Introduction

Functional MRI (fMRI), a non-invasive tool for mapping neuronal activation, has been proven beneficial to both fundamental neuroscience research and clinical diagnosis through many human studies. fMRI studies of animal subjects however are greatly limited by the need for anesthesia to prevent motional artifacts. Anesthetic agents not only diminish the fMRI responses, but also restrict the applications to observation of simple activation.

We present here the first functional study on conscious rabbits at 4.7 Tesla. Head and body movements were well controlled with the restraining and training protocol. Cerebral activation in response to a visual stimulus was measured with BOLD technique using single-shot echo planar imaging (EPI) sequence. Results from the intra- and inter- animal experiments consistently confirmed prior knowledge of the rabbit visual system, and demonstrated that the BOLD fMRI technique is highly reproducible on conscious animal subjects.

Methods

Subject and restraint

New Zealand white (NZW) and Dutch Belted (DB) female rabbits were used in this study. Rabbits were wrapped in a soft cloth bag that was tied at the neck and tail. The imaging RF coil and an acrylic plate were secured to four surgically implanted headbolts, and the plate was secured to the cradle with nylon machine nuts. All of the subjects were habituated to this setup for 2-3 hours per day for approximately two weeks prior to conducting fMRI studies.

Visual stimulus paradigm

Four green LEDs (2 x 2mm) flashing at 8Hz were used for visual stimulation. The imaging protocol consisted of a single trial paradigm of rest (darkness), stimulated state (flash), followed by rest (darkness). This trial was repeated 20 to 50 times with 10 sec duration of non-imaging rest between trials.

BOLD fMRI on conscious rabbits

A single-slice T2*-weighted gradient-echo EPI sequence was used to acquire functional data with the following parameters: TEoff 32 ms, slice thickness 2 mm, FOV 60 x 60 mm2, matrix size 64 x 64, and spectral width 125kHz. The spatial resolution of fMRI data was 0.935 x 0.935 x 2 mm3, and the temporal resolution was 1.5 sec. The imaged coronal slice was centered at approximately 11 mm posterior to bregma to contain the primary visual cortex. The acquired data was transferred to a Sun Ultra workstation and processed off line using software developed with IDL. The activation map was generated from the averaged fMRI data with three different approaches: subtraction, cross correlation with a pre-defined function, and t-test.

Results and Discussion

Functional activation of primary visual cortex: right and left eye stimuli

The functional maps (derived from the averaged data of 50 trials) overlaid on a high resolution anatomical image (Fig. 1a and b) indicate significant activation in four pixels with cross correlation coefficient higher than 0.67 and t-test p value less than 0.0001. The time course profiles of activated and non-activated sides (corresponding to solid and dashed lines, respectively) are shown in Fig. 1c and d, along with the timing diagram of stimulation (gray box). The MRI signal intensity increases about 4% during stimulation period, and the time delay for the hemodynamic response (on-response) is 3.75 sec.

Intra- and inter-animal fMRI reproducibility

Intra- and inter-animal experiments were performed to evaluate the reproducibility of fMRI technique on conscious animals. Responses in V1 contralateral to the stimulated eyes were observed in all the performed experiments. Similar amplitudes of response were observed in the same rabbit (n=4) with mean value 4.1% and standard deviation 0.6%. Comparable response amplitudes were obtained in inter-animal studies (n=8) with mean value 3.6% and standard deviation 0.7%. These data indicate high intra- and inter- animal reproducibility.

Conclusions

The first fMRI study on conscious rabbits is presented. Neuronal activation in response to visual stimulation was described with high spatial (938 x 938 μm2) and temporal resolution (1.5 sec). The signal to noise ratio of fMRI with BOLD contrast was improved by averaging the data from multiple trials on the same subject. The present technique is proved to be highly reproducible in both intra- and inter-animal studies, and is an appropriate model system for cognitive animal research.

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