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Effect of Citalopram on Emotion Processing in Humans: A Combined 5-HT_{1A} [^{11}C]CUMI-101 PET and Functional MRI Study

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A subset of patients started on a selective serotonin reuptake inhibitor (SSRI) initially experience increased anxiety, which can lead to early discontinuation before therapeutic effects are manifest. The neural basis of this early SSRI effect is not known. Presynaptic dorsal raphe neuron (DRN) 5-HT_{1A} receptors are known to have a critical role in affect processing. Thus we investigated the effect of acute citalopram on emotional processing and the relationship between DRN 5-HT_{1A} receptor availability and amygdala reactivity. Thirteen (mean age 48 ± 9 years) healthy male subjects received either a saline or citalopram infusion intravenously (10 mg over 30 min) on separate occasions in a single-blind, random order, crossover design. On each occasion, participants underwent a block design face-emotion processing task during fMRI known to activate the amygdala. Ten subjects also completed a positron emission tomography (PET) scan to quantify DRN 5-HT_{1A} availability using [^{11}C]CUMI-101. Citalopram infusion when compared with saline resulted in a significantly increased bilateral amygdala responses to fearful *vs* neutral faces (left $p = 0.025$; right $p = 0.038$ FWE-corrected). DRN [^{11}C]CUMI-101 availability significantly positively correlated with the effect of citalopram on the left amygdala response to fearful faces ($Z = 2.51$, $p = 0.027$) and right amygdala response to happy faces ($Z = 2.33$, $p = 0.032$). Our findings indicate that the initial effect of SSRI treatment is to alter processing of aversive stimuli and that this is linked to DRN 5-HT1A receptors in line with evidence that 5-HT1A receptors have a role in mediating emotional processing.
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INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed medications for anxiety and depressive disorders worldwide (Olfson and Marcus, 2009). However, despite being used to treat anxiety disorders, a subgroup of patients experience an initial increase in anxiety after initiation of SSRI treatment (Gollan *et al*, 2012; Sinclair *et al*, 2009). Although this generally ameliorates over a few weeks, it can be clinically problematic as these patients with high anxiety are less likely to reach remission (Gollan *et al*, 2012). The neural basis of this early effect of SSRIs on anxiety and subsequent heterogeneity in treatment response is not known.

Serotonin, or 5-hydroxytryptamine (5-HT), is thought to be critical for affect regulation in the brain (Dayan and Huys, 2008), and SSRIs are thought to act primarily by altering 5-HT function. The administration of single doses of citalopram, a commonly used SSRI, in healthy human subjects is associated with enhanced startle responses and fear recognition (Browning *et al*, 2007; Burghardt *et al*, 2004; Grillon *et al*, 2007) and altered serotonin release (Selvaraj *et al*, 2012b). Functional magnetic resonance imaging (fMRI) studies have revealed that depressed patients have exaggerated amygdala reactivity as measured using blood-oxygen-level-dependent (BOLD) responses when presented with emotions of negative valence (fearful or sad faces), and 8 weeks of SSRI treatment attenuates this to 'normalize' the amygdala responses (Sheline *et al*, 2001). Bigos *et al* (2008) using a double-blind balanced crossover study design found that citalopram 20 mg infusion compared with saline in healthy male participants ($N = 8$) caused

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concentration-dependent increases in human amygdala reactivity to aversive facial stimuli (Bigos *et al*, 2008). However, in contrast, Del-Ben *et al* (2005) used a covert (aversive) face emotion recognition task and found attenuated amygdala response to fear after a 7.5 mg citalopram infusion compared with saline in male volunteers ($N=12$).

5-HT_{1A} receptors are a key regulator of brain 5-HT activity through inhibitory autoreceptors located presynaptically on 5-HT dorsal raphe neurons (DRNs), as well as on postsynaptic neurons in projection sites (Barnes and Sharp, 1999). Activation of the DRN 5-HT_{1A} receptors causes hyperpolarization and reduces 5-HT neuronal firing, which results in decreased 5-HT release from the 5-HT nerve terminals in the synapses. Acute SSRI administration increases 5-HT by blocking 5-HTT, which then activates raphe 5-HT_{1A} autoreceptor and thus reducing neuronal firing. Raphe 5-HT_{1A} activation causes internalization, which immediately returns to baseline level (Riad *et al*, 2001), and this phenomenon is not observed in postsynaptic 5-HT_{1A} receptors (Riad *et al*, 2001). 5-HT_{1A} receptors have been consistently shown to modulate anxiety-related behavior in animal models. Specifically, 5-HT_{1A} receptor knockout mice exhibit increased fear-related behavior (Ramboz *et al*, 1998; Richardson-Jones *et al*, 2011) and an altered fear response (Gross *et al*, 2000). A common functional variation (C(-1019)G) in the human 5-HT_{1A} gene (HTR_{1A}) is associated with increased 5-HT_{1A} autoreceptor expression and decreased threat-related amygdala reactivity (Fakra *et al*, 2009). Finally, psychotropic drugs such as buspirone and vilazodone with 5-HT_{1A} receptor-binding properties have been found to be clinically useful for anxiety symptoms (Akimova *et al*, 2009; Gommoll *et al*, 2015; Sramek *et al*, 1999).

