Systemic inflammation alters central 5-HT function as determined by pharmacological MRI

Yvonne Couch \textsuperscript{a,b}, Chris J. Martin \textsuperscript{b}, Clare Howarth \textsuperscript{b}, Josie Raley \textsuperscript{b}, Alexandre A. Khrapitchev \textsuperscript{b}, Michael Stratford \textsuperscript{b}, Trevor Sharp \textsuperscript{a}, Nicola R. Sibson \textsuperscript{a,b,⁎}, Daniel C. Anthony \textsuperscript{a}

\textsuperscript{a} Department of Pharmacology, University of Oxford, Mansfield Rd, Oxford, OX1 3QT, UK
\textsuperscript{b} CR-UK/MRC Gray Institute for Radiation Oncology and Biology, Department of Oncology, University of Oxford, Churchill Hospital, Oxford, OX3 7LJ, UK

Abstract

Considerable evidence indicates a link between systemic inflammation and central 5-HT function. This study used pharmacological magnetic resonance imaging (phMRI) to study the effects of systemic inflammatory events on central 5-HT function. Changes in blood oxygenation level dependent (BOLD) contrast were detected in selected brain regions of anaesthetised rats in response to intravenous administration of the 5-HT-releasing agent, fenfluramine (10 mg/kg). Further groups of rats were pre-treated with the bacterial lipopolysaccharide (LPS; 0.5 mg/kg), to induce systemic inflammation, or the selective 5-HT\textsubscript{2A} receptor antagonist MDL100907 prior to fenfluramine. The resultant phMRI data were investigated further through measurements of cortical 5-HT release (microdialysis), and vascular responsivity, as well as a more thorough investigation of the role of the 5-HT\textsubscript{2A} receptor in sickness behaviour. Fenfluramine evoked a positive BOLD response in the motor cortex (\(+15.9 ± 2\%\)) and a negative BOLD response in the dorsal raphe nucleus (\(-9.9 ± 4.2\%\)) and nucleus accumbens (\(-7.7 ± 5.3\%\)). In all regions, BOLD responses to fenfluramine were significantly attenuated by pre-treatment with LPS (\(p < 0.0001\)), but neurovascular coupling remained intact, and fenfluramine-evoked 5-HT release was not affected. However, increased expression of the 5-HT\textsubscript{2A} receptor mRNA and decreased 5-HT\textsubscript{2A}-dependent behaviour (wet-dog shakes) was a feature of the LPS treatment and may underpin the altered phMRI signal. MDL100907 (0.5 mg/kg), 5-HT\textsubscript{2A} antagonist, significantly reduced the BOLD responses to fenfluramine in all three regions (\(p < 0.0001\)) in a similar manner to LPS. Together these results suggest that systemic inflammation decreases brain 5-HT activity as assessed by phMRI. However, these effects do not appear to be mediated by changes in 5-HT release, but are associated with changes in 5-HT\textsubscript{2A}-receptor-mediated downstream signalling pathways.

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Introduction

Evidence indicates that abnormalities in brain 5-HT function, responses to citalopram for example, persist in patients who are clinically recovered from depression, but at risk of relapse (Bhagwagar \textit{et al.}, 2002). Understanding biological variation in the 5-HT system caused by key risk factors is central to the search for effective antidepressant treatment. Inflammation is thought to be an important risk factor in the aetiology of depression (Hughes \textit{et al.}, 2012). Furthermore, antidepressant drugs are known to reverse inflammation-induced depression, suggesting that cytokine production may directly affect the 5-HT system. For example, therapy with the cytokine interferon-α results in 25% of patients suffering from a depressive episode as an adverse effect which is subsequently managed therapeutically by 5-HT targeted antidepressants (Horikawa \textit{et al.}, 2003). Similarly, potent broad-spectrum inflammatory agents, such as the bacterial endotoxin lipopolysaccharide (LPS) and attenuated \textit{Mycobacterium bovis}, have been shown to induce depressive-like symptoms, often known as sickness behaviours, in rodents and induce changes in the 5-HT system (O’Connor \textit{et al.}, 2009). Sickness behaviours are defined behavioural adaptations in response to an invading pathogen and are observed in both human patients and in rodents (Hart, 1988). They are now thought to arise from an interaction between the immune system and the 5-HT system (O’Connor \textit{et al.}, 2009). This interaction between 5-HT and the immune system is bidirectional in nature — clinically depressed patients with no obvious systemic infection often present with high levels of circulating cytokines and reduced tryptophan levels (Alesci \textit{et al.}, 2005; Levine \textit{et al.}, 1999). Reduced tryptophan levels indicate a potential link between the systemic immune system and the central 5-HT system, however, the mechanisms by which this link is established remain unclear.

