed epileptic responses 171 min after the initial DEX administration under DEX + ISO at 0.5%. Therefore, the rest of the experiments were performed under intravenous DEX at 50 µg/kg/h and ISO (0.1–0.5%, typically ~0.3%) to avoid the possible development of epileptic responses.

**DEX with N2O (Experiment 1.2)**

Inhaled N2O is widely used with other anesthetics to enhance analgesic effects in clinical, veterinary and research settings. However, N2O is an N-methyl-D-aspartate receptor antagonist (Jevtovic-Todorovic et al., 1998; Mennerick et al., 1998) that is known to reduce hemodynamic responses (Norup Nielsen & Lauritzen, 2001; Gsell et al., 2006). Indeed, inhaled N2O suppresses somatosensory evoked hemodynamic responses in combination with ISO at ~1.4% in rats (Masamoto et al., 2007; Kim et al., 2010). Thus, the effect of supplementary inhaled N2O on LFP and CBF responses was also tested in seven DEX-sedated rats (Fig. 3). As a control, evoked LFP and CBF responses were first recorded under DEX + ISO in O2-enriched air (30%O2 and 70%N2). ISO was then discontinued, and the inspired air was replaced with a mixture of 30%O2 and 70%N2O. No significant changes in vascular physiological parameters were found (Table 3). Additionally, N2O inhalation did not change the baseline CBF (see control vs. test panels in Fig. 3A, and left panel in Fig. 3B). However, the evoked LFP responses at 6 and 9 Hz under N2O were significantly smaller than those in the control condition ($P = 0.0262$ for 6 Hz, $P = 0.0478$ for 9 Hz, $n = 7$ rats, Mann–Whitney U-test). Similarly, the evoked CBF responses at 6, 9 and 12 Hz under N2O were significantly smaller than those under the control condition ($P = 0.0111$ for 6 Hz, $P = 0.0006$ for 9 Hz, $P = 0.0379$ for 12 Hz, $n = 7$ rats, Mann–Whitney U-test).

**Fig. 2.** CBF and LFP responses in DEX-sedated rats without supplemental ISO. (A, B) Examples obtained from two rats. Evoked responses are prone to induce epileptic activity after ~120 min from the initial DEX administration. First row – intravenous infusion of DEX plotted as a function of time from the initial bolus injection of 50 µg/kg (0 min). Open arrowheads on the time scale indicate the beginning of a block of five stimulation runs (each consisting of 10 s of forepaw stimulation with a 70-s ISI). Arrows with filled arrowheads indicate a block containing epileptic responses. Traces of MABP (second row), CBF (third row) and LFP (fourth row) responses from the blocks indicated by the arrows in the first row are shown. The time elapsed from the DEX bolus injection is shown above the MABP trace. Gray vertical bars in each panel indicate 10-s stimulation periods, and the numbers under the LFP traces indicate the stimulation frequencies, which were delivered in a pseudo-randomised order. Open arrowheads in the CBF and LFP traces point to apparent epileptic responses without any change in MABP.
Upon replacement of the O₂/N₂O breathing mixture with ISO and O₂-enriched air, both LFP and CBF responses fully recovered (see recovery, right panel in Fig. 3A and B), although the CBF response at 9 Hz in the recovery condition was significantly larger than that under control conditions ($P = 0.0221$, $n = 7$ rats, Mann–Whitney U-test).

Overall, N₂O significantly suppressed CBF responses to a larger extent than LFP responses (middle vs. right panel in Fig. 3B). For instance, the CBF response to 9-Hz stimulation under DEX + N₂O was only 28% (median) of the response under DEX + ISO, whereas the LFP response was 87% of the control. These results suggest that supplementary ISO (< 0.5%) is a more suitable combination with DEX than N₂O.

DEX with ISO (Experiment 1.3)

The use of supplementary ISO, a known vasodilator, did not seem to affect the baseline CBF level under DEX anesthesia (compare Figs 2 and 3). Similarly, at a level of 0.5%, ISO did not appear to decrease the amplitude of the evoked LFP response; however, the amplitude of the CBF response was slightly smaller that obtained under DEX-only sedation (compare Figs 2 and 3), although it was still robust. As compared with ISO only, DEX + ISO significantly increased evoked response amplitudes. At a median time of 10.2 min (IQR, 16.0 min) after the initial DEX administration, the LFP and CBF responses to 10 s of 8-Hz forepaw stimulation under DEX + ISO were 1.5-fold and 3.7-fold larger, respectively, than those under ISO only (both LFP and CBF responses became significantly larger; $P = 0.0313$, $n = 6$ rats, Wilcoxon signed rank test). No significant difference in MABP was found between these two conditions ($P = 0.1563$, $n = 6$ rats, Wilcoxon signed rank test). Our data indicate that DEX + ISO significantly enhances evoked hemodynamic responses.