An inverse relationship between 5-HT_{1A} receptor binding in the dorsal raphe and amygdala reactivity has been reported in healthy human subjects (Fisher *et al*, 2006). In a combined fMRI and positron emission tomography (PET) imaging study using the 5-HT_{1A} receptor tracer [^{11}C]-CUMI-101, we similarly found DRN 5-HT_{1A} receptor binding to be inversely related to amygdala BOLD responses to fear *vs* neutral faces (Selvaraj *et al*, 2014). The above findings suggest that DRN 5-HT_{1A} receptors may have a critical role in regulating amygdala reactivity during aversive emotion processing.

Citalopram is one of the most selective SSRI compared with fluoxetine, paroxetine, sertraline, or fluvoxamine and has high affinity to serotonin transporter (5-HTT) without any significant affinity for other serotonergic (5-HT_{1A}, 5-$HT1_B$, or 5-$HT2_{A/C}$), adrenergic, cholinergic, or other neurotransmitters and (Hyttel, 1994) a single administration of citalopram 1 mg/kg in rodents increases 5-HT levels in the raphe but not in the frontal cortex. A 10 mg/kg increases 5-HT release to 400% in the raphe but only 170% in the frontal cortex (Invernizzi *et al*, 1992). This dose-dependent and differential regional effect of SSRI on 5-HT release is consistent with 5-HT_{1A}-mediated negative feedback mechanism (Chaput *et al*, 1986; Gartside *et al*, 1995; Riad *et al*, 2001). Thus SSRI induced 5-HT release in 5-HT neuronal projection regions could be a balance of SSRIs' ability to block 5-HTT at local neuronal terminals and to decrease DRN neuronal firing (Fuller, 1994; Gartside *et al*, 1995; Hjorth and Auerbach, 1996; Richardson-Jones *et al*, 2011). Interestingly, mice selectively engineered to express lower 5-HT_{1A} autoreceptor levels compared with those with higher DRN 5-HT_{1A} autoreceptor levels had increased raphe firing rate, greater 5-HT release in fronto-limbic regions, and produced robust response to SSRI in reducing the aversive behavior (Richardson-Jones *et al*, 2010). In addition to 5-HT_{1A} autoreceptor-mediated negative feedback, postsynaptic 5-HT_{1A} heteroreceptor and 5-HT_{1B} mediate the inhibitory actions and 5-HT_{2A} mediates the excitatory actions of 5-HT on target neurons in the prefrontal and limbic cortices along with other 5-HT receptors such as 5-HT_3, 5-HT_4, and 5-HT_7 and also regulate 5-HT neuronal firing and release through postsynaptic feedback (Sharp *et al*, 2007).

Citalopram is the only SSRI available in intravenous form and is relatively well tolerated by volunteers in clinical studies (Attenburrow *et al*, 2001). Intravenous citalopram 10 mg has been successfully used as a probe to study brain serotonin function in clinical studies (Attenburrow *et al*, 2001; Bhagwagar *et al*, 2004). In addition, we have used intravenous citalopram in PET imaging studies to characterize the specificity of serotonin transporter radioligand [^{11}C] DASB occupancy (Hinz *et al*, 2008) and to study serotonin displacement (Selvaraj *et al*, 2012b).

In the present study, we aimed to investigate the effect of acute citalopram infusion on the neural processing of aversive emotional stimuli and to determine its relationship with DRN 5-HT_{1A} receptors as measured with [^{11}C]-CUMI-101 in healthy human subjects. We hypothesized that intravenous citalopram would increase amygdala reactivity to fear *vs* neutral faces. It is not known how the DRN 5-HT_{1A} is related to the effect of acute citalopram on emotion processing. Based on our work and other studies (Richardson-Jones *et al*, 2010; Richardson-Jones *et al*, 2011; Selvaraj *et al*, 2014; Selvaraj *et al*, 2012b), we hypothesized that subjects with higher DRN 5-HT_{1A} receptor availability would show a greater increase in amygdala response to emotional facial expressions following intravenous citalopram infusion.

MATERIALS AND METHODS

A total of 13 healthy male participants took part in the citalopram and saline infusion fMRI study. All participants had undergone Structured Clinical Interview for DSM IV Disorders (Spitzer *et al*, 2004) screening interview administered by study investigators to ascertain past and current psychiatric and medical history. Inclusion criteria were male and female subjects, aged 35 – 65 years, in good physical health, and capable of giving informed consent. Exclusion criteria were contraindication to PET scanning (pregnancy or breast feeding was an absolute contraindication), current or past history of major psychiatric disorder, present or recent (previous 3 months) use of psychotropic medication, current significant illicit substance/alcohol misuse or current significant other co-morbidity, and no MRI contraindications. Electrocardiogram was carried out before the infusion to rule out any prolonged corrected QT interval. All the subjects had urine drug screen on all scan days to check for illegal drug use. All subjects also completed validated subjective scales to assess mood and anxiety and also a visual analog scale (VAS) to quantify side effects, if any. The

subjects were paid a small honorarium for taking part in the study. The study was approved by the local research ethics committee. The PET and fMRI scans were carried out at the MRC London Institute of Medical Sciences, Hammersmith Hospital, London, UK.

Research Design

Thirteen subjects first took part in a PET scan experiment in which healthy subjects received either a placebo (saline) or citalopram infusion before a [^{11}C]CUMI PET scan to index 5-HT$_{1A}$ receptor availability (Supplementary Figure 1). The results of this experiment are described in our previous publication (Selvaraj *et al*, 2012b). Subjects then went on to participate in the new fMRI experiment reported here. Of the 13 subjects who took part in the PET experiment, 3 dropped out, leaving 10 subjects who completed the fMRI component as well and we recruited an additional 3 new subjects who only participated in the fMRI component. We used the data from the 5-HT$_{1A}$ [^{11}C]CUMI PET placebo (saline) scan as an index of baseline DRN 5-HT$_{1A}$ availability (Supplementary Figure 1).