The mechanisms by which inflammatory molecules affect 5-HT, its signalling pathways, and subsequent mood, are currently under debate. Danzer and colleagues have suggested that proinflammatory stimuli, such LPS or interleukin-1β (IL-1β), increase activity in the

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Moreover, clinical studies have demonstrated that 5-HT augmentation (O’Connor et al., 2009) evokes region-specific increases in BOLD are not yet known, our previous studies demonstrated relations of neuronal function (Rauch et al., 2008; Preece et al., 2009). Solutions of 5-HT function at the preclinical level (Ramamoorthy et al., 1995; Zhu et al., 2010), which is considered an aggregate index of excitation.

To determine the effect of systemic inflammation on the phMRI response to fenfluramine, animals were injected intraperitoneally with 0.5 mg/kg LPS (E. coli strain O111:B4; Sigma-Aldrich) in sterile pyrogen-free saline to induce a systemic inflammatory response 6 h prior to fenfluramine administration (n = 6). Previous data show that 0.5 mg/kg LPS is required to produce a robust febrile response (Derrijck et al., 1993). Control animals (n = 5) were injected with vehicle, instead of LPS, 6 h prior to fenfluramine administration. Two further control groups were included that received either LPS (n = 3) or saline (n = 4) 6 h prior to phMRI during which vehicle was injected in place of LPS. Two further control groups were included that received either LPS (n = 3) or saline (n = 4) 6 h prior to phMRI during which vehicle was injected in place of fenfluramine. The four groups are denoted as follows: LPS-Fen, Veh-Fen, LPS-Veh and Veh-Veh.

In a further group of animals the effects of blocking the 5-HT_{2A} receptor on the phMRI response to fenfluramine were assessed. In these animals, a single dose of MDL100907 (0.5 mg/kg) was administered i.p. via an indwelling cannula 15 min prior to either fenfluramine (n = 6) or vehicle (n = 3) administration. The MRI acquisition was started 15 min prior to MDL100907 administration and a further 15 min of baseline data were acquired following MDL100907 injection and prior to fenfluramine/vehicle administration (total baseline data = 30 min). In these groups image acquisition post-fenfluramine (or vehicle) was shortened to 75 min owing to the longer baseline period. The data were compared to animals injected with either fenfluramine (n = 5) or vehicle alone (n = 4). These animals were not pre-injected with vehicle given that the previous experiment had demonstrated no effect of saline administration on the BOLD signal and, hence, the baseline period was only 15 min in duration. Thus, the data from this experiment are shown from 15 min prior to either fenfluramine or vehicle injection for all groups. Again, the baseline period for analysis was taken as 16 min (8 images) in total to allow for the fenfluramine injection.

Analysis of the fMRI datasets was carried out using AFNI (http://afni.nimh.nih.gov/afni/) and FEAT (http://www.fmrib.ox.ac.uk) software packages (Jenkinson et al., 2012; Woolrich et al., 2009; Smith et al., 2004). Datasets were corrected for any motion artefacts using AFNI, and spatial smoothing performed in FEAT using a Gaussian kernel of FWHM 1 mm. Statistical analysis was performed using a general linear model within FEAT. The design matrix consisted of a simple temporal model of the expected response, with 0 specifying the baseline period and 1 or $-1$ specifying signal increases or decreases during the post-drug period respectively. To generate activation maps, we used the cluster-based thresholding approach described in Smith et al. (2008).
et al. (2004). Images were converted to Z-statistics and thresholded to identify voxel clusters. A Z-statistic threshold of $Z = 3.51$ was used to define contiguous clusters and the significance level of each clusters (from Gaussian random field theory, Friston et al., 1995) was compared with a cluster probability threshold of $p < 0.05$. A colour-coded z-statistic map was then overlaid onto the corresponding phMRI gradient echo image. With reference to the anatomical images and a stereotaxic atlas (Paxinos et al., 1980), regions of interest (ROI) were manually defined on the functional datasets for anterior motor cortex, nucleus accumbens and dorsal raphe nucleus. The average BOLD signal intensity changes across all pixels over time within these ROIs was determined, expressed as percentage change from baseline signal intensity, and plotted as a function of time.

