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Presynaptic Serotonergic Regulation of Emotional Processing: A Multimodal Brain Imaging Study

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Abstract

Background—The amygdala is a central node in the brain network that processes aversive emotions, and is extensively innervated by dorsal raphe nucleus (DRN) serotonin (5-HT) neurons. Alterations in DRN 5-HT_{1A} receptor availability cause phenotypes characterised by fearful behaviour in preclinical models. However it is unknown whether 5-HT_{1A} availability is linked specifically to the processing of aversive emotions in humans, or whether it modulates connectivity in brain networks involved in emotion processing. To test this we investigated the relationship between DRN 5-HT_{1A} availability and amygdala reactivity to aversive emotion; and functional connectivity within the amygdala-cortical network.

Methods—We studied 15 healthy human participants who underwent positron emission tomography scanning with [¹¹C]CUMI-101, a 5-HT_{1A} partial agonist radioligand, and functional magnetic resonance imaging of brain responses during an incidental emotion processing task including happy, fearful and neutral faces. Regional estimates of 5-HT_{1A} binding potential (BP_{ND}) were obtained by calculating total volumes of distribution for presynaptic DRN and amygdala. Connectivity between the amygdala and cortico-limbic areas was assessed using psychophysiological interaction analysis with the amygdala as the seed region.

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Conflict of Interest

Sudhakar Selvaraj, Elias Mouchlianitis, Paul Faulkner, Federico Turkheimer and Oliver Howes report no competing interests.

Jonathan Roiser has been a member of a media advisory board for Lundbeck and consults for Cambridge Cognition Ltd.

Philip Cowen has been a member of advisory boards of Eli Lilly, Servier and Lundbeck and has been a paid lecturer for Eli Lilly, Servier, Lundbeck and GlaxoSmithKline.

Results—Analysis of the fear vs. neutral contrast revealed a significant negative correlation between amygdala response and DRN BP_{ND} ($r=-0.87$, $P<0.001$). DRN 5-HT_{1A} availability positively correlated with amygdala connectivity with middle frontal gyrus, anterior cingulate cortex, bilateral precuneus and left supramarginal gyrus for fearful (relative to neutral) faces.

Conclusions—Our data show that DRN 5-HT_{1A} availability is linked specifically to the processing of aversive emotions in the amygdala and the modulation of amygdala-cortical connectivity.

Keywords

positron emission tomography (PET); serotonin (5-HT); functional connectivity; emotion; amygdala; neuroimaging; functional magnetic resonance imaging; 5-HT_{1A} receptor; [11C]-CUMI-101; face processing; dorsal raphe nucleus

Brain serotonin (5-HT) is a key neuromodulator of emotional processing, particularly in the processing of aversive events (1, 2). 5-HT neurons originating from midbrain-brainstem raphe nucleus modulate neural responses through at least 14 different 5-HT receptor subtypes (3). 5-HT_{1A} receptors are inhibitory G-protein-coupled receptors located presynaptically on raphe 5-HT cell bodies as autoreceptors where they regulate neuronal firing and 5-HT release in projection sites (4). 5-HT_{1A} receptors are also located postsynaptically as heteroreceptors on non-5-HT neurons in limbic and cortical regions where they regulate local inhibitory responses (4).

5-HT_{1A} knock-out mice exhibit phenotypes characterised by anxiety and heightened fear responses (5–7). Early postnatal restoration of forebrain 5-HT_{1A} heteroreceptors in knockout mice has been shown to normalize fear behavior (8). More recent studies show that either complete knock-out or early postnatal blockade of presynaptic 5-HT_{1A} autoreceptors alone, when compared to heteroreceptors; increases fear behavior in behavioural paradigms (7, 9). These findings suggest that the 5-HT_{1A} autoreceptor plays a key developmental role in anxiety and fear behaviour by regulating 5-HT tone early in the development (7). Neuroimaging studies in patients with depression and anxiety disorders have generally shown lowered 5-HT_{1A} binding in the presynaptic dorsal raphe nucleus (DRN) (10–14) (though see (15)) and in post-synaptic limbic and cortical regions (10, 11, 13, 14, 16) compared to healthy controls. These clinical findings support a critical role of both pre and postsynaptic 5-HT_{1A} receptors in the expression of aversive emotions. However, the precise neural mechanisms by which 5-HT_{1A} regulates aversive emotional processing in humans are unclear.

Converging evidence from brain lesion, electrophysiological and neuroimaging studies suggest that the amygdala plays a critical role in the processing and regulation of aversive emotions, especially fear (17–20). Face processing has been widely used as a functional magnetic resonance imaging (fMRI) paradigm to study emotion (21). A large body of imaging literature suggests that the processing of facial emotion additionally recruits an extended functional network of cortico-limbic regions including the temporo-occipital cortex, fusiform gyrus, prefrontal cortex, insula and anterior cingulate cortex, with the amygdala as a central node (22, 23). Current neural models propose interactions between

amygdala, prefrontal and anterior cingulate cortex in the regulation of emotional processing (24–26). Specifically, decreased amygdala reactivity has been found to be associated with increases in connectivity with prefrontal regions (27, 28).