In this new fMRI experiment, all participants received either saline or an intravenous infusion of 10 mg citalopram over 30 min in a single-blinded (participants), random order crossover design on alternate days. About 15–30 min after the end of infusion, the subjects underwent the fMRI emotion processing task (Selvaraj *et al*, 2012a). Blood samples were collected for citalopram levels at ($t=0$) and after the infusion ($t=45$ min). Mood was assessed before and after each scans using VAS to ascertain subjective affective responses on emotions (including anxiety, sadness, happiness, anger, and irritability) across sessions. VAS scale was divided into a 10-point scale for each emotion. There was a gap of at least 1 week between the two scans (mean and SD was 30 (42.9) days).

Measurement of Neural Response to Emotional Stimuli

fMRI data acquisition. MRI was performed on 3 T scanner (3 T Intera Philips Medical Systems (Best, The Netherlands) to acquire T2*-weighted transverse echoplanar images (EPI). A total of 132 whole-brain EPI volumes were collected with 44 slices acquired in an even–odd interleave in a descending direction (TR = 3 s; TE = 30 ms; slice thickness = 3.25 mm; 2.19×2.19 mm^2 in-plane resolution; phase encoding direction = anterior → posterior; field of view = 280 mm^2; matrix size 128×128). Real-time reconstruction, *z*-shimming correction, and a slice tilt of − 30° to the anterior commissure–posterior commissure line were used to minimize orbitofrontal and temporal signal dropout as a result of magnetic field inhomogeneities due to air tissue susceptibility differences in these regions (Weiskopf *et al*, 2006; Weiskopf *et al*, 2007). A whole-brain 3D-MPRAGE scan was acquired (TR = 9.6 ms, TE = 4.5 ms, flip angle = 8°, slice thickness = 1.2 mm, 0.94×0.94 mm^2 in-plane resolution, 150 slices) after the EPI scans.

fMRI task. A well-characterized incidental facial emotional processing task was employed as described in our previous studies (O'Nions *et al*, 2011; Selvaraj *et al*, 2014). Subjects were shown a series of faces on a projector screen and asked to respond by classifying if each face was male or female. Emotional faces representing a single emotion (ie, happy, fearful, or neutral) were presented in 16 s blocks of eight faces, with a total of 12 blocks (4 per emotion). Subjects were instructed to fixate on a cross during a 16 s rest period between stimulus blocks.

Measurement of 5-HT$_{1A}$ Receptor Availability

PET scan acquisition. All PET scans were performed on the GE Discovery RX PET/CT scanner with a PET axial field of view of 15.7 cm and 47 reconstructed transaxial image planes. [^{11}C]CUMI-101 is a selective 5-HT$_{1A}$ radioligand with high signal-to-noise ratio in the brain. CUMI-101 has higher affinity (Ki = 0.15 nM) and better selectivity for 5-HT$_{1A}$ receptor than 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (Kumar *et al*, 2013; Kumar *et al*, 2007). It was initially developed as 5-HT$_{1A}$ partial agonist ligand with specific binding to high affinity 5-HT$_{1A}$ receptors and thus to be more sensitive to study 5-HT release than older antagonist radiotracers, such as [^{11}C] WAY-100635 (Milak *et al*, 2011). However, the exact nature of [^{11}C]CUMI-101 intrinsic activity as 5-HT$_{1A}$ receptor agonist or antagonist is not clear (Hendry *et al*, 2011; Kumar *et al*, 2013; Shrestha *et al*, 2014). [^{11}C]CUMI-101 was administered via injection into an antecubital vein as a smooth bolus over 30 s. The dynamic PET scan was acquired over 90 min in (simultaneous) frame and list mode (Selvaraj *et al*, 2012a).

Graphical analysis of reversible radioligand binding together with the metabolite-corrected plasma input function was used to quantify the binding potential BP$_{ND}$ in regions of interest (ROIs; Selvaraj *et al*, 2012b). The specific binding was quantified as BP$_{ND}$ (37) where: BP$_{ND}$ = (V_T target region − V_T reference region)/V_T reference region. V_T is the volume of distribution (ml/cm^3) defined as the ratio of the tracer concentration in the region to the metabolite-corrected plasma concentration at equilibrium (Innis *et al*, 2007).

fMRI analysis. The fMRI preprocessing and analysis were carried out in FSL using FEAT (Smith *et al*, 2004) and mirrored the analyses reported in our previous study (Selvaraj *et al*, 2014). Functional MRI data for individual runs were high pass filtered at 0.0078 Hz and motion corrected using a 6 degree of freedom (DOF) rigid body transformation (MCFLIRT). Finally, data were smoothed with an 8 mm FWHM Gaussian kernel prior to a two-stage standard space transformation. fMRI data and individual high-resolution T1 images were registered to a 2 mm MNI standard template using a 12 DOF linear transformation (FLIRT) followed by nonlinear warping of T1 images to standard space (FNIRT). Both linear and nonlinear transformations were concatenated and applied to first-level, native space statistical images before higher-level analyses.