**Brain microdialysis**

Microdialysis probes were fabricated in-house using stainless steel cannulae (23G, Cooper's Needle Works Ltd, United Kingdom) with a semi-permeable membrane tip (3 mm effective tip length; 200 mm LD, 40,000 MW cut-off, 60 Å pore size, Hospal AN 69). Animals were prepared as described above ($n = 5$ or 6 per group). Following tracheotomy and vessel cannulations, animals were placed in a stereotaxic frame (Stoetling Co., USA), the skull was exposed and a burr-hole drilled over the region of skull overlying the right anterior motor cortex was thinned to translucency using a dental drill. A laser Doppler probe (Perimed) was fixed to the animal’s skull with a cluster probability threshold of $p < 0.05$. A colour-coded z-statistic map was then overlaid onto the corresponding phMRI gradient echo image. With reference to the anatomical images and a stereotaxic atlas (Paxinos et al., 1980), regions of interest (ROI) were manually defined on the functional datasets for anterior motor cortex, nucleus accumbens and dorsal raphe nucleus. The average BOLD signal intensity changes across all pixels over time within these ROIs was determined, expressed as percentage change from baseline signal intensity, and plotted as a function of time.

**High pressure liquid chromatography**

Dialysate samples were analysed using HPLC with electrochemical detection and separated with an ACE column (C18, 3 μm, 125 × 3 mm + ACE C18 guard, 10 × 3 mm run at 35°C). Samples were carried by an eluent (12.5% methanol, 130 mM NaH2PO4, 0.85 mM Na2EDTA, 0.1 mM 1-octanesulphonic acid, pH 3.55) pumped with a flow rate of 0.6 ml/min (Waters 2695 HPLC Pump). Samples were detected using a glassy carbon electrode held at +0.75 V (Dionex ED40). The dialysate content was determined with reference to daily-calibrated standard solutions in 0.06 M perchloric acid (5 pmol 5-HT and 5-HIAA). Chromatograms were displayed and analysed using Waters Empower 2 software.

**Laser Doppler flowmetry**

Six hours after either LPS or saline injection, animals ($n = 5$ per group) were placed in a stereotaxic frame (Kopf Instruments), and a region of skull overlying the right anterior motor cortex was thinned to translucency using a dental drill. A laser Doppler probe (Perimed Probe 403, Jarfalla, Sweden) was positioned above the visible cortical surface. Carbon fibre stimulating electrodes were inserted through burr hole made in the skull overlying the contralateral MCx and advanced over the cortical surface to a position overlying the anterior motor cortex (Austin et al., 2003). Electrical stimuli were delivered to the contralateral MCx and recordings of stimulus evoked cerebral blood flow changes were recorded using the laser Doppler probe. For each laser Doppler experimental session, 2-s stimuli of 5, 10, 20, 30 and 40 Hz were presented in a randomised sequence (10 repeats at each frequency) with a stimulus pulse width of 0.3 ms and an inter-stimulus interval (ISI) of 25 s. All stimulus presentation was controlled through a 1401 Plus (CED Ltd., UK) running custom-written code.

**Autoradiography**

Fresh frozen brain tissue was cut coronally at 12 μm. Adjacent tissue sections were incubated with either 2 nM [3H]Ketanserin or 2 nM [3H] with 10 μM methylsergide. Sections were then placed in a cassette with Kodak BioMax MR film and exposed for approximately 6 weeks. Analysis was performed using MCID-Core software.