The amygdala and its cortico-limbic connections are densely innervated by DRN 5-HT neurons (29). In humans, amygdala reactivity to fearful faces has been reported to be inversely correlated with DRN 5-HT_{1A} availability (30). It is not clear, however, if this relationship is specific to negative emotion *per se* or faces (which activate the amygdala even with neutral expressions), because shapes rather than neutral facial expressions were used as control stimuli in this prior study (30). Additionally, the role of DRN 5-HT_{1A} receptors in modulating functional connectivity between amygdala and cortico-limbic regions during the processing of emotional stimuli has yet to be investigated. Therefore we used positron emission tomography (PET) and fMRI to investigate whether DRN 5-HT_{1A} receptor availability predicts amygdala reactivity during aversive emotional processing using an incidental emotion task that featured happy, fearful and neutral faces (23). We predicted that presynaptic raphe 5-HT_{1A} availability would correlate negatively with amygdala responsiveness and this correlation would be specific for fearful faces. We also examined the relationship between the DRN 5-HT_{1A} and amygdala-cortical functional connectivity. We predicted that DRN 5-HT_{1A} availability would positively correlate with connectivity between amygdala and prefrontal regions during fearful emotion processing, in line with the preclinical evidence that autoreceptor knock-out mice show heightened fear responses, and the role of amygdala-frontal networks in emotion processing.

Materials and Methods

Subjects

The study was approved by the hospital research ethics committee. Fifteen healthy subjects (M:F 13:2; mean age 42.4±11.4 years) without any current or past history of psychiatric or neurological illness or had current or previous history of alcohol or substance dependence were recruited by advertisement and gave written informed consent to participate. Eleven subjects reported tobacco smoking, of which, six had already stopped smoking.

fMRI Experimental Design

A well characterized incidental emotion task was employed (23). During the task, participants saw a series of faces on the screen for 2s and classified each face as male or female. A block design was used, each 16s block consisting of 8 faces representing a single emotion (happy, fearful, neutral). Between blocks, there was a 16s baseline period during which subjects fixated on a cross presented centrally.

fMRI Data Acquisition

A 3T Intera system (Philips) was used to acquire 44 T2*-weighted transverse echoplanar images (EPI) per volume with blood oxygenation level dependent (BOLD) contrast. EPIs comprised 3 mm-thick axial slices, no between slice gap. They were acquired with 1.95 mm*1.95 mm voxel dimensions in-plane; repetition time=3 s; echo time=30 ms; phase encoding direction = anterior-posterior; field of view=576 mm²; matrix size 112x112; A

total of 132 EPI volumes were collected, with slices acquired in an even – odd interleaved descending direction. Real-time reconstruction, z-shimming correction and a slice tilt of -30° to the anterior–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 (31, 32). The EPI scans were followed by the acquisition of a whole-brain 3D-MPRAGE scan (TR = 9.6 ms, TE = 4.5 ms, flip angle 8° , slice thickness = 1.2 mm, $0.94 \text{ mm} \times 0.94 \text{ mm}$ in plane resolution, 150 slices).

fMRI Analysis

Data were analyzed using Statistical Parametric Mapping software (SPM8, <http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) in Matlab 7.9. The first three volumes were removed from the analysis to allow for T1 equilibration. Preprocessing of the image volumes included spatial realignment to the fourth image volume to correct for movement, followed by spatial normalization to Montreal Neurological Institute (MNI) space, using the linear and nonlinear normalisation parameters estimated from warping each participant's structural image to the T1-weighted MNI template. These re-sampled images (voxel size $3 \times 3 \times 3 \text{ mm}$) were smoothed spatially with an 8 mm full width at half maximum Gaussian kernel (final smoothness approximately $12 \times 12 \times 12 \text{ mm}$).

Box-car regressors of duration 16 s were constructed for each facial emotion (happy, fearful, neutral), with fixation constituting an implicit baseline. Neural responses to all experimental conditions were modelled by convolving each block with the canonical haemodynamic response function. Faces given incorrect gender judgments were modelled in a separate regressor. To account for residual movement artifacts after realignment, the model included six regressors representing the estimated movement parameters. Voxel-wise parameter estimates were obtained by maximum-likelihood estimation in the context of the general linear model (GLM), using a temporal high-pass filter (cut-off 128 s) to remove low-frequency drifts, and modelling temporal autocorrelation across scans with an autoregressive AR (1) process. Contrasts were constructed at the first (subject) level representing: 1) all faces (relative to implicit baseline); 2) fearful faces relative to neutral faces; 3) happy faces relative to neutral faces. At the second (group) level contrasts and correlations were performed with one-sample t-tests on the contrast images for each subject, using the standard summary statistics approach to random-effects analysis.

PET data acquisition

PET data were acquired on a GE Discovery RX PET/CT scanner with an axial field-of-view of 15.7 cm and 47 reconstructed transaxial image planes (33). The dynamic PET scan was acquired over 90 mins in list mode and then reconstructed into frames. For further details of the PET scan procedure and 5-HT_{1A} partial agonist radiotracer [¹¹C]CUMI-101 synthesis see (34). [¹¹C]CUMI-101 was administered via injection into an antecubital vein as a smooth bolus over 30 seconds. The range of injected radioactivity was between 352.7 and 395.1 MBq (mean: 379.8 MBq, SD: 10.9 MBq), mean specific activity was 71.9 GBq· μmol^{-1} (SD=31.4 GBq· μmol^{-1}), and the radiochemical purity of the injected [¹¹C]CUMI-101 was high (mean= 97.2%, SD 1.4%). The injected mass of cold [¹¹C]CUMI-101 varied between 0.97 μg and 4.95 μg with a mean value of 2.7 μg (SD=1.0

µg). Arterial blood samples were taken to derive the arterial input function for [¹¹C]CUMI-101.

PET Image analysis

The PET dynamic scans were analysed in SPM5 as previously described (34)(see supplementary material). The presynaptic DRN was defined in the midbrain area on the summed PET images of each individual (34). DRN was manually defined as a fixed size region (215 mm³) by placing a 5*3 rectangle ROI on the midline sagittal plane, aligned with the brain stem at 30 degree angle at midbrain level in front of cerebral aqueduct on each individual's summated PET image. A slice on both sides of this midline slice in sagittal plane was then selected (total of 3 slices). To test whether the correlation between the DRN and amygdala reactivity is specific to presynaptic 5-HT or not, amygdala was studied as the main post synaptic 5-HT region of interest. Amygdala and cerebellum were defined on the coregistered MRI using a probabilistic brain atlas template (35) (see supplementary material). Finally, cerebellar grey matter was used as the reference region.