Task regressors for happy, fearful, and neutral face blocks were modeled using a double-gamma function convolved with a 16 s square wave. Motion parameter estimates were included in the model to account for residual motion artifacts. All regressors were temporally filtered to match fMRI data preprocessing parameters (Hallquist *et al*, 2013). Time series data were prewhitened (FILM) prior to

modeling. As per Selvaraj *et al* (2014), three contrasts were calculated at this level to compare: (1) faces *vs* baseline; (2) fearful *vs* neutral faces; and (3) happy *vs* neutral faces. At the second level of analysis, each first-level contrast was submitted to a fixed-effect analysis, which computed a contrast estimate for each subject comparing citalopram *vs* placebo. Second-level contrast estimates were submitted for a final mixed-effect analysis using FSL's FLAME2 tool, which employs Bayesian estimation of mean contrast estimates to determine the group-level effect of citalopram on face (average within contrasts 1, 2, and 3) and valence processing (contrast 2 *vs* contrast 3).

To constrain the number of simultaneous tests, we defined two spherical ROIs, in the left and right amygdala, by setting a 6 mm radius around the MNI coordinates ($x = \pm 21$, $y = -6$, $z = -15$) adapted from our previous study (O'Nions *et al*, 2011; Selvaraj *et al*, 2014) using the same paradigm. This ensured an unbiased ROI definition. Voxelwise corrections for family-wise error inflation were applied using Gaussian random field (GRF) theory-based height thresholding of *Z*-statistical maps at $p < 0.05$ (corrected). The values reported in the text represent the mean lower-level contrast estimates for citalopram and placebo extracted from voxels showing a significant citalopram *vs* placebo difference and thus represents a potential selection bias (Kriegeskorte *et al*, 2009). Therefore, mean condition and contrast estimates across the independently defined spherical amygdala ROIs are presented in Table 1.

PET data analysis. Subjects' structural MRIs were segmented (into gray/white matter/cerebrospinal fluid) using the segmentation tool in SPM (www.fil.ion.ucl.ac.uk/spm) and were re-sliced ($1 \times 1 \times 1\,mm^3$) and co-registered to the corresponding subject's denoised, head movement-corrected, and summed PET image using SPM5. Amygdala, postsynaptic cortical regions, and cerebellum were defined using a probabilistic brain atlas template (Hammers *et al*, 2003). The atlas was spatially normalized to the coregistered individual MRI scans with deformation parameters obtained from the normalization to the standard MNI T1 template in SPM. The normalized brain atlas was resliced to the individual's PET space and fused with the individual gray matter map to obtain a gray matter template for the amygdala and postsynaptic cortical regions. These were then used to sample the dynamic PET to obtain the regional time–activity courses. The presynaptic DRN was manually defined as a fixed-size region ($215\,mm^3$) in the midbrain area on the summed PET images of each individual (Bose *et al*, 2011a; Selvaraj *et al*, 2014; Selvaraj *et al*, 2012b). Finally, cerebellar gray matter was used as the reference region (Selvaraj *et al*, 2012b). See Selvaraj *et al* (2012b) for further details of the [11C]CUMI PET analysis.

Multi-modal PET-MR analysis. The third-level analysis described in the fMRI analysis above was repeated for the 10 subjects who had also completed the PET imaging protocol. To estimate the relationship between fMRI changes associated with citalopram and DRN 5-HT_{1A} receptor availability, the [^{11}C]CUMI-101-binding potential (BND) values from the DRN were included as a continuous predictor variable in the analysis. Extracted data represent lower-level contrast estimates summarizing mean parameter estimate differences as in the fMRI-only analysis. Correlation coefficients were computed between DRN [^{11}C]CUMI-101 BP_{ND} and the citalopram *vs* placebo contrast estimates for the independent amygdala ROI (r_{AMYG}) to display general strength and direction of association. Extracted *Z*-statistics represent the mean of significant *Z*-scores with corresponding GRF theory-based *p*-values (Beckmann *et al*, 2003; Jenkinson *et al*, 2002; Woolrich *et al*, 2001). Owing to the possible presence of an outlier (ie, DRN [^{11}C]CUMI-101 BND > 2.4 SD of mean; q.v., Figure 2b and c), these analyses were repeated with FEAT's outlier deweighting tool. Statistical results were identical. Furthermore, our review of the specific outlier case found that [^{11}C]CUMI-101 availability in other brain regions (eg, amygdala) and the first-level fMRI results were comparable to other participants' PET/first-level results. We deemed the case to exhibit a real physiological effect and the final reported statistics reflect its inclusion.

RESULTS

Thirteen male subjects (mean (SD) age = 48 ± 9 years) completed the emotion processing task on both days. Study participants generally tolerated intravenous citalopram well

Table 1 Condition and Contrast Parameter Estimates to Face Stimuli During Placebo (PBO) and Citalopram (CITA) Infusions in the Amygdala

	Left amygdala, *M* (SD)		Right amygdala, *M* (SD)	
	PBO	**CITA**	**PBO**	**CITA**
Condition				
Neutral faces	17.98 (39.60)	13.89 (32.69)	15.73 (27.56)	20.08 (38.40)
Fearful faces	20.26 (35.23)	30.92 (30.59)	15.06 (41.77)	22.23 (32.38)
Happy faces	7.57 (37.08)	30.53 (53.00)	11.79 (35.13)	19.07 (22.35)
Contrast (level 1)				
All *vs* baseline	50.61 (85.23)	77.72 (82.18)	46.79 (98.94)	66.56 (76.02)
Fearful *vs* neutral	1.46 (25.78)	16.97 (37.33)	−1.88 (23.92)	3.11 (36.42)
Happy *vs* neutral	−12.14 (54.84)	16.79 (60.70)	−5.40 (30.81)	−1.54 (29.46)

with either no or minimal self-limiting adverse effects, which included mild nausea, hot flush, lightheadedness, and tiredness. One subject reported mild nausea after saline (placebo). None of the subjects stopped the scan procedures or dropped out of study during the study day. There were no significant differences in behavioral measures between sessions on subjective VAS affective state measures (paired *t*-tests, all $ps > 0.1$), especially no significant change in anxiety measures. The mean serum citalopram concentration at 45 min after the start of infusion is 757.45 μg/l (SD 802.75). There was no significant correlation was observed between serum citalopram concentration and citalopram effect on amygdala reactivity to fearful faces ($p > 0.1$).

