Subcortical fMRI Activations in Awake Animals

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Synopsis

fMRI studies in awake and behaving animals could offer many unique advantages. We demonstrated herein that BOLD fMRI responses under awake conditions are reasonably robust by using three well-established stimulation models, namely: global stimulation via seizure induction, and focal stimulation using forepaw stimulation and olfactory stimulus. Larger BOLD percent changes were observed under awake conditions compared to the anesthetized conditions. Most importantly, activations were observed in additional brain structures (predominantly in the subcortical regions and higher order functions) that were not detected in the anesthetized preparations.

Introduction

There has been increasing interest in fMRI studies with awake monkeys [1], rabbits [2], and rats [3]. fMRI of awake animals offers many distinct advantages over anesthetized models. *First*, the effects of anesthesia can be avoided. *Second*, neural activity is not compromised, which yields increased fMRI signal changes and potentially improved detection of activation. *Finally*, subcortical and higher order cognitive functions can be stimulated in an awake model. Such studies would be very difficult if not impossible under anesthesia. The disadvantages of performing awake fMRI studies are potential motion artifact and stress.

In this study, we showed that the BOLD fMRI responses under awake conditions are reasonably robust. BOLD fMRI responses under awake conditions were investigated using three widely-used stimulation models, namely: global stimulation via seizure induction, focal stimulation using forepaw and olfactory stimulation. These studies were compared with those under anesthesia. Reasonably robust and enhanced fMRI responses were observed in conscious animals. Furthermore, subcortical activations and high order functions were observed under awake conditions and were abolished by anesthesia. Potential movement artifacts and functional contrast-to-noise ratios were discussed.

Method

Sprague-Dawley rats were secured in a rat restrainer with ear-, nose-, tooth-, shoulder-bars, and a body restraining tube. For global stimulation, seizure was induced using γ-butyrolactone seizure model (200 mg/kg, ip) under awake (n=3) and anesthetized (2% isoflurane, n=3) conditions [4]. For focal stimulation, forepaw and olfactory stimulation were used. Electrical forepaw stimulation under awake (n=2) and anesthetized (α-chloralose, n=6) conditions was achieved with electrodes inserted under the skin of one forepaw. Olfactory stimulation was done by presenting a Q-tip saturated with iso-amylacetate (n=3). Rectal temperature was maintained at 37±1^{o}C and respiration rate was monitored. Blood gases were measured and maintained within normal physiological ranges in the anesthetized preparation.

MR experiments were performed on a 4.7T/40cm magnet using a surface coil or an actively-decoupled two-coil system. Anatomical (RARE) images were acquired with TR=2s, effective TE=65ms, matrix=256x256, FOV=2x2 or 3x3cm^2, 1.5-mm slices. and four averages. BOLD measurements were made using single-shot, gradient-echo EPI with matrix=64x64, FOV=2x2 or 3x3 cm^2, and 1.5-mm slices, TE=20-25ms, and TR=1 or 2s. Activation maps were calculated using cross-correlation analysis and superimposed on anatomy or echo-planar images. Time courses were generated from activated pixels.

Results & Discussion

In general, the baseline fluctuations of MR signals were higher under awake relative to the anesthetized conditions. This was not necessary due to increased movement artifact, which usually manifested into large spikes in the MR signal time courses in cases where the animals were not properly restrained. When the animals were properly restrained, few or no large spikes in the MR signal time courses were observed. The larger baseline fluctuations of MR signals were likely due to increased basal neural activity and/or physiological "processes" associated with being awake.

Seizure: A large, global BOLD signal increase was observed following seizure induction under awake conditions (**Fig 1**). In contrast, the BOLD responses under anesthetized conditions did not yield a substantial number of activated pixels at the same percent (or statistical) threshold. This is consistent with the notion that isoflurane anesthesia suppresses seizure activity. These data demonstated that the awake model offers a unique opportunity to study seizure using fMRI [4].

Olfactory stimulation: One of the most common stimulation paradigms for fMRI study in animal model is olfactory stimulation in rats. Under anesthesia, only BOLD activation in the olfactory bulb (primary sensory area) was detected (data not shown). No subcortical BOLD activity under olfatory stimulation has been reported in the literature. In marked contrast, activations in the subcortical areas (such as anterior olfactory nucleus and the medial-dorsal thalamus), in addition to olfactory bulb activation, were detected in the awake conditions (**Fig 2**).

Forepaw stimulation: Another common stimulation paradigm for fMRI study in animal model is the electrical forepaw stimulation in rats. Under anesthetized conditions, only the BOLD responses from the primary somatosensory cortex were observed across many laboratories. In contract, the awake conditions, BOLD responses in the secondary somatosensory cortex and subcortical structures (such as the caudoputamen) were detected in addition to activation in the primary sematosensory contex (**Fig 3**). The time courses showed larger percent changes under awake condition, with only moderate increase in baseline signal fluctuations.

Comparable or improved functional contrast-to-noise ratios were observed under the awake relative to the anesthetized conditions.

Fig. 1. Seizure **Fig. 2.** Olfactory stimulation

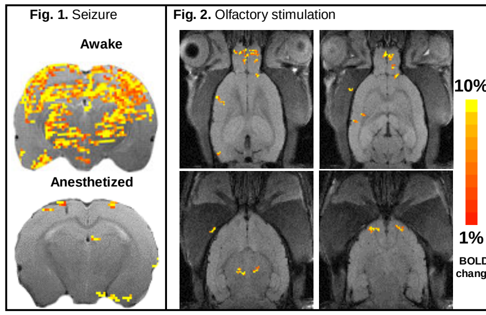
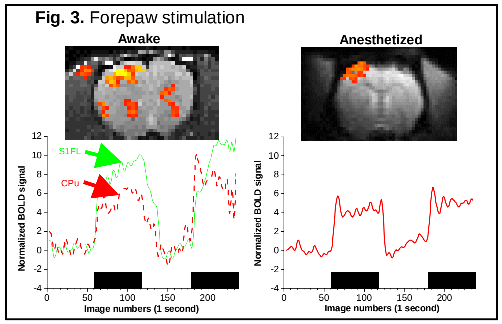


Fig. 3. Forepaw stimulation



Conclusion

These results demonstrate that BOLD fMRI changes in awake animals are reasonably robust, though at a marginally higher failure rate. Potential motion artifact and stress associated with performing awake fMRI could be alleviated with proper restraint and acclimation; these factors are currently under investigation. The ability to study *subcortical and higher order cognitive* functions justifies further development of awake animal models for fMRI studies.

References [1] Logothetis, *Nat Neurosci* 1999, 2:555. Zhang, *Brain Res* 2000, 852: 290. Ferris, *NeuroRep* 2001, 12:2231. Vanduffel et al. *Neuron* 2001, 32:565. [2] Wyrwicz et al. *MRM* 2000, 44:474. [3] Lahti et al. *J Neurosci Meth* 1998, 82:75. Peeters et al., *MRI* 2001, 19:821. [4] Tenney, ISMRM 2003.