Isoflurane supplement prevents epileptic activity in fMRI studies under medetomidine anesthesia

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Target audience: Researchers carrying out animal or pharmacological functional MRI studies.

Purpose: To investigate the effects of dexmedetomidine (DEX) – the active ingredient of medetomidine (MED) 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.

Methods: Twenty five male Sprague-Dauley rats weighting 260 – 450 g were used; 21 for optical and electrophysiological studies and 4 for fMRI. The right femoral artery and vein were catheterized for measuring arterial blood pressure (MABP) and administering drugs, respectively. A cranial window was made for recording neural activity, vessel diameter and CBF. After surgery under 2% isoflurane (ISO), a DEX bolus of 50 μg/kg were injected intravenously (IV) and the ISO was turned off or reduced to <0.5%. Fifteen minutes after the bolus injection, continuous infusion of DEX started at a rate of 50 μg/kg/h. Forepaw stimulation pulses with width of 1 ms, current of ≤1.5 mA and frequencies of 3 – 12 Hz were delivered for 10 s. No MABP change due to the stimulation was observed. The cortical surface was illuminated by yellow-green light (570 ± 10 nm) to capture images for measuring the diameters of pial vessels. A microelectrode with the tip diameter of 5 μm was placed at a depth of ~0.3 mm below the cortical surface of the forepaw area to record neural activity. Neural activity was recorded using an electrophysiological data acquisition system (Plexon, Inc.) at a 1 kHz sampling rate. Then we determined multiple unit activity (MUA) and LFP activity. A needle-type laser Doppler flow (LDF) probe with tip diameter of 450 μm (PeriFlux 4001, Perimed) measured parenchymal CBF. Functional MRI experiments were performed on Varian 9.4 T with a 2.3-cm diameter surface coil. Four coronal slices covering the primary somatosensory area (S1) was acquired with GE EPI with the following parameters: matrix=64×64×4, FOV = 2.3 × 2.0 × 0.8 cm^2, TE/TR = 20/1000 ms.

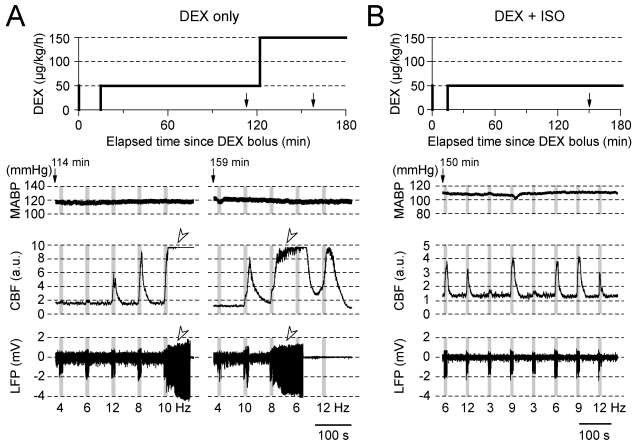

Figure 1: MABP, CBF and LFP responses in DEX sedative rats without (A) and with (B) supplemental isoflurane in two representative rats. First row in (A and B) plots infusion rate of DEX as a function of time from the initial bolus injection of 50 μg/kg. Arrows in A indicate a block (each consisting of 10-s forepaw stimulation and 70-s ISI) containing epileptic responses. The time elapsed from the DEX bolus injection is shown above the MABP traces. Gray vertical bars in each panel indicate 10-s stimulation periods, and the numbers under the LFP traces indicate the stimulation frequency, which were delivered in a pseudo-randomized order. Open arrowheads in CBF and LFP traces in (A) point to apparent epileptic responses without any change in MABP. Evoked LFP becomes epileptic ~2 hours after DEX bolus injection, while no epileptic LFP was observed with a supplement of ISO (B).