Effect of Citalopram on Amygdala Reactivity to Aversive Faces

For the all faces *vs* baseline contrast, citalopram infusions resulted in a significantly increased BOLD response bilaterally in the amygdala with a larger cluster in the left hemisphere: right amygdala ($k = 2$, peak MNI coordinates: ($x = 16$, $y = -10$, $z = -18$); $M_{PBO} = 12.31$, $SD_{PBO} = 110.48$; $M_{CITA} = 82.32$, $SD_{CITA} = 106.10$), $Z = 2.46$, $p = 0.039$ (FWE-corrected); left amygdala ($k = 11$, peak MNI coordinates: ($x = -20$, $y = -4$, $z = -12$); $M_{PBO} = 33.30$, $SD_{PBO} = 94.71$; $M_{CITA} = 75.06$, $SD_{CITA} = 81.33$), $Z = 2.33$, $p = 0.046$ (FWE-corrected) (Figure 1a and b). See Table 1 for individual condition and contrast parameter estimates across the spherical amygdala ROIs.

To test the hypothesis that intravenous citalopram would specifically increase the response to fearful faces, we repeated the citalopram contrast analysis for the fearful *vs* neutral faces contrast estimates and again identified increased bilateral amygdala activation associated with citalopram. Statistical differences were identified in both the right amygdala ($k = 14$, peak MNI coordinates: ($x = 24$, $y = -6$, $z = -20$); $M_{PBO} = -7.31$, $SD_{PBO} = 24.15$, $M_{CITA} = 13.74$, $SD_{CITA} = 25.65$), $Z = 2.59$, $p = 0.025$ (FWE-corrected), and the left amygdala ($k = 18$, peak MNI coordinates: ($x = -22$, $y = -6$, $z = -18$); $M_{PBO} = 4.25$, $SD_{PBO} = 26.64$, $M_{CITA} = 23.43$, $SD_{CITA} = 50.89$), $Z = 2.29$, $p = 0.038$ (FWE-corrected) (Figure 1a and c). There were no significant differences for the happy *vs* neutral faces contrast in the left amygdala after citalopram, though a significant increase was observed in the right amygdala ($k = 3$, peak MNI coordinates: ($x = 24$, $y = -4$, $z = -16$); $M_{PBO} = -13.70$, $SD_{PBO} = 31.01$, $M_{CITA} = 9.07$, $SD_{CITA} = 16.27$), $Z = 2.12$, $p = 0.042$ (FWE-corrected) (Figure 1a and d). Despite qualitative hemispheric differences between the fearful and happy face response, a direct comparison only demonstrated a trend-level effect of greater fearful face response modulation by citalopram compared with the happy face response in the left amygdala ($k = 2$, peak MNI coordinates: ($x = -20$, $y = -10$, $z = -12$); $M_{FEARFUL} = 3.50$, $SD_{FEARFUL} = 24.73$, $M_{HAPPY} = -5.24$, $SD_{HAPPY} = 18.42$), $Z = 2.02$, $p = 0.062$.

There was no significant correlation observed between serum citalopram concentration and citalopram effect on amygdala reactivity to fearful faces ($p > 0.1$).