However, this robust CBF response under DEX + ISO anesthesia was not sustainable over a long experimental time window, despite preserved LFP responses (Fig. 4). In general, the CBF response was robust over the initial 120–180 min. The baseline CBF level tended to decrease over time, becoming unstable. In addition, MABP tended to decrease over time, although it remained within a normal physiological range (80–120 mmHg). Thus, it was necessary to decrease the supplementary ISO level during the course of experimental recording to maintain MABP and obtain robust evoked CBF responses. When the supplementary ISO level was decreased below 0.1%, evoked responses occasionally became epileptic, and thus the ISO level was always maintained above 0.1% (typically, ~0.3%).
TABLE 3. MABP and inspired gas concentrations in experiment 1.2

<table>
<thead>
<tr>
<th></th>
<th>Control (DEX + ISO)</th>
<th>Test (DEX + N₂O)</th>
<th>Recovery (DEX + ISO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recording onset time</td>
<td>96.0 (10.8)</td>
<td>120.0 (8.3)</td>
<td>141.0 (9.8)</td>
</tr>
<tr>
<td>from initial DEX</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>administration (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>96.5 (3.3)</td>
<td>98.2 (4.8)</td>
<td>113.8 (11.2)</td>
</tr>
<tr>
<td>ISO (%)</td>
<td>0.3 (0.1)</td>
<td>0.07 (0.03)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>O₂ (%)</td>
<td>30.8 (2.3)</td>
<td>30.3 (2.4)</td>
<td>30.3 (1.9)</td>
</tr>
<tr>
<td>N₂O (%)</td>
<td>0.04 (0.00)</td>
<td>67.1 (2.1)</td>
<td>3.7 (1.8)</td>
</tr>
</tbody>
</table>

Values are medians (IQRs) from seven rats. No significant difference was found in MABP medians between control, test and recovery conditions [Kruskal–Wallis test, \( \chi^2(2, N = 21) = 4.9647, P = 0.0835 \)]. Similarly, no significant difference was found in inspired O₂ medians between control, test and recovery conditions [Kruskal–Wallis test, \( \chi^2(2, N = 21) = 0.2301, P = 0.8913 \)].

Spontaneous neuronal activity (Experiment 2.1)

Under DEX + ISO anesthesia, MUA was more phasic and the LFP waveforms were broadened (Fig. 5A), suggesting more synchronous neuronal activity. The mean spike firing rate was significantly increased from 3.5 to 6.9 spikes/s (Fig. 5B) by DEX administration (\( P = 0.00049362, n = 9 \) rats, Mann–Whitney U-test). To quantify the effect of ISO-only and DEX + ISO conditions on LFP activity, LFP power spectrum bands were compared (Fig. 5C). DEX increased delta band power significantly (\( P = 0.0040, n = 9 \) rats, Mann–Whitney U-test), whereas it significantly decreased beta and gamma band power (\( P = 0.0005 \) for beta, and \( P = 0.0067 \) for gamma, Mann–Whitney U-test).

Baseline vessel diameter (Experiment 2.2)

To examine whether basal CBV is changed by DEX, pial vessel diameters were measured before and after DEX administration (Fig. 6). DEX constricted both arteries and veins (Fig. 6A). The constriction reached a plateau 30 min after the initial bolus administration (Fig. 6B). This observation was consistent in 15 of the 16 rats studied. The medians of arterial and venous diameters at 67.5 min (IQR, 7 min) after initial DEX administration were 77% (\( n = 94 \)) and 74% (\( n = 331 \)), respectively, of those under ISO only (Fig. 6C). No significant difference was found between arterial and venous diameter changes (\( P = 0.7546, \) Mann–Whitney U-test), suggesting that systemic DEX administration equally constricts pial arteries and veins. The degree of constriction was related to the vessel diameter, so that vessels of larger diameter constricted by greater amounts (Spearman’s rank correlation, \( r_{331} = -0.4031, P = 0.00064899 \) for artery; \( r_{331} = -0.1487, P = 0.0067 \) for vein).