Graphical analysis of reversible radioligand binding together with the metabolite-corrected plasma input function was used to quantify the binding in brain tissue relative to the radioligand concentration in arterial plasma. Regional estimates of the total volume of distribution (V_T) were obtained from the slope of the linear part of the plot (36). V_T is the volume of distribution (mL/cm³) defined as the ratio of the tracer concentration in the region to the metabolite corrected plasma concentration at equilibrium (37). The threshold parameter for the graphical analysis was set at $t^*=20$ min. i.e., the transformed data from frames 13 to 22 were fitted to a straight line. The specific binding was quantified as BP_{ND} (37) where:

$$BP_{ND} = (V_T \text{ target region} - V_T \text{ reference region}) / V_T \text{ reference region}$$

Integration of PET and fMRI data

Our first aim was to assess the relationship between DRN 5-HT_{1A} availability and amygdala BOLD reactivity. We defined the amygdala ROI using a sphere with a radius of 6-mm centered on the peak left amygdala activation from a prior study employing an identical paradigm (23), MNI coordinates: [x=-21, y=-6, z=-15]. First, these coordinates were used for small volume correction (SVC) for the all faces vs baseline contrast in order to confirm significant amygdala activation to faces in our participants. We then extracted contrast estimates from the amygdala peak voxel identified in this analysis, for both fear vs neutral and happy vs neutral contrasts, and correlated them with DRN BP_{ND} values in SPSS. O'Nions et al 2011 (23) did not report a right amygdala ROI. Whilst it would have been possible to define and examine a right amygdala ROI (for example by flipping the x-coordinate as reported (23)) as well, however this would be based on the assumption that the activation is the same on the right side. To avoid relying on this assumption, we restricted our analysis to an ROI defined by the data reported by O'Nions et al. Note that although we did not set out to test a laterality hypothesis, specificity to left amygdala reactivity has been reported previously using facial stimuli (38), while fMRI and PET studies show a pattern of left amygdala lateralization in emotional processing (for review see (39)).

Our second aim was to assess whether amygdala-cortical functional connectivity is modulated by DRN 5-HT_{1A} availability. We tested this using a psychophysiological interaction (PPI) model with the left amygdala as the seed region. For each subject a sphere of 6-mm radius was defined around the amygdala peak voxel defined from the O'Nions et al (2011) coordinates described above, from which the average time series (first eigenvariate) of BOLD signal was extracted. At the first-level, three regressors were created in a GLM representing: (i) the deconvolved BOLD signal time-course in the seed region (the physiological factor); (ii) the task effect, defined by all faces vs baseline, fear vs neutral and happy vs neutral contrasts (the psychological factor, in separate models); (iii) the interaction of (i) and (ii) the PPI. Group-level voxel-wise SPMs were generated for the all faces vs baseline, fear vs neutral and happy vs neutral contrasts for the PPI regressor and we entered DRN BP_{ND} values as a covariate in the one-sample t-test analysis of the PPI for the fear vs neutral and happy vs neutral GLMs. For the amygdala ROI analysis we used small volume family-wise-error correction (SV-FWE) at voxel level (initial threshold $P=0.001$ uncorrected), while for the PPI covariate analysis we used whole-brain family-wise-error correction (WB-FWE) at the voxel level at $P=0.05$.

To evaluate the strength of 5-HT_{1A} modulation to amygdala-cortical connectivity we used a leave-one-subject-out (LOSO) cross-validation method that reduces possible effects of non-independence (40) (see supplementary material). Briefly, we constructed a GLM (as described above) that included all the subjects ($n=15$) and identified suprathreshold clusters. We then iteratively constructed LOSO GLMs which included every subject except for one ($n=14$). For each of the LOSO GLMs we identified activated voxels within the anatomical neighborhood of regions found significant in the full GLM. Given our reduced degrees of freedom we used a liberal threshold of $P=0.001$ uncorrected. Finally, we used those voxels to extract parameter estimates from the subject that was left out for each LOSO GLM and correlated them with DRN BP_{ND} values.

Residuals were tested for normality using the Shapiro-Wilkes Test. Having established that the residuals were normally distributed, Pearson's r was used for residuals that were normally distributed. To assess valence-specificity in the amygdala ROI analysis, correlation coefficients from the amygdala and DRN BP_{ND} correlations for each of the valence-specific contrasts were compared using Steiger's Z . Cook's distance test was used to assess the effect of potential outliers. A data point was considered as an outlier if their Cook's distance value was higher than four standard deviations over the mean of group's Cook's distance mean value.

Results

Relationship between 5-HT_{1A} availability and amygdala BOLD response to emotional stimuli

Analysis for the all faces vs baseline contrast revealed activation in a number of regions previously reported to participate in face processing (Table 1) including a significant left amygdala *activation* (MNI coordinates: $[x=-18\ y=-7\ z=-17]$, $Z=4.31$, $P<0.001$, SV-FWE) (Figure 1, *Panel A*). The amygdala response to fearful (relative to neutral) faces at this voxel was negatively correlated with DRN BP_{ND} ($r=-0.87$, $r^2=0.75$, $P<0.001$; see Figure 1, *Panel*

C), which remained nominally significant after excluding one participant with low DRN BP_{ND} ($r=-0.53$, $r^2=-0.28$, $P=0.037$). There was no significant relationship between amygdala responses to happy (relative to neutral) faces and DRN BP_{ND}, ($r=-0.34$, $r^2=-0.11$, $P=0.17$). These correlations differed significantly (Steiger's $Z=2.88$, $P<0.01$), suggesting that presynaptic 5-HT_{1A} availability modulates amygdala reactivity specifically to fearful facial stimuli. No significant correlation was detected between amygdala BOLD response and amygdala 5-HT_{1A} availability ($P>0.1$).