Results and Discussion: Robust, stimulation frequency-dependent CBF responses were observed under DEX-only anesthesia, consistent with earlier fMRI studies (1,2). However, epileptic responses to the 10-s stimulation were observed in runs at >120 min after the initial DEX administration (see open arrow heads above the LFP and CBF traces in Fig. 1A left column). To attempt to avoid this epileptic effect, the DEX dose was increased from 50 to 150μg/kg/h after 120 min from the initial dose. However, epileptic LFP and CBF responses were still elicited as soon as the stimulation started (Fig. 1A right column). 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. Supplementing with inspired ISO at 0.1 - 0.5% successfully mitigated the stimulation-evoked epileptic response (Fig. 1B). The use of supplementary ISO, a known vasodilator, did not seem to affect the baseline CBF level under DEX anesthesia (e.g., compare Figs. 1, A and B). Importantly, at a level of 0.5%, ISO did not decrease the amplitude of the evoked LFP response; however, the amplitude of the CBF response was slightly smaller than that obtained under DEX-only sedation (compare Figs. 1A and 1B).

DEX infusion induced constrictions of both arteries (marked as 'A' in Fig. 2A) and veins (marked as 'V'). The constriction reached plateau 30 min after the initial bolus administration (Fig. 2B). During the 10 s forepaw stimulation, vessel diameter increased not only in pial arteries, but also in veins (Fig 2C), although the diameter changes of arterial vessels were larger than those of venous vessels. The venous diameter change occurred as fast as the arterial diameter change, but returned to baseline slower than the artery. The observation of venous CBV increase induced by 10-s stimulation is quite different from previous observations under isoflurane and alpha-chloralose (3,4).

Forepaw stimulation evoked robust BOLD responses for >3 hours in forepaw cortical area in four DEX-ISO anesthetized rats studied by fMRI. After approximately 2 – 3 hours from the initial DEX administration, baseline signal oscillations became apparent and the evoked responses became unclear in two rats. Stimulation frequency-dependent BOLD responses were observed and the maximal response amplitude was reached at a frequency of 10 Hz, consistent with the LDF-based CBF results in this work as well as with earlier BOLD-fMRI report under MED only conditions (1).

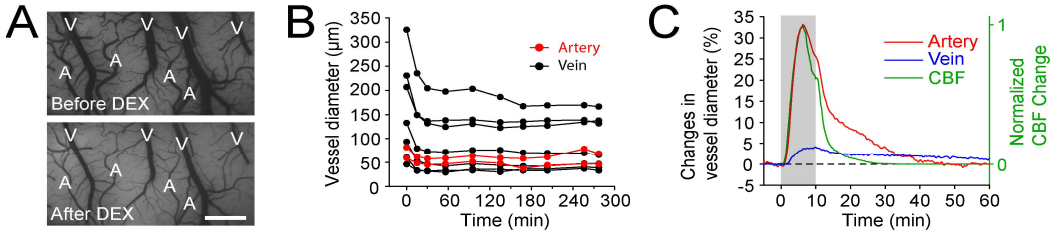

Figure 2: (A) Pial vessel images from one rat. 'A', artery; 'V', vein; scale bar, 1 mm. (B) Changes in arterial and venous vessel diameters versus time after the DEX bolus injection at time = 0. (C) Time courses of normalized CBF, arterial diameter and venous diameter changes (averaged over 11 animals). Gray bar indicates the 10-s stimulation period.

Conclusions: We have demonstrated that forelimb stimulation under continuous administration of DEX of 50 μg/kg/h for approximately more than 2 hours elicited epileptic LFP responses with concomitant large increases in CBF (Fig. 1). The addition of supplementary isoflurane (e.g., ~0.3%) suppressed the generation of the epileptic activity. DEX constricts arterial and venous vessels similarly, and induces both arterial and venous vessel dilations during stimulation (5). The DEX+ISO combination yields robust CBF and BOLD fMRI responses, and is a suggested anesthesia for 2-3 hr fMRI studies.

References: (1) Zhao F. *et al.*, NeuroImage **39**, 248-260 (2008). (2) Pawela C.P. *et al.*, NeuroImage, **46**, 1137-47 (2009). (3) Kim T. et al., JCBFM, **27**, 1235-47 (2007). (4) Zong X. et al., Neuroimage, **60**, 2238-46 (2012). (5) Fukuda et al., Eur J Neurosci, doi:10.1111/ejn.12024.