Detecting single cortical column activation under super high spatial resolution at 9.4 T using single-shot half k-space GR-EPI

R. Li[1], P. Hettinger[2], Y. Cho[1], C. P. Pawela[1], J-G. Yan[2], A. Jesmanowicz[1], A. Hudetz[3], H. Matloub[2], and J. Hyde[1]
[1]Biophysics, Medical College of Wisconsin, Milwaukee, WI, United States, [2]Plastic Surgery, Medical College of Wisconsin, Milwaukee, WI, United States,
[3]Anesthesiology, Medical College of Wisconsin, Milwaukee, WI, United States

Introduction

The representation of the rat forepaw occurs in a brain area known as the rat forepaw barrel subfield (FBS), which has been mapped to the cortical-column level using electrophysiological techniques (1). Electrical stimulation using electrodes placed on the forepaw results in fMRI activation of the FBS (2), but to date not at the cortical-column level. Electrophysiological studies show that the middle phalange of each digit is represented by a single cortical column (1). In the present work, small electrodes have been constructed that are designed to excite only the middle phalange of the rat digit, and fMRI experiments have been carried out at 9.4T using cubic 300 micron voxels to address the question of whether or not it is possible to excite and detect a single cortical column in rat brain under this high resolution. The issue in question for BOLD contrast is possible signal loss from partial voluming of the microcirculation in rat brain. Both physiology and histology studies have shown that each column of the nearby whisker barrel cortex contains a single, central penetrating vein. The regular organization of the nearby FBS suggests a similar vasculature, which supports the hypothesis that single-column fMRI resolution of the FBS should be achievable. In this study, we use single-shot half k-space gradient-recalled echo-planar imaging (EPI) (3) to study single cortical column activation caused by direct cutaneous stimulation of an individual rat digit. In this study, we show not only that this highly refined sensory system can be activated at the columnar level and detected at 0.3 mm^3 resolution, but also that the cortical column representation of all eight digits of the same rat can be distinguished from each other and each digit has a unique representation pattern.

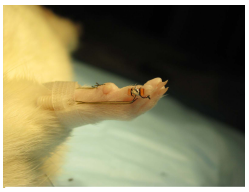

Fig. 1. A customized electrode was put on the rat digit that can precisely stimulate the middle phalange on each finger of the rat to activate a single cortical column (eight total in each rat).

Method

Animal preparation: Five Sprague-Dawley rats were used in this study. For all the animals, the right femoral vein was cannulated for drug delivery. A tracheotomy was done to maintain respiration during the scan. Eight customized finger electrodes were attached on the ulnar and radial side of the middle phalanges of the rat digits to stimulate the end braches of the peripheral sensory nervous system (Fig. 1). Rats were ventilated during the scan, and all physiological parameters were monitored and properly maintained. **Anesthesia:** Isoflurane (1.4%) was administered during the surgical portion of the procedure. Once the rat was transferred to the scanner, the isoflurane was turned off. A continuous infusion of pancuronium bromide (2 mg/kg/hr) and Domitor (0.1 mg/kg/hr) was used during the fMRI acquisition. **Stimulation protocol:** Electrical stimulation of 10 Hz, 2 mA with a constant duration of 3 ms was used. The stimulation sequence began with an OFF period of 40 s followed by three repetitions of ON for 20 s and OFF for 40 s (total scan time = 3 min, 40 s). There was a four-minute resting time between two stimulations for rats to recover. Digits were stimulated sequentially without moving the animal. **fMRI parameter:** BOLD contrast fMRI was done using a 9.4 T small-animal scanner (AVANCE; Bruker, Billerica, MA). A RARE anatomy image was acquired with ten continuous slices with 1 mm thickness. The third slice was located over the anterior commissure. Normal EPI BOLD-contrast fMRI data were acquired with parameters TR = 2 s, TE = 18.4 ms, matrix size 96 × 96 using single-digit stimulation. This normal fMRI activation served as a navigator for the high-resolution functional imaging later. After this, the system was further tuned and shimmed to locate the iso-center on top of the activation site. Finally, a half k-space gradient-recalled EPI sequence with 24 over-scan lines was used under a bandwidth of 400 kHz (Fig. 2). Other parameters were: TE = 18.4 ms, TR = 2 s, FOV = 38.2 mm, slice thickness = 0.3 mm, matrix size = 128 × 128, and a total of 15 slices. The center slice (slice 7) was also located over the activation site. Data acquired were processed using customized reconstruction software. Functional data were analyzed using AFNI software. Activation was determined with a P-value threshold of 0.01 for an individual rat and was displayed on the anatomical images.