Relationship between dorsal raphe 5-HT_{1A} availability and amygdala functional connectivity

Analysis of the all faces vs baseline PPI revealed a large network of regions in which connectivity with the amygdala was significantly altered during face processing (see Table 2).

Analysis of the fear vs neutral PPI contrast revealed that amygdala connectivity was positively correlated with DRN 5-HT_{1A} availability in a number of regions: middle frontal gyrus (MNI coordinates: $[x=33\ y=35\ z=43]$, $Z=5.79$, $P<0.001$ WB-FWE); ACC (MNI coordinates: $[x=-3\ y=29\ z=25]$, $Z=5.05$, $P=0.013$ WB-FWE); left precuneus (MNI coordinates: $[x=-6\ y=70\ z=37]$, $Z=5.02$, $P=0.015$ WB-FWE), right precuneus (MNI coordinates: $[x=3\ y=-73\ z=40]$, $Z=4.96$, $P=0.021$ WB-FWE); and left supramarginal gyrus (MNI coordinates: $[x=-54\ y=-49\ z=40]$, $Z=4.32$, $P=0.043$ WB-FWE).

The LOSO analysis revealed the following correlation strengths: middle frontal gyrus ($r=0.96$, $r^2=0.92$); ACC ($r=0.93$, $r^2=0.86$); left precuneus cortex ($r=0.93$, $r^2=0.86$), right precuneus cortex ($r=0.92$, $r^2=0.84$); and left inferior parietal lobule $r=0.91$, $r^2=0.84$). For all regions there was an outlier point with low BP_{ND}. When removing the outlier all the correlations remained nominally significant: middle frontal gyrus ($r=0.87$, $r^2=0.76$, $P<0.001$); ACC ($r=0.76$, $r^2=0.58$, $P<0.001$); left precuneus cortex ($r=0.84$, $r^2=0.70$, $P<0.001$); right precuneus cortex ($r=0.77$, $r^2=0.60$, $P<0.001$); left supramarginal gyrus ($r=0.76$, $r^2=0.60$, $P=0.0011$). For the happy vs neutral contrast there were no suprathreshold voxels in which amygdala connectivity was modulated by DRN 5-HT_{1A} availability.

As a final step, we assessed the PPI covariate model removing the outlier subject. None of the above regions survived voxel-wise whole brain FWE correction at $P=0.05$. However, using cluster-level correction (initial threshold $P=0.001$ (uncorrected)) two clusters survived FWE correction at $P=0.05$: right lateral occipital cortex (MNI coordinates: $x=-39\ y=-70\ z=31$, $Z=4.19$, $k=77$, $P=0.004$) and right precuneus (MNI coordinates: $x=9\ y=-70\ z=34$, $Z=4.07$, $k=44$, $P=0.049$). A left precuneus cluster also approached significance (MNI coordinates: $x=-9\ y=-70\ z=34$, $Z=4.22$, $k=38$, $P=0.082$). All three clusters overlapped with regions identified in the model that included the outlier.

Discussion

The present study has two main findings: first, we found that lower dorsal raphe nucleus 5-HT_{1A} receptor availability was strongly negatively associated with greater amygdala response to fearful but not to happy faces during incidental emotional processing. Our

findings confirm and extend a previous finding that lower DRN 5-HT_{1A} availability was associated with greater amygdala response to faces (30) by showing that lower DRN 5-HT_{1A} availability is specifically linked to negative, in this case fearful, emotion. Note that here we only examined a left amygdala correlation with DRN 5-HT_{1A} availability while (30) reported bilateral correlations (also see (41)). The left amygdala ROI was chosen in our study a priori based on a study that used exactly the same paradigm, and showed left amygdala activation for emotional processing (23). Although there is evidence for a left amygdala specificity in emotional processing (39), as we did not examine the right side the lateralization issue needs to be addressed by future studies.

Our data are consistent with studies of 5-HT_{1A} autoreceptor knockout mice, which exhibit increased fearful behaviour (7). In addition, direct injection of a 5-HT_{1A} agonist into the DRN has been reported to reduce anxiety in mice (42, 43). Taken together with these results from rodent studies, our findings indicate that DRN presynaptic 5-HT_{1A} receptors play a key modulatory role in amygdala reactivity to fearful stimuli in humans.

The second main finding was that DRN 5-HT_{1A} availability positively correlated with amygdala connectivity with the ACC, middle frontal gyrus, supramarginal gyrus and precuneus when viewing fearful faces (relative to neutral) faces. This correlation also seems to be specific to aversive emotions as no such correlation was detected in the analysis of connectivity during happy (relative to neutral) face processing. Our data seem to be consistent with models of amygdala connectivity (negatively modulated) with prefrontal and parietal cortex constituting a network that plays a crucial role in emotional processing (24, 25). Our data suggest that dorsal raphe 5-HT_{1A} availability may also modulate a wider network involving amygdala and fronto-cingulate-parietal regions involved in emotion processing. It is to be noted that, whilst supportive, our results showing the DRN 5-HT_{1A} availability correlations with amygdala and its connectivity do not prove a causative link.

The exact neural mechanism of how DRN 5-HT_{1A} receptors might modulate the interaction between amygdala and its cortical connections remains to be elucidated. Dorsal ACC is thought to be involved in the regulation of attention and affective states (25) and also plays a key role in the appraisal, expression and regulation of negative emotion (44). Middle frontal gyrus is thought to be involved in the top-down control of emotional states (45). The precuneus is implicated in the recognition of familiarity of faces (46) and retrieving information regarding their mental states (47). Finally, inferior parietal lobe lesions affect facial emotion recognition (48). These areas have extensive anatomical connections with the amygdala (49, 50) and serotonergic innervation from the DRN (51).

