

fMRI of the rat's whisker-to-barrel pathway

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Introduction

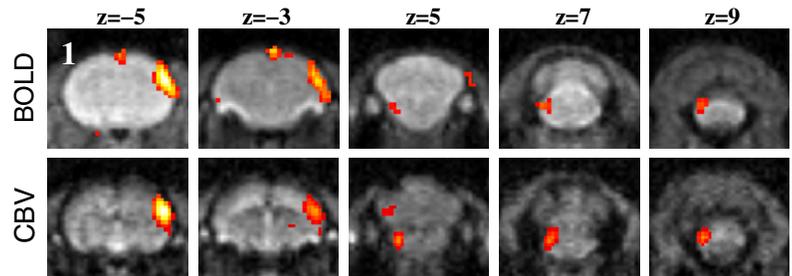
Functional MRI of experimental animals (predominantly rodents) is used to improve our understanding of haemodynamic changes induced by brain activation [1,2]. However, the majority of animal fMRI has focussed solely on primary sensory cortex (predominantly somatosensory), and less often, on secondary sensory areas, such as SII and cerebellar cortex. All these areas are superficially located in the brain, and thus they are imaged conveniently using surface coils [3]. Nevertheless, a number of deep brain structures are responsible for relaying neuronal information between the stimulation site and the cortex, including thalamic nuclei and the trigeminal system [4]. Although such areas have been studied using traditional invasive techniques such as electrophysiology [4] and autoradiography [5], their fMRI response is largely unknown. This work performs whole-brain fMRI of the rat brain in an attempt to measure the haemodynamic responses of the whisker-to-barrel pathway induced by electrical stimulation of the rat's whiskers.

Materials and Methods

Animal preparation: Hooded Lister rats 250-300 g were anaesthetised with urethane (1.25g/kg i.p.), tracheotomised, artificially ventilated and cannulated for mean arterial blood pressure (MABP) monitoring and intravenous infusions. Phenylephrine (0.13-0.26mg/hr) was infused to maintain MABP between 100-110mmHg. Rectal temperature was maintained at 37°C using a homeothermic blanket. **Whisker stimulation:** The entire whisker pad on the left of the rat's snout was stimulated electrically by inserting two stainless steel electrodes underneath the skin ventral to whisker rows A and D. A square-wave current (1.6mA; pulse-width 0.3ms) was applied at 5Hz for 40s. This sequence was presented 6 times with an inter-stimulus-interval (ISI) of 180s. **fMRI:** A 3T, 16cm horizontal bore Magnex magnet equipped with a Magnex 10cm-id self-shielded gradient (10kHz/mm max per axis), and an MRRS console was used. A home-built, quadrature, 8-strut birdcage coil (length: 40mm, diameter: 35mm) was used in transmit/receive mode. The open space between the coil's struts allowed free access to the rat's head, which was immobilised with a home-built Perspex stereotactic frame. BOLD and CBV fMRI was performed over 12, 2mm-thick contiguous transverse slices using fat-suppressed, single-shot, asymmetric spin-echo (ASE) echo-planar imaging (EPI) with the following parameters: 64x64 image matrix, FOV=3x3cm, TE=24ms, ASE delay=5ms, TR=2s. For CBV fMRI, 10 mg/kg of MION (in 1 ml) was injected intravenously 15 minutes before data acquisition. **Data analysis:** SPM99 (<http://www.fil.ion.ucl.ac.uk/spm>) was used. First, the EPIs were realigned and spatially smoothed with a 3d Gaussian kernel (FWHM=1.5* voxel size). The paradigm was convolved with SPM's haemodynamic response function and was high-passed filtered (cut-off= 2*ISI=360s). T-contrast was used and the paradigm was weighted by 1 (BOLD) and -1 (MION). The uncorrected p-value threshold was 0.05 (FWE). The SPM's VOI facility was used to extract time-series of activated clusters. The MION time-series were transformed as in [2] to provide relative CBV changes.

Results

Figure 1 shows activation clusters overlaid on EPIs for BOLD and CBV data for two rostral slices and three caudal slices to illustrate primary sensory cortex (S1) and brainstem trigeminal (BS) activation, respectively (slice position is shown on the top of the figure in mm). BS activation is ipsilateral to the stimulated whiskers whereas S1 is contralateral. The average extent (in voxels) of S1 and BS clusters were: for BOLD: S1=73, BS=25, and for CBV, S1=89, BS=55. The CBV data also revealed thalamic (z=-3) and cerebellar (z=5) clusters.



Figures 2, 3 plot average responses of BOLD and CBV signal, respectively, for S1 (solid lines) and the BS (dashed lines) in 7 sessions from 3 animals; error bars represent s.e.m. Clearly, strong BOLD and CBV responses were observed in the BS, which were almost 70% of the magnitude of the respective cortical responses. Since ASE EPI was used, these measurements reflect haemodynamic changes in microvasculature.

Discussion & Conclusions

By using a small volume coil we have removed a significant limitation of previous whole brain fMRI studies, namely the inability to detect activation robustly in deep structures of the rat brain due to the use of surface coils [3]. As a result, robust microvascular responses were measured simultaneously in the two extreme stations of the whisker-to-barrel pathway (cortex and brain stem) at a moderate (for animal studies) field strength (3T). The thalamic nuclei were detected inconsistently; due to their small spatial extent, increased spatial resolution may be necessary for consistent observation of their responses [3].

This work demonstrates simultaneous detection of cortical and brain stem activation in the anaesthetised rat using fMRI, and will enable investigations of the relationship between neural activity and haemodynamic responses in the main brain regions along the rat's somatosensory pathway. Although a consistent relationship is assumed to exist over the whole brain, this assumption may not necessarily hold because of heterogeneous patterns of vascularisation and baseline activity. Thus, the acquisition of such data is crucial to enable a more accurate understanding and interpretation of fMRI data.

References [1] Hyder F et al, *NMR Biomed* 21: 413-431 2001 [2] Mandeville JB et al, *Magn Reson Med* 39: 615-624 1998 [3] Keilholz SD et al, *Magn Reson Med* 52: 89-99 2004 [4] Sosnik R et al, *J Neurophysiol* 86: 339-353 2001 [5] Adachi K et al, *Am J Physiol* 267: H2155-2162 1994

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