Pharmacological modulation of the serotonergic system in rat brain detected by fMRI

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Introduction

Serotonin (5-hydroxytryptamine [5-HT]) is one of the three main types of monoamine neurotransmitters in the brain and is involved in many processes, including regulation of cerebral blood flow, sleep, learning and mood. Moreover, it is clear that pharmacological manipulation of brain 5-HT levels can produce striking therapeutic effects in a number of psychiatric illnesses, and that abnormalities in brain 5-HT function persist in patients with recurrent depression[1]. In addition, decreased circulating concentrations of the amino acid L-tryptophan, the precursor for 5-HT, is a significant risk factor for depression[2]. Functional MRI offers the potential to investigate the effects of pharmacological manipulation on brain function. However, to date, relatively few studies, in either human or rodent brain, have investigated the functionality of the 5-HT system. In this study, fMRI was use to investigate blood oxygenation level dependent (BOLD) contrast changes in the rat brain after administration of the 5-HT-releasing agent, d-fenfluramine, both in naïve animals and following depletion of brain 5-HT levels with a tryptophan hydroxylase inhibitor.

Methods

Animal Preparation: Male Sprague-Dawley rats (250-300g) were anaesthetised with 2.5% halothane in 60%N_2O/40%O_2. Animals were tracheotomised, mechanically ventilated, and halothane levels reduced to 1-1.5% for the remainder of the experiment. Core body temperature was regulated, and the left femoral artery and vein were cannulated for MABP measurement, withdrawal of blood samples and i.v. administration of drugs. Animals were positioned in a quadrature birdcage resonator with an in-built stereotaxic frame.

MRI: MRI was performed on a 7T horizontal-bore magnet with a Varian Inova spectrometer. Anatomical images were acquired using a T_2-weighted fast spin echo sequence (TR=3sec, TE=45msec, FOV=3cmx3cm, matrix=128x128). For the fMRI run, sets of 5 coronal images (1.5mm thick) spanning the forebrain were acquired using a T_2*-weighted FLASH sequence (TE=25msec, TR=500msec, 40º flip angle, FOV=8cmx4cm, matrix=256x128, acq. time=2min) with dynamic first order shim updating for each slice. Baseline images were acquired for 15min, followed by a bolus administration of fenfluramine (10mg/kg i.v.; n=6) or vehicle (n=6) and a further 85min of acquisition. In a third group, fenfluramine-injected animals were pretreated with the tryptophan hydroxylase inhibitor p-chlorophenylalanine (PCPA); 300mg/kp i.p., 48h and 24h before study (n=5).

Data Analysis: Analysis was carried out using AFNI (http://afni.nimh.nih.gov/afni/) and FEAT (http://www.fmrib.ox.ac.uk) software packages. Datasets were corrected for motion artefacts and spatially smoothed. Activation maps were generated and thresholded at a significance level of P=0.05. ROI's were manually defined from the anatomical scans for the medial prefrontal and motor cortices, nucleus accumbens, septum, caudate, thalamus, dorsal raphe nucleus and hippocampus. Data were compared using repeated measures ANOVA followed by a Dunnett 2-sided t test.

Results

Compared to saline control injections, d-fenfluramine resulted in significant (P<0.01) reductions in BOLD signal intensity in a number of cortical and subcortical regions, including the prefrontal cortex, nucleus accumbens, septum and caudate. In animals pretreated with PCPA to deplete 5-HT levels the BOLD responses to fenfluramine were either completely (prefrontal cortex [fig.1a], septum, caudate) or partially (nucleus accumbens [fig.1b]) attenuated. In addition, d-fenfluramine caused a smaller but significant (P<0.05 compared to saline injected animals) increase in the BOLD response in the motor cortex, which was not attenuated by pretreatment with PCPA (fig. 1c). No significant BOLD responses were observed in either the dorsal raphe nucleus or hippocampus.

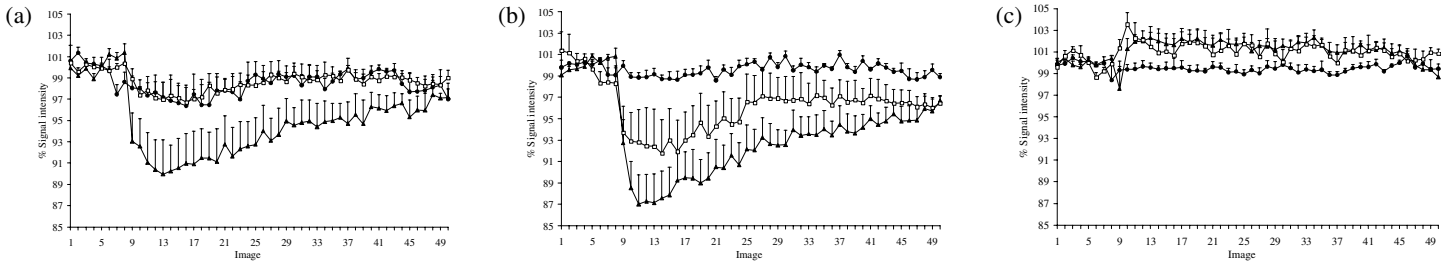

Fig.1. Graphs showing the average BOLD signal intensity changes across all pixels over time within the (a) prefrontal cortex (b) nucleus accumbens and (c) motor cortex. Values are expressed as % change from baseline signal intensity. ●=control, ▲=fenfluramine, □=PCPA + fenfluramine.

Discussion

All of the regions demonstrating significant BOLD responses receive serotonergic input from the raphe nuclei in the brainstem. The reductions in BOLD response in the prefrontal cortex, nucleus accumbens, septum and caudate are in accord with a previous study demonstrating regional reductions in cerebral blood volume (CBV) following administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT[3]. The attenuation of the responses to fenfluramine in the prefrontal cortex, septum and caudate following PCPA treatment indicates that these effects are 5-HT-mediated. In contrast, the increased BOLD response in the motor cortex is contrary to the previously reported decrease in CBV induced by 8-OH-DPAT in this region[3], suggesting that this effect may be mediated via a different 5-HT receptor subtype. Alternatively, the partial attenuation or persistence of the responses to fenfluramine in the nucleus accumbens and motor cortex following 5-HT depletion, suggest that other factors may play a part in the responses observed in these regions. These finding demonstrate the utility of pharmacological fMRI as an approach to investigating the functionality of the serotonergic neurotransmitter system *in vivo*.

References

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