Evidence from preclinical animal studies suggests that lower DRN 5-HT_{1A} availability is associated with increased 5-HT neuronal firing and therefore increased 5-HT transmission at projection sites (7, 52). DRN 5-HT_{1A} has also been shown to influence the development of neural circuits regulating aversive emotions (7, 9). We therefore speculate that in those individuals with lower DRN 5-HT_{1A} receptor availability, lesser connectivity between the amygdala and dorsal PFC/parietal regions may reflect a developmental compensatory mechanism to regulate aversive emotion processing. Altered DRN 5-HT_{1A}, presumably via

its effect on amygdala reactivity, could thus disrupt functional connectivity during emotional processing and contribute to the development of affective and anxiety disorders.

It is interesting to note that individual differences in postsynaptic regional 5-HT_{1A} availability did not correlate with amygdala responses during emotional processing. These results are in agreement with a recent selective knock-out mouse study indicating that presynaptic, but not post-synaptic, 5-HT_{1A} receptors play a significant role in the establishment of anxious and fearful behaviours (7). There may also be other 5-HT mechanisms involved in the aversive processing including 5-HT transporter at presynaptic level (38) or 5-HT_{2A} at postsynaptic level (53). Rhodes et al reported significant negative correlation between the left amygdala BOLD signal changes to fearful faces and left amygdala 5-HT transporter availability (38) and Fisher et al found inverse correlation between medial prefrontal cortex 5-HT_{2A} ligand binding and right amygdala reactivity to threat (54). Furthermore, 5-HT transporter genetic polymorphisms (SLC6A4) have also been associated with amygdala reactivity (55); short allele 's' carriers, relative to long allele 'l' carriers, showed higher amygdala BOLD response to fearful faces. Together with these findings, our results emphasize the important role of 5-HT transmission in regulating emotional processing.

Our study has some limitations. Firstly, the PET measure of 5-HT_{1A} availability depends on receptor density and affinity (see methods) and could be affected by synaptic 5-HT levels. Based on our previous [¹¹C]-CUMI-101 displacement study in the healthy subjects (34), we have evidence that [¹¹C]-CUMI-101 can reliably detect changes in endogenous 5-HT release in humans in cortical regions. However both the amygdala and dorsal raphe did not show statistically significant changes after low dose intravenous citalopram. Based on this we conclude that there is not likely to be a major effect of physiological differences in serotonin on [(11)]-CUMI binding, but we cannot rule out an effect. Secondly, DRN is a relatively small brain region which can lead to imprecise estimates of binding potential; however the test retest variability of [¹¹C]-CUMI-101 in a human study for DRN was 16% and intra-class correlations were >0.8, indicating good reliability (56). Though the sample size in this study is reasonable for a combined PET/fMRI experiment, it is not possible to rule out possible moderating effects of gender or age in our results (57, 58). However there seem to be inconsistencies among 5-HT_{1A} PET studies investigating the effect of gender on brain 5-HT_{1A} availability. For instance, Moses-Kolko et al (59) reported gender differences in age dependent changes in 5-HT_{1A}. They observed decreased 5-HT_{1A} of about 4.5% per decade of age primarily in men. However other studies either did not observe these differences (58, 60) or found opposite results, that is, age-dependent raphe decrease only seen in females (61). Similarly, there are gender differences in the emotion processing; for example, a meta-analysis found that male subjects activated limbic and prefrontal cortical areas more than the female subjects (62). It is therefore clear that a larger sample may be required for a comprehensive examination of the above potential moderators of 5-HT_{1A} receptor availability. Finally, it is important to note that the outlier subject had both the lowest dorsal raphe BP_{ND} as well the lower PPI parameter estimate. However, the results remained significant when this participant was excluded.

Implications

Our findings linking 5-HT_{1A} availability specifically to fearful emotion processing have implications for understanding emotional disorders. Studies in major depression have found lowered DRN 5-HT_{1A} availability (10–12) and separately, increased amygdala reactivity to fearful faces which attenuates after antidepressant treatment (63, 64). Our finding that lower DRN 5-HT_{1A} availability was associated with lesser connectivity between the amygdala and a prefrontal-precuneus cortical network suggests that DRN 5-HT_{1A} play a wider network level modulatory role in emotion processing. Overall the findings therefore suggest that the low 5-HT_{1A} availability in depression would lead to altered processing of fearful stimuli in line with theories that biased processing of stimuli underlies depression (65, 66). However, clearly it will be important to assess the relationship between 5-HT_{1A} availability and amygdala reactivity in patients with mood disorders to see whether the same relationships between 5-HT_{1A} availability and amygdala response are seen in anxiety and depression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

- Dayan P, Huys QJ. Serotonin, inhibition, and negative mood. *PLoS Comput Biol*. 2008; 4:e4. [PubMed: 18248087]
- Deakin JF, Graeff FG. 5-HT and mechanisms of defence. *J Psychopharmacol*. 1991; 5:305–315. [PubMed: 22282829]
- Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav*. 2002; 71:533–554. [PubMed: 11888546]
- Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology*. 1999; 38:1083–1152. [PubMed: 10462127]
- Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, et al. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci U S A*. 1998; 95:14476–14481. [PubMed: 9826725]
- Parks CL, Robinson PS, Sibille E, Shenk T, Toth M. Increased anxiety of mice lacking the serotonin_{1A} receptor. *Proc Natl Acad Sci U S A*. 1998; 95:10734–10739. [PubMed: 9724773]
- Richardson-Jones JW, Craige CP, Nguyen TH, Kung HF, Gardier AM, Dranovsky A, et al. Serotonin-1A autoreceptors are necessary and sufficient for the normal formation of circuits underlying innate anxiety. *J Neurosci*. 2011; 31:6008–6018. [PubMed: 21508226]
- Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, et al. Serotonin_{1A} receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature*. 2002; 416:396–400. [PubMed: 11919622]
- Donaldson ZR, Piel DA, Santos TL, Richardson-Jones J, Leonardo ED, Beck SG, et al. Developmental Effects of Serotonin 1A Autoreceptors on Anxiety and Social Behavior. *Neuropsychopharmacology*. 2013
- Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C, Meyer J, et al. Brain serotonin_{1A} receptor binding measured by positron emission tomography with [¹¹C]WAY-100635: effects of depression and antidepressant treatment. *Arch Gen Psychiatry*. 2000; 57:174–180. [PubMed: 10665620]