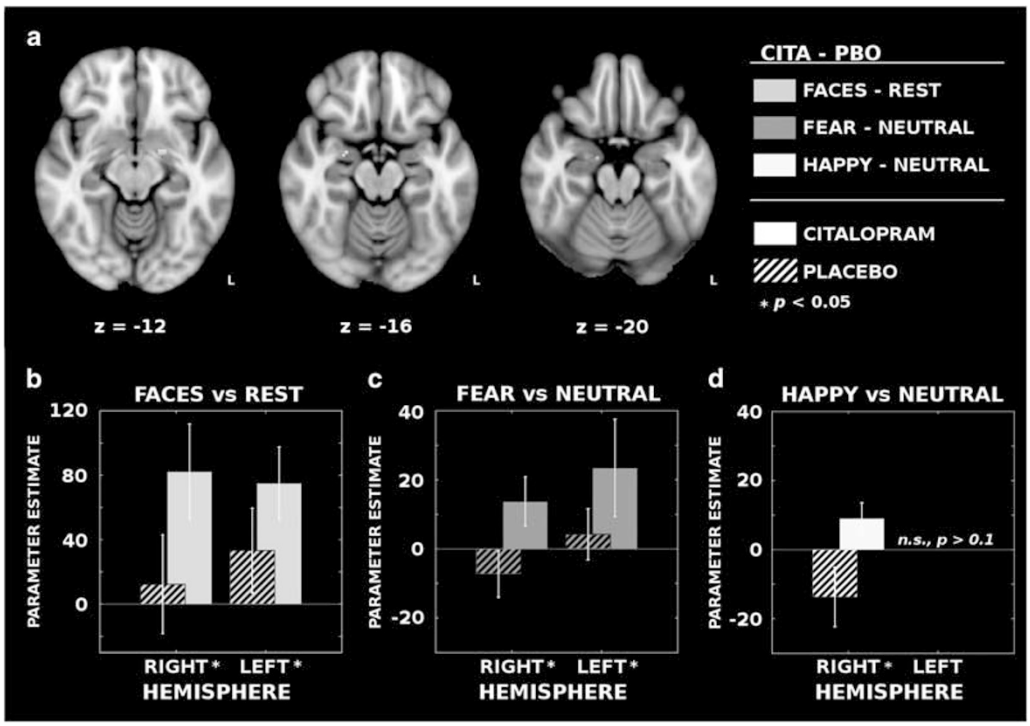

Figure 1 Functional imaging reveals bilateral patterns of increased face-dependent activation with citalopram infusion. (a) Intravenous citalopram (CITA) significantly increased activation bilaterally for the all faces *vs* rest (blue) and fearful *vs* neutral face (red) contrasts when compared with placebo (PBO). Trend-level activation increases were also observed in the right amygdala for the happy *vs* neutral face contrast (yellow). (b; cyan) Mean parameter estimates (± SEM) extracted across the voxels with significant differences in the second-level contrasts (ie, CITA–PBO). Within each hemisphere, the blood-oxygen-level-dependent (BOLD) signal elicited by faces increased after CITA infusions compared with PBO (striped bars indicate PBO estimates). (c; red) A similar pattern of activity was observed in the left hemisphere for the fearful *vs* neutral face contrasts as well; however, (d; yellow) the right hemisphere effects for fearful *vs* neutral and happy *vs* neutral faces are less clear owing to a task-related deactivation in the PBO condition. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

Relationship between Citalopram Induced Changes in Amygdala Reactivity and DRN 5-HT_{1A} Receptor

Of the total of 13 subjects, 10 completed the [^{11}C]CUMI-101 PET imaging protocol as well. 5-HT_{1A} receptor BP_{ND} values from the DRN (mean = 1.63, SD = 0.34) were included as a continuous predictor in the citalopram contrast model, and analyses were repeated within this subset to identify associations within the amygdala. There was a significant positive association between DRN 5-HT_{1A} and the all faces *vs* baseline citalopram contrast in the right amygdala, ($k = 1$, peak MNI coordinates: ($x = 16$, $y = -8$, $z = -12$); $M_{PBO} = 36.57$, $SD_{PBO} = 67.94$, $M_{CITA} = 33.07$, $SD_{CITA} = 56.66$), $r_{AMYG}(10) = 0.05$, $Z = 2.59$, $p = 0.032$, (FWE-corrected).

A significant positive association was identified within the left amygdala for the fearful faces *vs* neutral faces contrast. The citalopram *vs* placebo difference in the response to fearful faces *vs* neutral faces was larger in participants with greater DRN [^{11}C]CUMI-101 binding ($k = 11$, peak MNI coordinates: ($x = -16$, $y = -10$, $z = -14$); $M_{PBO} = 0.74$, $SD_{PBO} = 38.78$, $M_{CITA} = 5.29$, $SD_{CITA} = 35.19$), $r_{AMYG}(10) = 0.38$, $Z = 2.51$, $p = 0.027$ (FWE-corrected) (Figure 2a, c and e). There was a smaller positive association between DRN [^{11}C]CUMI-101 binding and the citalopram *vs* placebo difference in response to fearful *vs* neutral faces in the right amygdala ($k = 1$, peak MNI coordinates: ($x = 24$, $y = -10$, $z = -14$); $M_{PBO} = 4.51$, $SD_{PBO} = 27.91$, $M_{CITA} = -8.51$, $SD_{CITA} = 48.77$), $r_{AMYG}(10) = 0.06$, $Z = 2.16$, $p = 0.042$, FWE-corrected.

For the happy *vs* neutral face contrasts, DRN values demonstrated positive associations in the right amygdala ($k = 6$, peak MNI coordinates: ($x = 24$, $y = -10$, $z = -14$); $M_{PBO} = -2.85$, $SD_{PBO} = 28.63$, $M_{CITA} = -12.60$, $SD_{CITA} = 40.37$), $r_{AMYG}(10) = 0.21$, $Z = 2.33$, $p = 0.032$, (FWE-corrected) (Figure 2a, b and d), and again, a smaller effect in the left amygdala ($k = 1$, peak MNI coordinates: ($x = -16$, $y = -10$, $z = -14$); $M_{PBO} = -13.53$, $SD_{PBO} = 60.41$, $M_{CITA} = -7.38$, $SD_{CITA} = 32.36$), $r_{AMYG}(10) = 0.34$, $Z = 2.29$, $p = 0.033$, (FWE-corrected). DRN [^{11}C]CUMI-101-binding associations did not differ significantly between fearful and happy faces. Collectively, this pattern of results suggests that greater [^{11}C]CUMI-101-binding potential in the DRN is positively associated with the degree of increase in BOLD signaling to emotional faces induced by citalopram infusion. However, the specificity of the amygdala response to particular valences is still unclear.

