# Mapping functional changes in rat brain in response to altered serotonergic function using BOLD fMRI

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## Introduction

Dysfunction in the brain serotonergic (5-HT) system is thought to underlie human depression[1]. Selective Serotonin Re-uptake Inhibitors (SSRIs) are widely used antidepressant drugs whose therapeutic onset are delayed for several weeks whilst neuroadaptive changes take place in the brain, after which the previously disturbed serotonergic neurotransmission is restored[2]. A suggested way of hastening the action of SSRIs is to combine them with 5-HT receptor antagonists. This effect has been shown with $5HT_{1A}$ autoreceptor antagonists in rats, but was more variable in the clinic[1,3,4]. 5-$HT_{2C}$ receptor antagonists may also accelerate the therapeutic onset of SSRIs and the mechanisms involved are under investigation[5].

## Aim

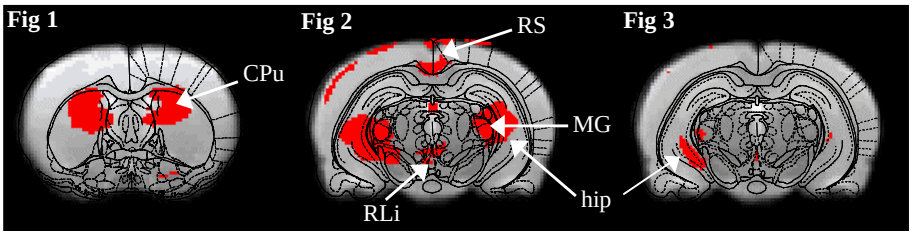
BOLD contrast fMRI is a rapidly developing technique that can be used to investigate indirect measures of neuronal activity in response to a pharmacological challenge *in vivo*[6]. The aim of the study was to map functional responses of whole rat brain to acute systemic administration of the SSRI citalopram[7] and the highly selective 5-$HT_{2C}$ antagonist SB242084[(8)] both alone and in combination using BOLD fMRI *in vivo*.

## Methods

Male Sprague Dawley rats (210–260g) were maintained under isoflurane (~2% with $N_2O/O_2$) anaesthesia and inserted with a subcutaneous cannula previously loaded with either vehicle (2-OH-propyl-β-cyclodextrin solution), citalopram (10mg/kg, Lundbeck), SB242084 (2.5mg/kg) or citalopram and SB242084 combined (n=8 rats/group). Rats were imaged using RARE implemented on a 2.35T Bruker Biospec Avance imaging system: Acquisition time=280s, NEX=8, TR=4379ms, TE=62.7ms, flip angle 90°, matrix dimensions=64x64, FOV=5cm). Basal scans of whole rat brain (30 X 1mm coronal slices) were collected for 1 hour after which drugs were injected, and scanning continued for a further 3 hours. FMRI data were pre-processed and analysed (drug versus basal) using Fixed Effects analysis (SPM99[(9)]). Due to the low number of rats per group and the inter-rat variability in response within the groups, SPM_T maps were thresholded to $p \leq 0.05$ and were converted into probability maps (Medx, Sensor Systems) which display the number of rats exhibiting BOLD responses in different brain areas. According to binomial distribution, in order for a response to be deemed significant ($p \leq 0.05$, i.e. not due to chance), a response must be observed in 6 or more rats in a group of 8.

## Results

Neither the vehicle nor the citalopram produced reproducible BOLD effects relative to basal. However, SB242084 produced significant positive BOLD responses (increases in blood flow relative to basal) in brain regions including the caudate putamen (CPu, fig 1) and the amygdala, and negative BOLD responses (decreases in blood flow relative to basal) were observed in CA2/CA3 hippocampal (hip) regions, the region of the raphe (RLi), the cingulate and retrosplenial (RS) cortices and the medial geniculate (MG, fig 2). The combination of citalopram and SB242084 produced less functional change than SB242084 alone; no positive BOLD effects were detected, and relatively small negative BOLD responses were found in areas including CA2/CA3 hippocampal fields (fig 3).




The effect of SB242084 (2.5mg/kg, s.c.) on positive BOLD (fig 1) and negative BOLD (fig 2). The effect of citalopram (10mg/kg, s.c.)+SB242084 (2.5mg/kg, s.c.) on negative BOLD.
Red regions shown are areas in which ≥ 6 rats exhibited a significant drug response versus basal ($p \leq 0.05$). Regions with BOLD in <6 rats not shown. Fig 1 Bregma -0.26mm, Fig2 &3 Bregma -5.30mm[(10)]

## Discussion

Functional responses to manipulation of the 5-HT system were detected using BOLD contrast fMRI. The lack of acute citalopram-induced effect in the present study may be due to 5-$HT_{1A}$ receptor-mediated reduction in 5-HT neuronal firing rate and consequently only a modest increase in 5-HT release in terminal regions, as previously documented in electrophysiological[3] and microdialysis[4] experiments. In contrast, SB242084 produced robust BOLD responses in rat brain regions rich in 5-$HT_{2C}$ receptors[11] indicating that SB242084 altered tonic activity via 5-$HT_{2C}$ receptors in these areas. The marked effect in the striatum supports a role for 5-$HT_{2C}$ receptors in the regulation of dopaminergic function[12]. The combination of the two drugs resulted in a smaller BOLD response than with SB242084 alone (fig 2 & 3). The lack of effect with citalopram may suggest that our BOLD technique lacks adequate sensitivity to detect effects that may occur, but that these effects are unmasked by co-treatment with the 5-$HT_{2C}$ antagonist.

## Conclusions

The present study demonstrated that acute citalopram produced no significant functional changes while the 5-$HT_{2C}$ receptor antagonist produced changes in brain areas innervated by 5-HT. Acute citalopram diminished the response to 5-$HT_{2C}$ receptor antagonism possibly by a competitive mechanism involving enhanced endogenous 5-HT levels. Thus, functional changes in the serotonergic system can be detected using BOLD fMRI in rats *in vivo*.

**References:** [1] Blier P (2003) Eur Neuropsychopharmacol, 13, 57-66 [2] Stahl SM (1998) J Affect Disord, 51, 215-235 [3] Arborelius L, Nomikos GG et al (1995) Naunyn Schmiedebergs Arch Pharmacol, 352, 157-165 [4] Hjorth S, Bengtsson HJ and Milano S (1996) Eur J Pharmacol, 316, 43-47 [5] Cremers TIFH, Bosker FJ et al (2003) Proc 10th Int Conf on In Vivo Methods, p28-30 [6] Leslie RA and James MF (2000) Trends Pharmacol Sci, 21, 314-318 [7] Hyttel J (1994) Int Clin Psychopharmacol, 9 suppl 1, 19-26 [8] Kennett GA, Wood MD et al (1997) Neuropharmacology, 36, 609-620 [9] Friston KJ, Holmes PA et al (1995) Hum Brain Mapp, 2, 189-210 [10] Paxinos G and Watson C (1996) The Rat Brain in Stereotaxic Coordinates 3rd Ed [11] Clemett DA, Punhani T et al (2000) Neuropharmacology, 39, 123-132 [12] De Deurwaerdere and Spampinato U (2001) Trends Pharmacol Sci, 22, 502-504 **Acknowledgments:** Y. Shah, S. Hogg and A. Mørk (Lundbeck, Copenhagen), Medical Research Council