11. Drevets WC, Frank E, Price JC, Kupfer DJ, Holt D, Greer PJ, et al. PET imaging of serotonin 1A receptor binding in depression. *Biol Psychiatry*. 1999; 46:1375–1387. [PubMed: 10578452]
12. Meltzer CC, Price JC, Mathis CA, Butters MA, Ziolk SK, Moses-Kolko E, et al. Serotonin 1A receptor binding and treatment response in late-life depression. *Neuropsychopharmacology*. 2004; 29:2258–2265. [PubMed: 15483563]
13. Nash JR, Sargent PA, Rabiner EA, Hood SD, Argyropoulos SV, Potokar JP, et al. Serotonin 5-HT1A receptor binding in people with panic disorder: positron emission tomography study. *Br J Psychiatry*. 2008; 193:229–234. [PubMed: 18757983]
14. Lanzenberger RR, Mitterhauser M, Spindelegger C, Wadsak W, Klein N, Mien LK, et al. Reduced serotonin-1A receptor binding in social anxiety disorder. *Biol Psychiatry*. 2007; 61:1081–1089. [PubMed: 16979141]
15. Parsey RV, Oquendo MA, Ogden RT, Olvet DM, Simpson N, Huang YY, et al. Altered serotonin 1A binding in major depression: a [carbonyl-C-11]WAY100635 positron emission tomography study. *Biol Psychiatry*. 2006; 59:106–113. [PubMed: 16154547]
16. Hirvonen J, Karlsson H, Kajander J, Lepola A, Markkula J, Rasi-Hakala H, et al. Decreased brain serotonin 5-HT1A receptor availability in medication-naïve patients with major depressive disorder: an in-vivo imaging study using PET and [carbonyl-11C]WAY-100635. *Int J Neuropsychopharmacol*. 2008; 11:465–476. [PubMed: 17971260]
17. Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron*. 2005; 48:175–187. [PubMed: 16242399]
18. Morris JS, Friston KJ, Buchel C, Frith CD, Young AW, Calder AJ, et al. A neuromodulatory role for the human amygdala in processing emotional facial expressions. *Brain*. 1998; 121(Pt 1):47–57. [PubMed: 9549487]
19. Anderson AK, Phelps EA. Lesions of the human amygdala impair enhanced perception of emotionally salient events. *Nature*. 2001; 411:305–309. [PubMed: 11357132]
20. Adolphs R, Tranel D, Damasio H, Damasio A. Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature*. 1994; 372:669–672. [PubMed: 7990957]
21. Morris JS, Frith CD, Perrett DI, Rowland D, Young AW, Calder AJ, et al. A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature*. 1996; 383:812–815. [PubMed: 8893004]
22. Fairhall SL, Ishai A. Effective connectivity within the distributed cortical network for face perception. *Cereb Cortex*. 2007; 17:2400–2406. [PubMed: 17190969]
23. O'Nions EJ, Dolan RJ, Roiser JP. Serotonin transporter genotype modulates subgenual response to fearful faces using an incidental task. *Journal of cognitive neuroscience*. 2011; 23:3681–3693. [PubMed: 21568644]
24. Ochsner KN, Gross JJ. The cognitive control of emotion. *Trends Cogn Sci*. 2005; 9:242–249. [PubMed: 15866151]
25. Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception I: The neural basis of normal emotion perception. *Biol Psychiatry*. 2003; 54:504–514. [PubMed: 12946879]
26. Davidson RJ. Anxiety and affective style: role of prefrontal cortex and amygdala. *Biol Psychiatry*. 2002; 51:68–80. [PubMed: 11801232]
27. Urry HL, van Reekum CM, Johnstone T, Kalin NH, Thurow ME, Schaefer HS, et al. Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *J Neurosci*. 2006; 26:4415–4425. [PubMed: 16624961]
28. Phan KL, Fitzgerald DA, Nathan PJ, Moore GJ, Uhde TW, Tancer ME. Neural substrates for voluntary suppression of negative affect: a functional magnetic resonance imaging study. *Biol Psychiatry*. 2005; 57:210–219. [PubMed: 15691521]
29. Jacobs BL, Azmitia EC. Structure and function of the brain serotonin system. *Physiol Rev*. 1992; 72:165–229. [PubMed: 1731370]
30. Fisher PM, Meltzer CC, Ziolk SK, Price JC, Moses-Kolko EL, Berga SL, et al. Capacity for 5-HT1A-mediated autoregulation predicts amygdala reactivity. *Nat Neurosci*. 2006; 9:1362–1363. [PubMed: 17013380]