Baseline CBF (Experiment 2.2)

Systemic administration of DEX significantly decreased baseline CBF in all 15 rats studied, as shown in Fig. 6D (\( P = 0.00022289, n = 15 \) rats, Wilcoxon rank sum test). The median CBF baseline in the DEX + ISO condition was 47% of that in the ISO-only condition, suggesting that intracortical vessels may also constrict.

Characterisation of neuronal and vascular responses under DEX + ISO anesthesia

The optimal stimulation frequency varies with the anesthetic used (Masamoto et al., 2007). Zhao et al. (2008) examined the dependence of the hemodynamic response amplitude on forepaw stimulation frequency in rats anesthetised with MED only, and found that the largest BOLD fMRI response was observed at 9 Hz, although no significant difference was found when the response was compared with those at 6–15 Hz. Here, we examined the impact of supplemental ISO administration on neurovascular responses as a function of forepaw stimulation frequency by measuring LFP and CBF and performing BOLD fMRI.
Effects of dexmedetomidine on neurovascular responses

Although the LFP and CBF responses had the same optimal frequency (i.e. 8–10 Hz), their tuning curves differed significantly [Friedman test, two-way analysis of variance by ranks, $\chi^2(1, N = 7) = 21.5753$, $P = 0.00000034020$], indicating a non-linear relationship between LFP and CBF responses. The relationship observed between the LFP and CBF responses (Fig. 7C) was well described by a power law function ($CBF = aLFP^b$, where $a = 1.03 \pm 0.09$ and $b = 3.84 \pm 0.70$, $R^2 = 0.94$). Under DEX-only conditions, CBF was well described by $aLFP^b$, where $a = 1.00 \pm 0.10$ and $b = 3.23 \pm 0.86$ ($R^2 = 0.90$) (data not shown), suggesting no change in neurovascular coupling resulting from supplemental administration of ISO.

Stimulation frequency-dependent BOLD fMRI (Experiment 3.2)

BOLD responses evoked by forepaw stimulation were measured from the somatosensory forepaw cortical area in four DEX + ISO-anesthetised rats for $>3$ h. After approximately 2–3 h from the initial DEX administration, baseline signal oscillations became apparent, and the evoked responses became unclear in two rats (not shown). Thus, frequency-dependent BOLD data were averaged over the initial 2 h after DEX bolus injection. Forepaw stimulation induced significant BOLD responses in the contralateral cortical somatosensory area for all frequencies (Fig. 8A). Time courses of frequency-dependent BOLD responses from all four rats are shown in Fig. 8B. In all four rats, similar frequency-dependent trends were observed. The commonly observed post-stimulus undershoot was present regardless of stimulation frequency in all four rats. To obtain stimulation frequency tuning, normalised BOLD signals over the 10-s stimulation period average and over the 20-s post-stimulus offset period average were plotted as a function of stimulation frequency (Fig. 8C). On average, the tunings from both the positive response and the post-stimulus undershoot appeared to be similar; the maximum response was observed at ~10 Hz. The optimal frequency found in BOLD fMRI studies was consistent with the LDF-based CBF results in this study as well as with an earlier MED-only BOLD fMRI report (Zhao et al., 2008).

Evoked pial vessel diameter changes (Experiment 3.3)

Arterial vessel dilation is a major component of the vascular response, whereas venous vessel dilation is minimal during 10 s of forepaw stimulation under either ISO or $\alpha$-chloralose anaesthesia (Hillman et al., 2007; Kim et al., 2007; Vazquez et al., 2010a; Zong et al., 2012). To examine whether this is the case for DEX + ISO anaesthesia, the diameter changes in pial arterial and venous vessels were measured (Fig. 9). As seen in a representative example (Fig. 9A), vessel diameter increased not only in pial arteries but also in veins during forepaw stimulation. The diameter changes of arterial vessels were larger than those of venous vessels. The maximal diameter changes in feeding arteries and draining veins of the active area from all 11 rats are shown in Fig. 9B; significant dilation of vessels was found in all arteries ($n = 38$; $3 (2.0)$ vessels/ rat (median and IQR)) during stimulation, and significant diameter changes were found in 85% of veins measured ($n = 80/94$; $7 (3.3)$ vessels/rat). Median increases in arterial and venous vessel diameters were 32.2% and 5.8%, respectively (Fig. 9B histogram), which were significantly larger than 0 ($P = 0.00001777979$ for artery ($n = 38$), $P = 0.00000000069148$ for vein ($n = 94$), Wilcoxon signed rank test). The change in arterial diameter appeared to be greater in smaller arteries, even though this finding was not statistically significant (Spearman’s rank correlation coefficient, $r_{38} = -0.2726$,