**Results**

**Effect of fenfluramine on BOLD in rat brain regions**

Physiological parameters were maintained within normal ranges throughout each experiment: body temperature = $37 ± 0.5°C$; $pCO_2 = 35–42$ mm Hg; $pO_2 ≥ 90$ mm Hg; pH = $7.4 ± 0.1$; mean arterial blood pressure = $120 ± 20$ mm Hg. Fenfluramine (10 mg/kg i.v.) evoked significant and prolonged changes in BOLD signal intensity in all three brain regions studied (Figs. 1 & 2A–C); anterior motor cortex (MCx); nucleus accumbens (NAc) and dorsal raphe nucleus (DRN). In particular, bilateral increases in BOLD signal intensity were evident in the MCx, whilst decreases in BOLD signal intensity were found in the NAc (Fig. 2B) and DRN (Fig. 2C). The maximum change observed was $+15.9 ± 2%$ of baseline in the MCx, $−7.7 ± 5.3%$ in the NAc and $−9.9 ± 4.2%$ in the DRN.
Effect of systemic inflammation on fenfluramine-induced BOLD responses

Pre-treatment with LPS (0.5 mg/kg i.p.) 6 h prior to fenfluramine administration significantly reduced the BOLD response to fenfluramine. In all brain regions examined, ANOVA across the first 46 min of the time-course data (16 min baseline + 30 min post-fenfluramine or vehicle; Figs. 2A–C) showed highly significant (time $p < 0.0001$ $F_{21,414} = 28.83$, group*time $p < 0.0001$ $F_{21,414} = 66.58$) differences in the BOLD time-course response to fenfluramine between the groups. However, in the MCx and DRN, whilst post-hoc Dunnett’s tests showed that whilst the Veh-Fen time-course was significantly different to the Veh-Veh group (MCx, DRN $p < 0.0001$), neither the LPS-Fen nor the LPS-Veh time-courses were significantly different to the Veh-Veh group. In the NAc both the Veh-Fen and the LPS-Fen groups were significantly different to the Veh-Veh group, albeit to differing degrees; $p < 0.0001$ and $p < 0.05$, respectively.

In the analysis of the averaged first 30 min of data acquired post-fenfluramine injection (see Materials & methods), highly significant (post-hoc Dunnett’s test, $p < 0.0001$) differences were again found between the Veh-Fen and Veh-Veh groups in all regions (Figs. 2D–F). However, in this case small, but significant, differences were also found between LPS-Fen and Veh-Veh groups in all regions (post-hoc Dunnett’s test MCx $p < 0.05$, NAC $p < 0.0001$ and DRN $p < 0.01$). In addition, the LPS-Veh group in the DRN showed a significant difference to the Veh-Veh group (post-hoc Dunnett’s test $p < 0.05$). These data suggest some residual effects of fenfluramine, although from the time course analysis these effects appear minimal with the BOLD responses to fenfluramine being largely abolished (Figs. 2A–C).

Effect of systemic inflammation on fenfluramine-evoked release of 5-HT

To determine whether attenuation of the fenfluramine-evoked BOLD response by LPS was due to decreased 5-HT release, in vivo microdialysis of the MCx was carried out under the same conditions as the phMRI experiments. The MCx was chosen for these studies based primarily on the fact that the largest BOLD changes in response to fenfluramine were found in this area. However, Mangiaviti et al. (2001); Herwig et al. (2002) have also shown that antidepressant treatment increases the excitability of the motor cortex, possibly via a 5-HT-related mechanism. Administration of fenfluramine (10 mg/kg i.v.) induced an increase in 5-HT in cortical dialysates (Fig. 3). This increase in 5-HT was greatest 40 min post-fenfluramine injection and 5-HT levels returned to baseline within the following 60 min. In comparison, fenfluramine also evoked an increase in 5-HT in animals pre-treated with LPS (0.5 mg/kg i.p., 6 h) and the magnitude 5-HT response and duration of effect was not significantly different to animals receiving fenfluramine alone (Fig. 3). Fenfluramine had no significant effect on the levels of the principal 5-HT metabolite 5HIAA, and this was not different in animals treated with LPS.