31. Weiskopf N, Hutton C, Josephs O, Deichmann R. Optimal EPI parameters for reduction of susceptibility-induced BOLD sensitivity losses: a whole-brain analysis at 3 T and 1.5 T. *Neuroimage*. 2006; 33:493–504. [PubMed: 16959495]
32. Weiskopf N, Hutton C, Josephs O, Turner R, Deichmann R. Optimized EPI for fMRI studies of the orbitofrontal cortex: compensation of susceptibility-induced gradients in the readout direction. *MAGMA*. 2007; 20:39–49. [PubMed: 17268781]
33. Kemp BJ, Kim C, Williams JJ, Ganin A, Lowe VJ. NEMA NU 2-2001 performance measurements of an LYSO-based PET/CT system in 2D and 3D acquisition modes. *J Nucl Med*. 2006; 47:1960–1967. [PubMed: 17138738]
34. Selvaraj S, Turkheimer F, Rosso L, Faulkner P, Mouchlianitis E, Roiser JP, et al. Measuring endogenous changes in serotonergic neurotransmission in humans: a [11C]CUMI-101 PET challenge study. *Mol Psychiatry*. 2012; 17:1254–1260. [PubMed: 22665264]
35. Hammers A, Allom R, Koepp MJ, Free SL, Myers R, Lemieux L, et al. Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. *Hum Brain Mapp*. 2003; 19:224–247. [PubMed: 12874777]
36. Logan J, Fowler JS, Volkow ND, Wolf AP, Dewey SL, Schlyer DJ, et al. Graphical analysis of reversible radioligand binding from time-activity measurements applied to [N-11C-methyl]-(-)-cocaine PET studies in human subjects. *J Cereb Blood Flow Metab*. 1990; 10:740–747. [PubMed: 2384545]
37. Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab*. 2007; 27:1533–1539. [PubMed: 17519979]
38. Rhodes RA, Murthy NV, Dresner MA, Selvaraj S, Stavrakakis N, Babar S, et al. Human 5-HT transporter availability predicts amygdala reactivity In vivo. *Journal of Neuroscience*. 2007; 27:9233–9237. [PubMed: 17715358]
39. Baas D, Aleman A, Kahn RS. Lateralization of amygdala activation: a systematic review of functional neuroimaging studies. *Brain Res Brain Res Rev*. 2004; 45:96–103. [PubMed: 15145620]
40. Esterman M, Tamber-Rosenau BJ, Chiu YC, Yantis S. Avoiding non-independence in fMRI data analysis: leave one subject out. *Neuroimage*. 2010; 50:572–576. [PubMed: 20006712]
41. Hariri AR, Drabant EM, Munoz KE, Kolachana BS, Mattay VS, Egan MF, et al. A susceptibility gene for affective disorders and the response of the human amygdala. *Arch Gen Psychiatry*. 2005; 62:146–152. [PubMed: 15699291]
42. Maier SF, Grahn RE, Watkins LR. 8-OH-DPAT microinjected in the region of the dorsal raphe nucleus blocks and reverses the enhancement of fear conditioning and interference with escape produced by exposure to inescapable shock. *Behav Neurosci*. 1995; 109:404–412. [PubMed: 7662151]
43. File SE, Gonzalez LE. Anxiolytic effects in the plus-maze of 5-HT1A-receptor ligands in dorsal raphe and ventral hippocampus. *Pharmacol Biochem Behav*. 1996; 54:123–128. [PubMed: 8728549]
44. Etkin A, Egner T, Kalisch R. Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends Cogn Sci*. 2011; 15:85–93. [PubMed: 21167765]
45. Amting JM, Greening SG, Mitchell DG. Multiple mechanisms of consciousness: the neural correlates of emotional awareness. *J Neurosci*. 2010; 30:10039–10047. [PubMed: 20668188]
46. Gobbini MI, Haxby JV. Neural systems for recognition of familiar faces. *Neuropsychologia*. 2007; 45:32–41. [PubMed: 16797608]
47. Cavanna AE, Trimble MR. The precuneus: a review of its functional anatomy and behavioural correlates. *Brain*. 2006; 129:564–583. [PubMed: 16399806]
48. Adolphs R. Recognizing emotion from facial expressions: psychological and neurological mechanisms. *Behav Cogn Neurosci Rev*. 2002; 1:21–62. [PubMed: 17715585]
49. Aggleton JP, Burton MJ, Passingham RE. Cortical and subcortical afferents to the amygdala of the rhesus monkey (*Macaca mulatta*). *Brain Res*. 1980; 190:347–368. [PubMed: 6768425]