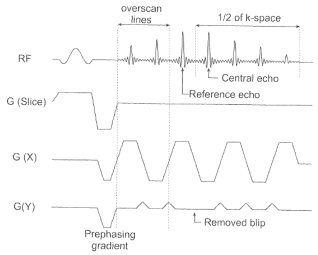

Fig. 2. Gradient-recalled half k-space EPI sequence used in this study (3).

Results

Figure 3a shows results of stimulating eight digits of a single rat. Localized activation can be seen in the S1FL region, representing sensory activation. Column activation can be clearly seen, penetrating the gray matter, and terminating at the top of the caudate putamen. In most slices, only one column was activated. There is a signal-to-noise ratio (SNR) drop when this high-resolution imaging is compared to the 1 mm slice thickness imaging (Fig 3b); however, cortical activation remains stable and localized. When comparing the cortical representation of eight digits, it can be seen that all activations are unique in shape, location inside the S1FL, spatial location across slices, and intensity. The small digit has a relatively small representation area compared to the other digits on both sides. No indication of sidedness was found. These localized activations at 0.3 mm^3 resolution were seen across all animals, but the activation patterns were slightly different across rats (data not shown due to limited space).

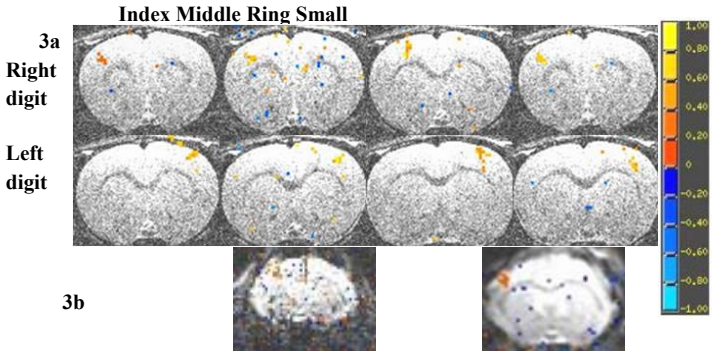

Fig. 3 (a) Cortical activation induced by single-digit stimulation at high resolution with a voxel size of 0.3 × 0.3 × 0.3 mm. Data were acquired from the same rat with P = 0.01. Distinct activations can be seen on all digits in the S1FL area. (b) Comparison of digital nerve representations between high resolution (left; voxel size 0.3 × 0.3 × 0.3 mm) and low resolution (right; voxel size 0.3 × 0.3 × 1 mm) EPI images. SNR of the high-resolution imaging is lower than the lower resolution, but cortical activation still can be seen.

Conclusion and Discussion

By applying the single-shot half k-space GR-EPI sequence, which allows high spatial resolution with relatively short TE value, we successfully pushed the functional imaging resolution to 0.3 mm to achieve single-column resolution. We also showed that each digit representation is unique and that high resolution methods are required not only to achieve single column resolution but also to distinguish adjacent columns arising from other fingers. Histology studies show that cortical columns in S1FL are generally accompanied by penetrating veins. Due to the nature of BOLD contrast, the activated voxel, ideally, should encompass the small columnar vein. This was found in some of our slices (for example, Fig. 3b [left]). This technique has some limitations. Relatively good surgical technique is needed to make all eight stimulators work on the same rat. Since the cortical activation is precise and unique on each digit of each rat, improving the SNR by averaging data across rats does not seem feasible.

References

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