Neurovascular coupling experiments

Laser-Doppler flowmetry (LDF) monitoring of stimulus-evoked cerebral blood flow (CBF) changes was made to verify that neurovascular coupling and functional hyperaemic responses remained intact post-LPS treatment (Figs. 4A–B). Six hours post-LPS administration (0.5 mg/kg i.p.) area under the curve measurements of the CBF response showed no significant differences to those obtained from control animals (Fig. 4C). Given that the BOLD signal measurements described above were acquired with a temporal resolution of 120 s, an integrated measure of total CBF response over time was used to characterise the stimulus evoked CBF signal change rather than a readout of the transient response maxima.

Effect of 5-HT$_{2A}$ receptor blockade on fenfluramine-induced BOLD responses

Our previous studies demonstrated that fenfluramine-evoked changes in activity-dependent gene expression involved 5-HT$_{2A}$ receptor activation (Hirani et al., 2003). For the time course analysis (15 min baseline + 31 min post-fenfluramine or vehicle) pre-treatment with the selective 5-HT$_{2A}$ receptor antagonist MDL100907 significantly attenuated the BOLD response to fenfluramine compared to untreated animals in the MCx and DRN (ANOVA group $F_{3,14} = 22.31$, time $F_{7,460} = 38.79$, group*time $F_{21,414} = 38.79$; post-hoc MCx $p < 0.0001$, DRN; $p < 0.0001$; Figs. 5A, C). In these regions both groups pre-treated with MDL (MDL-Fen and MDL-Veh) were significantly different to the animals given fenfluramine alone (post-hoc Tukey $p < 0.0001$), but not to the control animals given vehicle alone. In contrast, in the NAc MDL100907 did not appear to ameliorate the response to fenfluramine, with both MDL-Fen and fenfluramine alone groups showing significant differences to the MDL-Veh and vehicle alone groups (post-hoc Tukey $p < 0.0001$; Fig. 5B), but not to each other.

Similarly, in the MCx and DRN, analysis of the first 30 min post-fenfluramine (or vehicle) administration showed that the response to fenfluramine was largely abolished with MDL100907 pre-treatment (Fig. 5D, ANOVA group $p < 0.0001$ $F_{3,14} = 39.33$; post-hoc Tukey $p < 0.001$). No significant differences were found between the MDL-Fen, MDL-Veh and vehicle alone groups in the MCx (Fig. 5D). However, although only minor differences in the BOLD response time course were evident between the MDL-Fen, MDL-Veh and vehicle only groups in the DRN (Fig. 5C), these differences reached significance in the analysis of the first 30 min post-fenfluramine (post-hoc Tukey $p < 0.01$; Fig. 5F) suggesting some residual effect of

![Fig. 1. BOLD activation maps and ROIs. Representative z-score maps and Paxinos atlas images showing statistically significant BOLD responses in (A) anterior motor cortex, (B) nucleus accumbens and (C) dorsal raphe nucleus.](image-url)
fenfluramine. As for the time course analysis, no significant difference in the BOLD response of the NAc to fenfluramine was seen in the MDL-Fen group compared to the fenfluramine alone group in the analysis of the first 30 min post-fenfluramine injection (Fig. 5E). At the same time, the MDL-Fen group showed significant differences to both the vehicle only and MDL-Veh groups (post-hoc Tukey, p < 0.05 and 0.001, respectively; Fig. 5E) confirming minimal effects of 5-HT2A antagonism on the BOLD response to fenfluramine in the NAc.

To confirm that MDL100907 pre-treatment did not alter the baseline BOLD signal the initial 15 min of baseline in the two MDL100907 treated groups was compared with the 15 min of baseline acquisition post-MDL100907 administration. No significant differences were found.

Systemic inflammation and 5-HT2A receptor expression

Changes in BOLD responses after systemic inflammation and in response to 5-HT2A drugs may reflect post-synaptic changes in receptor expression. Tissue was cryosectioned for autoradiographic analysis using [3H]-ketanserin, a 5-HT2A ligand. Expression of the 5-HT2A receptor is largely restricted to the prefrontal areas of the brain, therefore cryosections did not extend beyond the start of the hippocampus (Lopez-Gimenez et al., 1997). 5-HT2A expression was measured in saline (Fig. 6B) and LPS (Fig. 6C) brains and was found to be significantly increased in the cingulate MCx (Student’s t-test; p < 0.05) and elevated, but not significantly different from saline challenged animals (p = 0.06), in the frontal MCx.

