A Ceiling Effect of BOLD Responses to Theophylline Infusion in a Rat Model

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Introduction. Pharmacological MRI (phMRI) has been quickly applied to drug development as new questions about brain physiology and neuropharmacological mechanisms become addressable simultaneously for the first time (1). However, introducing a drug into the system could potentially alter the coupling of neural activity with regional cerebral blood flow and/or the extraction of oxygen from blood, or may cause local or global cardiovascular changes unrelated to neural activity. Results of a few fMRI studies on human subjects that have manipulated baseline brain perfusion are contradictory (2) on whether different perfusions are confounding factors to BOLD response and its characteristics. In this animal study, the relationship among neuronal status, baseline of brain perfusion and BOLD signals perturbed by theophylline was investigated through invasive and non-invasive approaches on a well-established rat forepaw stimulation model.

Materials and Methods. *Animal preparation:* Twenty male Sprague-Dawley rats, weighing 300-350g, were employed. The details of animal preparation for fMRI study were described in Ref.#3. Briefly, all rats were tracheotomized under α-chloralose anesthesia (80 mg/kg) and artificially ventilated. All rats underwent five 10-min experimental runs (control, saline, and three theophylline treatments with 10mg/kg per i.v. infusion). Saline and theophylline were slowly infused (0.7 ml/10 min) starting at the beginning of each run, via a syringe pump. *fMRI experiments:* fMRI experiments (n=5) were performed on a Bruker 3T/60cm scanner using a custom-built 5-inch long, 3.5-inch diameter RF saddle coil producing a uniform transmission field, and a 2 cm, one-turn surface coil on the top of the rat head with high sensitivity of signal reception. The two-coil system was inserted into a homemade cylindrical local gradient coil, which, at 100 A, produced gradient fields of 21.30 Gauss/cm, 20.83 Gauss/cm, 41.20 Gauss/cm in the X, Y, and Z directions, respectively. A single-shot, gradient echo EPI sequence was used for functional imaging with FOV=3.5 cm, slice thickness=2 mm, image matrix=64 x 64, giving an in-plane image resolution of 550 x 550 µm, TR=2 sec, TE=27.2 ms, and bandwidth=±62.5 kHz. *EEG experiments:* Bipolar micro-electrodes were inserted into holes drilled into the contralateral somatosensory cortex and either the ispsilateral somatosensory cortex (n=4) or the contralateral visual cortex (n=4). A stainless steel machine screw in the caudal cranium served as a ground electrode. *LDF measurement*: Local CBF changes (n=7) in the ipsi and contralateral somatosensory cortices were measured during forepaw stimulation, with and without theophylline, with LDF probes. Rats were secured in a stereotaxic apparatus, the skull was thinned to transparency over somatosensory cortices with a pneumatic dental drill, and LDF probes were suspended over the transparent areas. *Data analysis: fMRI*: Voxels with cross-correlation ≥ 0.16 corresponding to $p < 0.05$ after Bonferroni correction in the ipsilateral somatosensory cortex were considered significantly activated, using AFNI. *EEG*: Sixty evoked responses were averaged from each rat at each condition. *LDF:* LDF data were averaged for one minute (10 sec before, 20 sec during, and 30 sec post stimulation) for each condition. The maximum LDF response during forepaw stimulation is reported as a percent of either the 10 second baseline just prior to each stimulation (open circle in Fig. 1b) or the baseline at the start of the experiment, prior to any stimulation at all (black dot in Fig. 1b). The mean increases for each condition was averaged over all rats.