**Fig. 5.** Intravenous DEX administration makes MUA phasic and broadens the LFP waveform. (A) Spontaneous MUA and LFP recorded for 5 min in one rat are compared with those recorded before (upper two traces) and 33 min after (lower two traces) DEX administration. A 5-s segment is shown for better visualisation. (B) Histogram of spontaneous spike firing rates (open bar, ISO; gray bar, DEX + ISO) with a bin size of 1 s. Open and gray arrowheads indicate the medians of spontaneous spike firing rates for ISO (3.5 spikes/s) and DEX + ISO (6.9 spikes/s) conditions, respectively. These medians are significantly different ($P < 0.05$). (C) Comparisons of the LFP power bands before and after DEX administration. Results are shown as box plots (open box, ISO; gray box, DEX + ISO). DEX significantly increased the power of the delta band, whereas it significantly decreased the power of the beta and gamma bands ($P < 0.05$). In B and C ($n = 9$ rats), recording was started at a median time of 31.0 min (IQR, 4.3 min) after initial DEX administration. Before and after DEX administration, the median ISO levels were 1.3% (IQR, 0.1%) and 32.2% and 5.8%, respectively (Fig. 9B histogram), which were significantly larger ($P = 0.00006$; Mann–Whitney U-test, Bonferroni-corrected) than responses at other frequencies (Fig. 7B).
No relationship was found between venous diameter and its change during stimulation ($r_{94} = 0.0687$, $P = 0.5104$).

Finally, the dynamic properties of CBF and vascular responses were examined by plotting normalised time courses of CBF along with arterial and venous diameter changes (Fig. 9C). The diameter changes in both arterial and venous vessels occurred as quickly as the CBF response. The time to return to baseline was observed first in CBF, then in arterial diameter, and finally in venous diameter. No post-stimulus undershoot was observed in CBF, arterial and venous diameter changes.

### Discussion

**DEX can elicit epileptic evoked responses**

We have demonstrated that forelimb stimulation under continuous administration of DEX at 50 μg/kg/h for more than approximately 2 h elicited epileptic LFP responses with concomitant large increases in CBF (Fig. 2). Increasing the dose of DEX (150 μg/kg/h) did not prevent the development of the epileptic response. However, the addition of supplementary ISO (e.g. ~0.3%) suppressed the generation of the epileptic activity. Seizure activity associated with α2-adrenergic receptor agonists (Mirski et al., 1994; Miyazaki et al., 1999; Rainger et al., 2009) can be triggered by several factors. First, if $O_2$ availability is greatly decreased because of CBF reduction (as may happen with administration of DEX), hypoxic injuries could lead to seizures (Jensen et al., 1991). However, $O_2$ did not decrease to hypoxic levels under DEX with or without ISO (Table 2), and $O_2$ appeared to be similar under DEX with or without ISO. Thus, hypoxia is probably not the mechanism causing the epileptic responses. Second, hyper-excitability induced by hyperglycemia (Schwechter et al., 2003) could cause an evoked epileptic response. However, although both ISO and DEX can induce hyperglycemia (Nakadate et al., 1980; DiTullio et al., 1984; Morgan & Montague, 1985; Hikasa et al., 1996; Kawano et al., 2008), the data showed...
that only DEX elicited epileptic responses. Thus, hyperglycemia is probably not the primary cause of the epileptic response. Finally, perturbation of central adrenergic effects by DEX is most probably involved in seizure expression. As lesion of the locus coeruleus – the central adrenergic nucleus and the main target of DEX (Guo et al., 1996) – causes seizures (Oishi & Suenaga, 1982), DEX administration may cause alternation of the net presynaptic inhibitory and postsynaptic excitatory balance (Samuels & Szabadi, 2008), eliciting evoked epileptic activity.

**DEX supplemented with ISO prevents evoked epileptic responses**

With supplementary ISO administration at levels > 0.1%, the evoked epileptic response was successfully suppressed in this study, possibly because of enhancement of the inhibitory action of GABA by ISO [for a review, see Campagna et al. (2003)]. As ISO at 0.1 minimum alveolar concentration (0.1–0.2%) produces hyperalgesia (Zhang et al., 2000), DEX in combination with ISO at between 0.3% and 0.5% is recommended for rat forepaw model studies (e.g., Zhao et al., 2012).