Effects of LPS on 5-HT2A-mediated behaviour

Whilst changes in 5-HT2A receptor expression and the administration MDL100907 strongly indicate that LPS might alter 5-HT2A signalling, it was important to demonstrate functional effects in a 5-HT2A-dependent behaviour. With this in mind, we used a direct 5-HT2A agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane...
DOI; 1 mg/kg subcutaneous) in the presence and absence of systemic inflammation. Subcutaneous DOI administration in laboratory animals elicits a very specific behavioural response; in rats it is known as wet dog shake (WDS) behaviour. Both LPS and DOI had a significant effect on WDS shake behaviour, there was also a significant interaction (two-way ANOVA; DOI p < 0.001 F1,19 = 124.3; LPS p < 0.001 F1,19 = 38.17; LPS:DOI p < 0.001; F1,19 = 29.31). Post-hoc analysis shows that when compared to vehicle, DOI induced significantly more WDS 6 h after a 0.1 ml i.p. injection of 0.9% saline (Bonferroni post-hoc; p < 0.001). Administration of a single dose of 0.1 ml LPS in saline (0.5 mg/kg i.p.; broken line; n = 5) 6 h prior to t = 0. Data were calculated as a percentage of baseline (mean ± SEM values are shown).

Discussion

The principal findings of the present study are that phMRI-measured brain responses to the 5-HT releasing agent fenfluramine were markedly reduced by prior administration of the systemic inflammatory agent LPS, and that mechanically these effects may be mediated by changes in post-synaptic receptor expression. Our data indicate not only that systemic inflammatory pathways affect central 5-HT function, but that functional MRI outcomes, used in both animals and man, can be altered by systemic immune system activation. Our parallel microdialysis experiments showed that LPS did not directly reduce fenfluramine-evoked 5-HT release, thereby ruling out a presynaptic 5-HT mechanism in this instance. Direct recordings of stimulus evoked blood flow responses further show that the effects of LPS on the fMRI response to fenfluramine were not due to disruption of vascular reactivity or neurovascular coupling mechanisms. Finally, results using 5-HT2A-targeting drugs indicate that some aspects of sickness behaviour may be mediated by the 5-HT2A receptor, whilst the fenfluramine evoked BOLD signal changes were attenuated by pre-treatment with the selective 5-HT2A receptor antagonist, MDL100907. Taken together, these data suggest that contrary to predictions from previous studies, the inhibitory effect of systemic inflammation on central 5-HT function is mediated by a post- and not presynaptic 5-HT mechanism, and that a downstream reduction in 5-HT2A receptor signalling may play a key role in this inhibition.

Decreased BOLD responses to fenfluramine after systemic inflammation

We have recently reported that the 5-HT releasing agent fenfluramine elicits a pattern of BOLD signal changes across several brain regions of the anaesthetised rat. This effect was attenuated by prior
5-HT depletion, suggesting that the BOLD response to fenfluramine is a surrogate marker of increased 5-HT release (Preece et al., 2009). Consistent with these data, human studies report that the selective 5-HT re-uptake inhibitor citalopram, also evokes BOLD responses (McKie et al., 2005). As shown previously, fenfluramine evoked region-specific changes in BOLD signal in the current study. Pre-treatment with LPS markedly attenuated the BOLD responses to fenfluramine. This finding is in accord with human fMRI studies, which show that changes in neuronal activity may be altered by states of heightened immune compromise (Brydon et al., 2008). Given the evidence that the fenfluramine-evoked BOLD changes are 5-HT-dependent (Preece et al., 2009), it seems likely that the effect of LPS on the BOLD responses to fenfluramine is also mediated by alteration of central 5-HT function.

Importantly, cortical in vivo microdialysis experiments demonstrated that LPS administration did not alter either basal extracellular 5-HT levels or its metabolite 5-HIAA, nor fenfluramine-evoked release of cortical 5-HT. In previous studies we have demonstrated that fenfluramine not only evoked cortical 5-HT release similar in magnitude to that observed here, but that the 5-HT response to fenfluramine was markedly attenuated in animals with depleted brain levels of 5-HT (Series et al., 1994). Thus, in the MCx, at least, decreased 5-HT release does not appear to underlie the LPS-induced suppression of BOLD responses to fenfluramine.