50. Roberts AC, Tomic DL, Parkinson CH, Roeling TA, Cutter DJ, Robbins TW, et al. Forebrain connectivity of the prefrontal cortex in the marmoset monkey (*Callithrix jacchus*): an anterograde and retrograde tract-tracing study. *J Comp Neurol*. 2007; 502:86–112. [PubMed: 17335041]
51. Hornung JP. The human raphe nuclei and the serotonergic system. *J Chem Neuroanat*. 2003; 26:331–343. [PubMed: 14729135]
52. Haddjeri N, Lavoie N, Blier P. Electrophysiological evidence for the tonic activation of 5-HT(1A) autoreceptors in the rat dorsal raphe nucleus. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2004; 29:1800–1806. [PubMed: 15127086]
53. Fisher PM, Meltzer CC, Price JC, Coleman RL, Ziolkowski SK, Becker C, et al. Medial prefrontal cortex 5-HT(2A) density is correlated with amygdala reactivity, response habituation, and functional coupling. *Cerebral cortex*. 2009; 19:2499–2507. [PubMed: 19321655]
54. Fisher PM, Price JC, Meltzer CC, Moses-Kolko EL, Becker C, Berga SL, et al. Medial prefrontal cortex serotonin 1A and 2A receptor binding interacts to predict threat-related amygdala reactivity. *Biology of mood & anxiety disorders*. 2011; 1:2. [PubMed: 22738071]
55. Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, et al. Serotonin transporter genetic variation and the response of the human amygdala. *Science*. 2002; 297:400–403. [PubMed: 12130784]
56. Milak MS, DeLorenzo C, Zanderigo F, Prabhakaran J, Kumar JS, Majo VJ, et al. In vivo quantification of human serotonin 1A receptor using 11C-CUMI-101, an agonist PET radiotracer. *J Nucl Med*. 2010; 51:1892–1900. [PubMed: 21098796]
57. Tauscher J, Verhoeff NP, Christensen BK, Hussey D, Meyer JH, Kecojovic A, et al. Serotonin 5-HT1A receptor binding potential declines with age as measured by [11C]WAY-100635 and PET. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2001; 24:522–530. [PubMed: 11282252]
58. Parsey RV, Oquendo MA, Simpson NR, Ogden RT, Van Heertum R, Arango V, et al. Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT1A receptor binding potential measured by PET using [C-11]WAY-100635. *Brain research*. 2002; 954:173–182. [PubMed: 12414100]
59. Moses-Kolko EL, Price JC, Shah N, Berga S, Sereika SM, Fisher PM, et al. Age, sex, and reproductive hormone effects on brain serotonin-1A and serotonin-2A receptor binding in a healthy population. *Neuropsychopharmacology*. 2011; 36:2729–2740. [PubMed: 21849982]
60. Stein P, Savli M, Wadsak W, Mitterhauser M, Fink M, Spindelegger C, et al. The serotonin-1A receptor distribution in healthy men and women measured by PET and [carbonyl-11C]WAY-100635. *Eur J Nucl Med Mol Imaging*. 2008; 35:2159–2168. [PubMed: 18542956]
61. Costes N, Merlet I, Ostrowsky K, Faillenot I, Lavenne F, Zimmer L, et al. A 18F-MPPF PET normative database of 5-HT1A receptor binding in men and women over aging. *J Nucl Med*. 2005; 46:1980–1989. [PubMed: 16330560]
62. Fusar-Poli P, Placentino A, Carletti F, Landi P, Allen P, Surguladze S, et al. Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. *J Psychiatry Neurosci*. 2009; 34:418–432. [PubMed: 19949718]
63. Sheline YI, Barch DM, Donnelly JM, Ollinger JM, Snyder AZ, Mintun MA. Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biol Psychiatry*. 2001; 50:651–658. [PubMed: 11704071]
64. Godlewska BR, Norbury R, Selvaraj S, Cowen PJ, Harmer CJ. Short-term SSRI treatment normalises amygdala hyperactivity in depressed patients. *Psychol Med*. 2012; 42:2609–2617. [PubMed: 22716999]
65. Bhagwagar Z, Cowen PJ, Goodwin GM, Harmer CJ. Normalization of enhanced fear recognition by acute SSRI treatment in subjects with a previous history of depression. *Am J Psychiatry*. 2004; 161:166–168. [PubMed: 14702268]
66. Roiser JP, Elliott R, Sahakian BJ. Cognitive mechanisms of treatment in depression. *Neuropsychopharmacology*. 2012; 37:117–136. [PubMed: 21976044]

Figure 1.

Correlation between left amygdala and dorsal raphe nucleus 5-HT_{1A} availability. Panel A shows summated [¹¹C]CUMI-101 BP_{ND} image from a representative subject in sagittal section. White circle shows the dorsal raphe. Panel B shows left amygdala activation for the contrast all faces versus baseline (coordinates: [x=-18, y=-7, z=-17], P=0.001 uncorrected, extent=10 voxels). Panel C shows the inverse correlation between dorsal raphe 5-HT_{1A} availability and the amygdala fear versus neutral contrast estimate. This relationship remained significant after removing the subject with the lowest 5-HT_{1A} receptor availability (r=-0.53, P=0.037)

Figure 2.

Correlations between dorsal raphe nucleus 5-HT_{1A} availability and psychophysiological interaction (PPI) parameter estimate from peak voxels that survived whole brain family-wise error correction for the fear vs neutral contrast, $P=0.05$. Panel A shows the correlation of dorsal raphe nucleus 5-HT_{1A} availability with the anterior cingulate cortex, Panel B with middle frontal gyrus, Panel C with left precuneus cortex, Panel D with right precuneus cortex and Panel E with left supramarginal gyrus.

Table 1

Coordinates of suprathreshold clusters for the All Faces vs Baseline contrast, $P=0.05$, whole brain family-wise error corrected

Region	No. of voxels	Coordinate			z-score
		x	y	z	
Right fusiform gyrus	1813	39	-55	-17	7.02
Left Occipital cortex		-15	-103	-11	7.01
Left Occipital cortex		-24	-103	10	5.06
right lateral occipital cortex	7	30	-61	46	5.19
left precentral gyrus	2	-33	-4	46	5.06
left putamen	2	-30	-7	10	4.95
left postcentral gyrus	4	-39	-22	46	4.91
left lateral occipital cortex	1	-42	-88	7	4.88
right precentral gyrus	1	45	8	28	4.88
right inferior lateral occipital cortex	2	54	-64	4	4.82
right middle frontal gyrus	1	33	2	58	4.81

Table 2

Coordinates of clusters from the all faces baseline psychophysical interactions PPI analysis (F-contrast), thresholded at $P=0.005$ uncorrected

Region	No. of Voxels	Coordinate			Z-score
		x	y	z	
Left orbitofrontal cortex	87	-36	38	-14	5.34
Left superior frontal cortex	1375	-15	32	37	5.07
Right temporo-occipitalcortex	1005	60	-49	10	5.06
Left lateral occipital cortex	199	-48	-58	19	4.77
Left amygdala	54	-18	-7	-17	4.69
Left precentral gyrus	287	-45	-22	52	4.61
Left insular cortex	128	-36	-19	10	4.39
Right precentral gyrus	132	39	-22	46	4.37
Right lateral occipital cortex	200	42	-64	34	4.32