Although supplemental ISO administration with DEX effectively suppressed the development of evoked epileptic responses, it did not seem to stably extend the effects of DEX; that is, robust evoked CBF responses were usually obtained for up to at most 3 h under DEX + ISO anesthesia (Fig. 4). This duration matches the duration of the sedative effect of DEX; the effective hypnotic duration for DEX at 50 μg/kg/h (intraperitoneal, or intravenous MED at 100 μg/kg/h) is ~3 h (Doze et al., 1989; Pawela et al., 2009). Maintaining a stable sedative level is important for experimentation, as well as for obtaining robust responses. Increasing the blood concentration of DEX (Pawela et al., 2009) would enhance the analgesic effect, but would not extend the sedative effect (Pertovaara et al., 1991, 1994; Ansah et al., 2000). The use of a higher ISO concentration could also help to enhance the analgesic effect rather than the sedative effect. Receptor desensitisation (Reid et al., 1994; Hayashi et al., 1995) or the competitive effect between α2-adrenergic receptors and α2-adrenergic receptors resulting from continuous DEX administration at a high dose can be related to the attenuation of the sedative effect. Although DEX is highly selective for presynaptic α2-adrenergic receptors, inhibiting norepinephrine release from presynaptic sites, it also exerts an excitatory effect through postsynaptic α1-adrenergic receptors when it exceeds a certain dosage (Doze et al., 1989; Schwinn et al., 1991), resulting in attenuation of α2-mediated effects. Thus, the use of relatively low concentrations of DEX (with ISO or other anesthetics) can potentially extend the experimental duration. Recently, the combination of 30 μg/kg/h DEX and 0.25% ISO was able to maintain stable fMRI responses for up to ~4 h (Lu et al., 2012).

**DEX enhances slow-wave synchronous neural activity and evoked neural responses**

The results show that LFP delta band power increased under DEX + ISO anesthesia, whereas beta and gamma band power decreased (Fig. 5), and this is consistent with previous electroencephalographic (EEG) observations of DEX-induced slow waves (Dumitrașcu et al., 2005). In addition, the latency of the evoked responses was reduced (Fig. 5), which is in accordance with previous reports (Zhao et al., 2000; Pawela et al., 2009). However, the suppressive effect of ISO on the evoked responses was not observed (Fig. 5). This might be due to the use of relatively low ISO concentrations and/or higher DEX concentrations in this study compared with previous reports (Guo et al., 1996; Zhang et al., 2000).
These discrepancies can be explained by hippocampal neural circuits (Angenstein et al., 2012). This suggests that ISO and MED interfere differently with evoked potential amplitudes. In the hippocampus, the BOLD response to identical stimulation changes between up and down states under anesthesia, whereas it is persistently in an up-like state in a wakeful state (Constantinople & Bruno, 2011). Thalamic activity does not seem to be important for the persistent up-like state in the wakeful state, but the locus coeruleus seems to be critical (Constantinople & Bruno, 2011). As DEX directly inhibits norepinephrine release from the locus coeruleus, slow-wave fluctuations (i.e. bimodal up and down states) can become more prominent. The increase in the low-frequency band power may also enhance resting state functional connectivity under MED or DEX anesthesia (Pawela et al., 2010; Zhao et al., 2008; Williams et al., 2010; Kalthoff et al., 2011; Nasrallah et al., 2012).

The results also show that the evoked LFP response under DEX + ISO was significantly larger than that under ISO only. A reduction in ISO concentration from 1.4% to < 0.5% is the likely reason for the enhancement of the LFP response (Banoub et al., 2003). Also, DEX has a small effect on sensory-induced neural responses (Li et al., 2003). The differential impacts of ISO and DEX on neural activity are not limited to evoked potential amplitudes. In the hippocampus, the BOLD response to identical stimulation depends on the previous stimulation history under MED, but not under ISO (Angenstein et al., 2010; Krautwald & Angenstein, 2012). This suggests that ISO and MED interfere differently with hippocampal neural circuits (Angenstein et al., 2010; Krautwald & Angenstein, 2012). These discrepancies can be explained by the fact that DEX does not involve the GABAergic system, but affects the neuromodulator epinephrine, unlike ISO. In other words, the neurovascular response under DEX should be different across brain regions (for example, see Fig. 5 in Nasrallah et al., 2012), because the sensitivity to DEX varies across brain regions, depending on α2-adrenergic receptor expression (Talley et al., 1996).