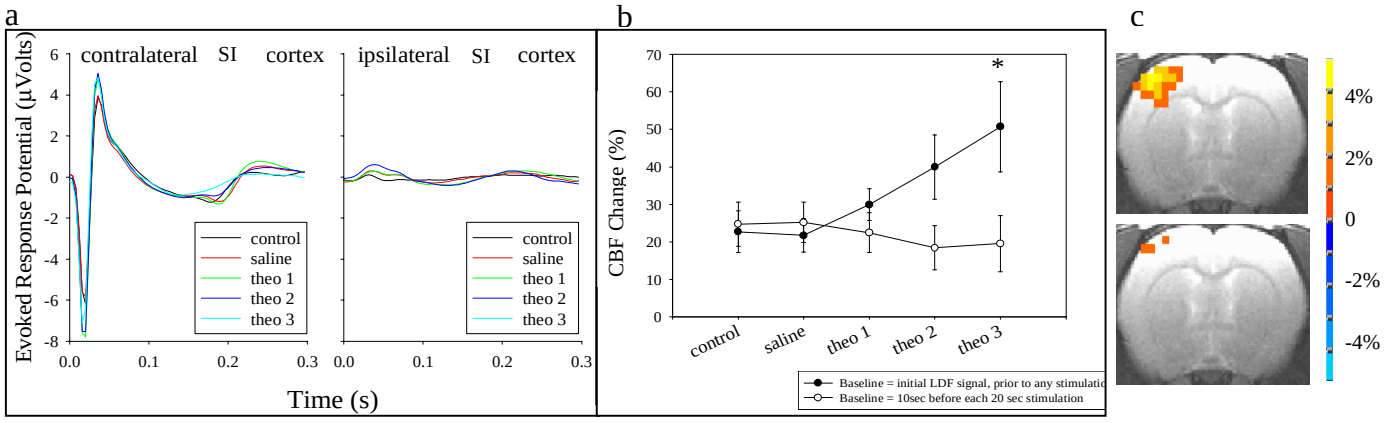

Fig. 1. A biphasic local field potential was found in the contralateral somatosensory cortex during forepaw stimulation, whereas a flat line was observed in the ipsilateral somatosensory cortex. No significant difference was found under control, saline infusion, or among three doses of theophylline (1mg/kg/min, i.v.) continuous infusion (Fig. 1a). A global perfusion increase was found when assessment of CBF changes was compared to the initial baseline before any stimulation (black dot in Fig. 1b). A relative perfusion decrease was found when comparing an immediate baseline, which is just prior to each stimulation (open circle in Fig.1b). *: $p<0.05$, significance between saline and theo 3. Activated voxels and intensity were significantly reduced after the third dose of theophylline infusion (bottom map in Fig. 1c) compared to control condition during rat forepaw stimulation (top map in Fig. 1c).

Results. Theophylline intravenous infusion did not significantly change the local field potential on the contralateral somatosensory cortex or ipsilateral during forepaw stimulation (Fig. 1a) or the contralateral visual cortex (not shown). The absolute global perfusion was increased (black dot in Fig. 1b) whereas the relative CBF decreased (open circle in Fig. 1b). The number of activated voxels and signal intensity significantly decreased after the second and third theophylline infusions (bottom map of Fig. 1c).

Discussion. The issues of how BOLD signals manipulated by global perfusion independent of neuronal activation were studied using theophylline, which is an adenosine-receptor antagonist in low dose, but a vascular dilator at high dose due to accumulation of cAMP (4). A high dose of theophylline (0.15 µmol/g, i.v.) in this study increases CBF without perturbing the local field potential in the contralateral somatosensory cortex. However, a significant decrease in BOLD signals during forepaw stimulation was found, accompanied by an increase in the absolute CBF. Our findings are consistent with a recent report that the BOLD response attenuated during finger-tapping task while global perfusion increase induced by acetazolamide (2). Two reasons could account for the observation shown in Fig. 1c: 1) selection of a baseline. Calculating BOLD contrast based on initial baseline or immediate baseline. Fig. 1c is obtained based on immediate baseline. A similar trend of relative CBF decrease was calculated as shown in Fig. 1b (open circle); 2) a ceiling effect of BOLD response which has limited dynamic BOLD range. Therefore, different vascular effects by pharmacological interventions need to be considered when using fMRI BOLD method to interpret drug-induced CNS alterations.

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