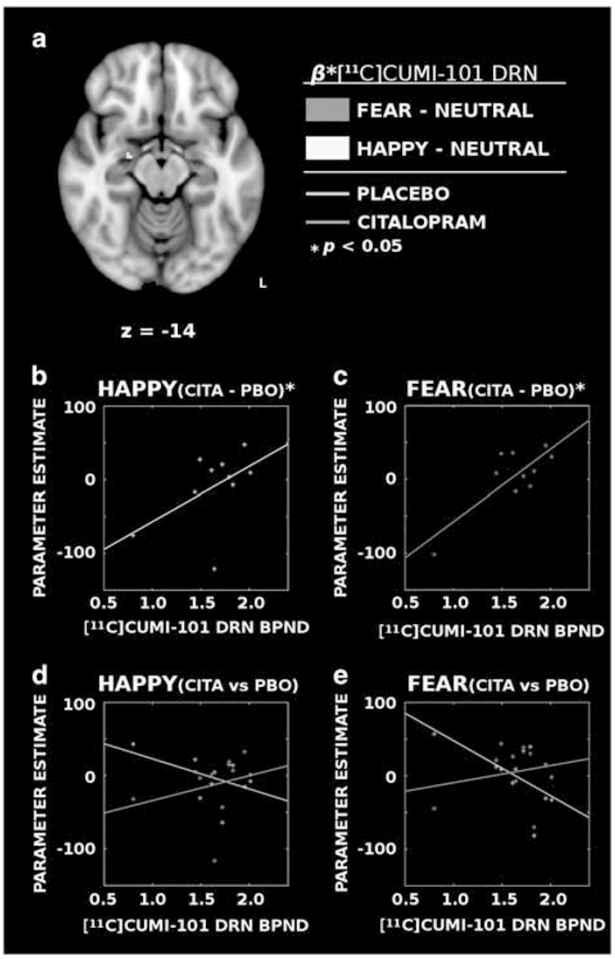

Figure 2 Dorsal raphe nucleus (DRN) 5-HT_{1A} availability was positively associated with the degree of modulation induced by citalopram infusion in the amygdala. (a) PET [^{11}C]CUMI-101-binding estimates demonstrated a functionally lateralized positive association with activity in the left amygdala between the response to fearful faces and DRN 5-HT_{1A} availability (red) and the right amygdala showing an association with happy faces (yellow). (b and c) Citalopram (CITA) minus placebo (PBO) differences show that individuals with greater DRN 5-HT_{1A} availability have reduced citalopram-induced modulation of the amygdala to happy (yellow) and fearful (red) faces in the right and left hemispheres, respectively. (d and e) Same as panels (b and c) but with citalopram and placebo data presented separately. Whereas individuals with less DRN 5-HT_{1A} availability show heightened amygdala responses during placebo infusions (cyan), the association largely disappears with citalopram (magenta), suggesting that individual differences in the response of the amygdala to emotional content is associated with differences in DRN 5-HT_{1A} availability. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

DISCUSSION

In this multimodal brain imaging study, we report the effect of acute intravenous citalopram on amygdala reactivity and its relationship with DRN 5-HT_{1A} receptor availability (as indexed by [^{11}C]CUMI-101 PET) in healthy human male subjects. The main findings of this study are that: (1) acutely citalopram increased the BOLD response bilaterally in the amygdala to fearful faces and in the right amygdala to happy faces with a trend-level left amygdala selectivity to fearful *vs* happy faces; and (2) DRN 5-HT_{1A} receptor availability is positively associated with the degree of increase in amygdala BOLD response to emotional faces (both fearful and happy *vs* neutral) induced by citalopram infusion. The current findings, when combined with other findings (Fisher *et al*, 2006; Richardson-Jones *et al*, 2010; Richardson-Jones *et al*, 2011; Selvaraj *et al*, 2014), support the critical role of presynaptic DRN 5-HT_{1A} receptors in regulating emotion processing.

Intravenous citalopram increased amygdala BOLD response bilaterally for fearful *vs* neutral faces. This finding is consistent with our *a priori* hypothesis regarding amygdala reactivity to SSRI and in agreement with Bigos *et al* (2008) but not with Del-Ben *et al* (2005). The differences could be due to differences in the nature of emotion task paradigms of using covert or explicit emotion faces task. Curiously, voxels demonstrating significant increases in BOLD response were not positively active for the placebo in the fearful and happy

faces *vs* neutral contrasts (Figure 1c and d). One possibility for this result is that our analytical approach highlighted those voxels that were found to be maximally different between the two sessions rather than which voxels are maximally activated by the task *per se*. However, parameter estimates extracted from the condition regressors presented in Table 1 demonstrate consistent positive activation across all conditions, suggesting that the amygdala was sensitive to faces but not uniquely sensitive to emotional faces during the placebo visit. Thus only during the citalopram infusion were amygdala responses sensitive to affect. Another possibility is that the voxels responsive to emotional faces during the placebo visit were already active at ceiling and the voxels identified in the present analysis represent an increase in the spatial extent of activation (ie, similar peak but wider spread). Future investigations may consider varying the valence of affective stimuli to better address this question of state-based reactivity *vs* activation span.