**DEX-induced vasoconstriction and CBF reduction**

We showed that intravenous DEX at 50 μg/kg/h decreased baseline CBF and constricted both pial arteries and veins with supplemental ISO administration (Fig. 6). CBF reduction and pial vessel constriction induced by DEX have been well documented for various species. Whereas the cerebral vasoconstriction is mediated by direct agonist binding to α2-adrenergic receptors on the cerebral vessels (Nakai et al., 1986), the degree of vasoconstriction depends on the dose, the delivery method (topical vs. systemic), and anesthetics used prior to DEX administration (Karlsson et al., 1990; Zornow et al., 1990; Bari et al., 1993; Fale et al., 1994; McPherson et al., 1994; Ishiyama et al., 1995; Asano et al., 1997; Ohata et al., 1999; Iida et al., 2006), as well as arterial carbon dioxide tension (Ganjoo et al., 1998). For instance, the reductions in CBF and pial arterial diameter in hypocapnic rats were larger than those in normocapnic rats.

Although the behavior of pial vessels is expected to be generally similar to that of parenchymal vessels, their adrenergic innervations differ. Pial vessels are innervated by nerve terminals derived from peripheral superior cervical sympathetic ganglia, whereas parenchymal vessels (including capillaries) are innervated by nerve terminals derived from central adrenergic neurons within the brain, such as the locus coeruleus (Hartman et al., 1972; Vaucher & Hamel, 1995;...
Paspalas & Papadopoulos, 1996; Cohen et al., 1997). In the peripheral vasculature, vasodilatation via sympathetic action and vasoconstriction mediated by smooth muscle receptors affected by DEX are known (for a review, see Kamibayashi & Maze, 2000). These different innervations might therefore explain the observation in one rat, although it is not clear why the dissociation between pial and parenchymal vessel diameter changes happened only in this rat (Fig. 6); that is, the dilation of pial vessels with DEX administration despite a decrease in baseline CBF, an indication of vasoconstriction in the parenchyma.

**DEX administration yields robust CBF responses and rapid vasodilatation**

Evoked CBF responses under DEX + ISO were 3.7 times larger than those under ISO only, and the increase in LFP activity was 1.5 times larger. The large and rapid nature of evoked hemodynamic responses under DEX + ISO anesthesia (200–300% changes, Fig. 7; ~2% in BOLD, Fig. 8) is the most notable difference from the hemodynamic responses obtained under other anesthetics. This may be a result of the relatively high basal vascular tone induced by DEX (Fig. 6). Additionally, the effect of DEX on astrocyte activity may be considered, as astrocytes may be key mediators of neurovascular coupling (Zonta et al., 2003; Takano et al., 2006). Cortical astrocytes can be directly activated by somatosensory stimulation via the norepinephrine-dependent locus coeruleus pathway in addition to the general glutamate-dependent thalamocortical pathway (Bekar et al., 2008). However, the locus coeruleus pathway is unlikely to have been involved in the present study, because it is activated only for pain stimulation. Thus, the enhancement of the hemodynamic response by DEX is not a result of activation of the additional pathway. The enhancement of energy metabolism in cortical astrocytes by DEX (Chen et al., 2000) might have implications for the enhancement of the hemodynamic response. Further studies are necessary to understand the impact of the central adrenergic system on neurovascular coupling.

With other anesthetics, sensory stimulation evokes rapid vasodilation in pial arteries, followed by a delayed and slow increase in...
venous CBV. This temporal character has been observed in γ-chloralose-anesthetised rats during 40 s of forepaw stimulation (Zong et al., 2012) and in wakeful mice during 30 s of vibrissa stimulation (Drew et al., 2011). Earlier studies performed under ISO only (Kim et al., 2007) showed that venous vessels did not dilate significantly during 10 s of stimulation. However, venous and arterial dilation were significant in response to 10 s of forepaw stimulation under DEX + ISO anesthesia, and the venous dilation was observed to be as fast as the arterial dilation, but took longer to return to baseline (Fig. 9). To our knowledge, dilation of pial veins and the increase in venous CBV in response to stimulation of such a short duration has not been found in animals anesthetised with isoflurane, γ-chloralose, or urethane (Hillman et al., 2007; Vazquez et al., 2010a; Drew et al., 2011). This discrepancy can be explained by the fact that DEX constricts pial veins as well as pial arteries, unlike other anesthetics. In addition, high basal vascular tone may make veins respond as fast as arteries. CBV increases resulting from venous dilation work to reduce the BOLD response, and a slow return to baseline in as fast as arteries. CBV increases resulting from venous dilation could be useful not only for designing fMRI experiments, as the effects of DEX can be easily reversed with anropin in rats: a laser Doppler study. Can. J. Anaesth., 40, 748–754.


