An fMRI study of naturalistic stimulation of the rat's whiskers

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Introduction

Functional MRI studies in experimental animals (mainly rats) extensively use the somatosensory system to improve our understanding of haemodynamic changes induced by brain activation. The large majority of studies use electrical stimulation of the rat's forepaw or whiskers, eliciting robust activations of the primary somatosensory cortex. However, electrophysiological studies have shown that air-puff whisker stimuli better resemble the movement profile observed during natural whisking [1] and evoke brainstem responses that resemble those observed in freely moving rats [2]. As such, the findings of studies that use electrical stimuli may not correspond to those using a more naturalistic stimulus. As a case in point, studies in our laboratory using optical imaging techniques found that haemodynamic responses elicited by air-puff whisker stimuli were enhanced during states of cortical arousal whereas responses elicited by electrical stimuli were reduced [3, 4]. As such naturalistic stimulation has not been studied with fMRI before, this work develops an air-puff stimulation protocol of the rat's whiskers and performs whole-brain fMRI of the rat brain in response to air-puff 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. *Air-puff whisker stimulation:* Whiskers on the right of the rat's snout were stimulated using pulses of compressed air (at 60 psi) ejected from a home-built pressure ejection system. These were delivered via a 1.5 m length of polythene tubing (3 mm OD, 2 mm ID) that was attached to a short (20 mm) length of PVC tubing (6 mm OD) that had been heat-stretched to create a tip with an inside diameter of ~ 1mm. The tip was placed approximately 20 mm anterior and 10 mm lateral to the rat's snout and aimed primarily at the large caudal whiskers. Stimuli were presented at 5 Hz for 40 s with pulse-width of 50ms. This sequence was presented 6 times with an inter-stimulus-interval of 180 s. *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. 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. 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. 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 [5] to provide relative CBV changes.

Results & Discussion

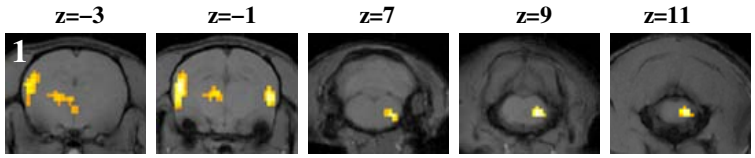

Figure 1 shows representative activation clusters overlaid on anatomical images for two rostral slices and three caudal slices, to illustrate cortical and brainstem trigeminal (BS) activation, respectively. BS activation is ipsilateral to the stimulated whiskers whereas primary somatosensory cortex (S1) is contralateral. Secondary somatosensory activation was also observed but not consistently. The average extent (in voxels) of S1 and BS clusters over all animals were 69 and 43, respectively. Although both S1 and BS showed robust activation in all animals studied, activation of thalamic nuclei was not consistently observed, presumably due to their size and relatively small magnitude of haemodynamic changes that are observed in these areas using autoradiographic techniques [6].

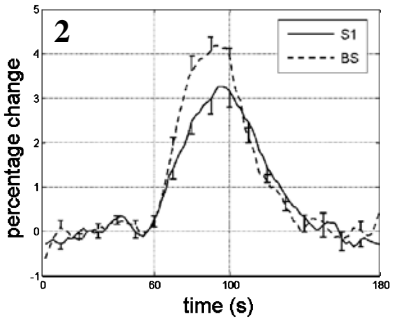

Figure 2 plots average CBV responses in 9 sessions from 3 animals (solid lines = S1; dashed lines = BS; error bars represent s.e.m.). On average, responses in the BS were larger than S1 (4.2 % vs. 3.2 %) in agreement with previously published data from autoradiographic studies (e.g. [6]). Since spin-echo EPI was used, these measurements reflect microvasculature CBV changes.

Conclusions

This work reports robust haemodynamic responses obtained with naturalistic stimuli in an fMRI setting. As such stimuli are known to better mimic sensory interactions that occur in behavioural conditions, they are well suited to investigations into the mechanisms of sensory transduction and response modulation. Consequently, fMRI techniques, obtaining data from the whole-brain, can now be applied to such investigations. One such application that our laboratory is interested in is understanding the origins of sensory enhancement that occurs in response to drugs of abuse [4], a phenomenon that is thought to play a role in cue-induced craving by altering the salience of sensory cues.

References [1] Sosnik R et al. *J. Neurophys.* 2001; 86: 339-353 [2] Nicolelis MA et al. *Science* 1995; 268: 1353-1358 [3] Berwick J et al. *Neurosci.* 2005; 132: 361-374 [4] Devonshire IM et al. *NeuroImage* 2004; 22: 1744-1753 [5] Mandeville JB et al, *Magn Reson Med* 1998; 39: 615-624 [6] Adachi K et al. *Am. J. Physiol.* 1994; 267: H2155-2162. **Acknowledgments** This work is supported by the MRC (G0200484, G9825307).