Since systemic inflammation is well known to affect the vasculature (Brydon et al., 2008), it was important to test whether LPS treatment disrupted mechanisms linking changes in neuronal activity to the hemodynamic responses. To this end, a model that we have previously used to study neurovascular coupling (Martin et al., 2006) was used to verify that functional hyperaemic responses remained intact in LPS treated animals. Although there was some variation in responses between LPS- and saline-treated animals, these experiments...
demonstrated that it was still possible to elicit robust haemodynamic responses to increased neuronal activity in the LPS treated animals. Previous studies have shown that endotoxin-induced inflammation significantly alters CBF and disrupts autoregulation (Rosengarten et al., 2008a, 2008b). Moreover it has been hypothesised that systemic endotoxin causes a direct uncoupling of the cerebral microvasculature from its neuronal input, but that this is not caused by inflammation-induced oedema (Rosengarten et al., 2008a, 2008b). In contrast, the current data show that in the MCx, at least, neurovascular coupling mechanisms remain intact during the acute phase of systemic inflammation. In the current study, a lower LPS dose was used (0.5 mg/kg vs. 1–5 mg/kg) than that of Rosengarten et al. and this may explain the discrepancy with earlier work.

**BOLD responses to fenfluramine: role of 5-HT2A receptors**

As shown previously (Preece et al., 2009), fenfluramine induced a positive BOLD response in the MCx, but negative responses in both the DRN and NAc. Pre-treatment with the selective 5-HT2A antagonist MDL100907 effectively blocked the BOLD response to fenfluramine in both MCx and DRN, whilst attenuation of the BOLD response in the NAc was only partial. These findings suggest a strong involvement of the 5-HT2A receptor in the BOLD response to increased 5-HT in the MCx and DRN, but the involvement of additional receptor(s) for 5-HT or other neurotransmitters in the NAc. Candidate excitatory 5-HT receptor subtypes in NAc that would not be blocked by MDL100907 include 5-HT2C, 5-HT4 and 5-HT6 receptors. Previous studies have detected non-5-HT-mediated changes in Fos response to fenfluramine in the caudate nucleus (Javed et al., 1998), and other studies report that fenfluramine releases catecholamines at higher doses (Balcioglu and Wurtman, 1998). Thus, although fenfluramine may primarily release 5-HT, a minor contribution from other transmitters is possible in the NAc.

The BOLD increase in MCx induced by fenfluramine most likely reflects activation of postsynaptic 5-HT2A receptors, which are excitatory and abundant in this region. In comparison, there is a low abundance of 5-HT2A receptors in the DRN suggesting that a component of this effect may be mediated by 5-HT2A receptors located outside the DRN. We have previously demonstrated evidence for a feedback loop from the anterior MCx to the DRN involving cortical glutamatergic neurones that synapse onto local GABAergic neurons in the DRN (Hajos et al., 1998; Varga et al., 2001), which is likely mediated by 5-HT2A receptors in MCx (Boothman et al., 2003; Sharp et al., 2007). This cortical-raphe control system can be altered under conditions of stress has been reviewed by Stein et al. (2007). Thus, the fenfluramine-evoked decrease in BOLD signal observed in the DRN might, in part, be triggered by cortical 5-HT2A receptors, which excite cortico-raphe projections leading to a reduction in neuronal activity in the DRN through activated local GABAergic neurons. The fenfluramine-evoked BOLD decrease observed...
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In summary, the current data suggest that the mechanisms underlying the effects of systemic inflammation on the BOLD response to fenfluramine are not pre-synaptic in origin, specifically through compromised 5-HT release, and that they do not reflect disruption of neurovascular coupling capacity. However, the pattern of LPS-induced effects is strikingly similar to those observed with a specific 5-HT2A receptor antagonist. These findings, in combination with inflammation-induced changes in 5-HT2A expression and 5-HT2A-mediated behaviours, suggest that the effects of systemic inflammation on central 5-HT function reflect, at least in part, modulation of 5-HT2A signalling pathways.

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**Conflict of interest statement**

The authors have no conflicts of interest, financial or scientific, to disclose.

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