Our data provide evidence that variations in DRN 5-HT_{1A} receptor availability are related to the SSRI effect on emotion processing. However, the mechanistic pathway of this relationship cannot be determined from the correlations we report. Our finding is, however, consistent with preclinical research on the role of presynaptic DRN 5-HT_{1A} receptors. Preclinical studies show that stimulation of 5-HT_{1A} receptors decreases the firing rate of 5-HT neurons (Sprouse and Aghajanian, 1987) and 5-HT release (Bosker *et al*, 1997; Bosker *et al*, 2001). Mice selectively expressing high DRN 5-HT_{1A} autoreceptors compared with low DRN 5-HT_{1A} autoreceptors have decreased 5-HT cell firing and therefore reduced 5-HT tone in the projection sites such as the amygdala (Richardson-Jones *et al*, 2011). Acute SSRI-induced increases in extracellular raphe 5-HT also activates 5-HT_{1A} autoreceptors, thereby decreases 5-HT firing and release in projection sites (Auerbach *et al*, 1995; Fuller, 1994; Gartside *et al*, 1995; Haddjeri *et al*, 2004; Romero and Artigas, 1997). Thus high DRN 5-HT_{1A} may be associated with low 5-HT in amygdala. Based on our findings, we speculate that individuals with high DRN 5-HT_{1A} autoreceptors are more sensitive to the autoreceptor activation and thus show greater amygdala response when given acute dose of SSRI. However, acute citalopram treatment also increases 5-HT levels in the amygdala in rodent models (Bosker *et al*, 2001) and elevated 5-HT in amygdala increases fear learning and acquisition (Bocchio *et al*, 2016; Deakin and Graeff, 1991). Furthermore, the net effect of synaptic 5-HT at projection sites may be influenced by regional variations in 5-HT transporters (Bose *et al*, 2011b). Finally, the modulatory effect of 5-HT on anxiety response depends upon a balance of excitatory (5-HT_{2A}) and inhibitory (5-HT_{1A} and 5-HT_{1B}) 5-HT signaling on cortical pyramidal and interneurons in the prefrontal and amygdala circuitry (Albert *et al*, 2014; Fisher *et al*, 2011). Therefore, multiple mechanisms may be involved in SSRI-induced amygdala response.

When taken together, these findings indicate that combined knowledge of 5-HTT and 5-HT_{1A} autoreceptor density may have predictive value in understanding antidepressant response. Based on our preliminary findings in healthy volunteers, we speculate that patients with high DRN 5-HT_{1A} receptor availability would be predicted to have more severe anxiety responses to SSRIs. Further research in patients with major depression might help clarify the contribution of this mechanism to the increase in anxiety levels reported by some patients after initiation of antidepressant treatment.

This study has a number of limitations; first, we only studied male participants. Future studies should assess whether gender has an effect on the relationship between DRN 5-HT_{1A} receptor and amygdala reactivity as some studies have reported associations between DRN 5-HT_{1A} autoreceptor availability and sex (Parsey *et al*, 2002). Second, although $N=13$ is comparable to the size of similar studies (Bigos *et al*, 2008; Del-Ben *et al*, 2005), the sample size for the combined PET/fMRI experiment does not permit investigation of additional potential moderators (eg, 5-HTT genetic polymorphisms) on the influence of the 5-HT_{1A} receptors on amygdala reactivity. This will have to be addressed in future larger studies. Furthermore, analysis of smaller sample sizes, as reported here, is known to provide inflated estimates of effect sizes (Button *et al*, 2013). As such, we have presented means and SDs from voxels showing significant differences and from our *a priori* amygdala ROI as a whole. A substantially larger sample (eg, $N>78$ for the fearful *vs* neutral face citalopram contrasts; cf., fmripower.org; (Mumford and Nichols, 2008) will be required to establish well-powered effect size estimates. Therefore, we put forward our own results with caution to be interpreted as indicating the presence of an association rather than a specific magnitude of effect. Third, correlation between DRN 5-HT_{1A} receptor availability and amygdala BOLD response to emotional faces after citalopram does not prove causality. Further experimental research studies will be needed to study the direct role of 5-DRN HT_{1A} in emotion processing in humans. Finally, the average interval between PET and fMRI data acquisition was 125 (SD 7.7) weeks (2.4 years). Although the PET and fMRI scans were performed at different time points, several lines of evidence indicate that the binding potential measures in this study would be stable over the timescale of the experiment. A previous 5-HT_{1A} [^{11}C]CUMI-101 study reported high test–retest reliability for raphe measurements, with an intraclass correlation coefficient of 0.8 (Milak *et al*, 2010), indicating that it can be reliably measured. A selective 5-HT_{1A} receptor antagonist ligand (^{18}F-MPPF) study that collected test–retest scans with a mean delay 27 weeks between scans in healthy volunteers reported high reliability of dorsal raphe-binding potential measurements (ICC 0.78) (Costes *et al*, 2007). This result suggests that 5-HT_{1A} PET measures from the dorsal raphe are reliable over time, albeit using a different tracer (^{18}F-MPPF). In addition, PET studies report no significant decline of 5-HT_{1A} availability (as indexed by [^{11}C]WAY--100635) in presynaptic or postsynaptic regions over time with age (age range of 24–53 years) in a large cohort ($N=61$; Rabiner *et al*, 2002), suggesting that ageing does not significantly affect 5-HT_{1A} availability. Thus brain 5-HT_{1A} receptor availability *in vivo* using PET provides stable measure of 5-HT_{1A} binding and the time interval between acquisitions of the PET/MRI scan data is less likely to have influenced the results. Nevertheless, we cannot exclude variation over time, although this would, if anything, be expected to increase noise and weaken the results.

CONCLUSION

An acute intravenous administration of citalopram increased amygdala reactivity to aversive emotion, and this was positively associated with DRN 5-HT_{1A} receptor availability. Our findings indicate that the initial effect of SSRI treatment is to alter processing of aversive stimuli and that this is linked to DRN 5-HT_{1A} receptors in line with evidence that 5-HT_{1A} receptors have a role in mediating emotional processing.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)