Title

Serotonergic neural network related to behavioral inhibition system

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Conflict of interests

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Abstract

Rationale The tendency to avoid punishment, called behavioral inhibition system, is an essential aspect of motivational behavior. Behavioral inhibition system is related to negative affect, such as anxiety, depression and pain, but its neural basis has not yet been clarified. *Objectives* To clarify the association between individual variations in behavioral inhibition system and brain 5-HT$_{2A}$ receptor availability and specify which brain networks were involved in healthy male subjects, using [^{18}F]altanserin positron emission tomography and resting-state functional magnetic resonance imaging. *Result*s Behavioral inhibition system score negatively correlated with 5-HT$_{2A}$ receptor availability in anterior cingulate cortex. A statistical model indicated that the behavioral inhibition system score was associated with 5-HT$_{2A}$ receptor availability, which was mediated by the functional connectivity between anterior cingulate cortex and left middle frontal gyrus, both of which involved in the cognitive control of negative information processing. *Conclusions* Individuals with high behavioral inhibition system displays low 5-HT$_{2A}$ receptor availability in anterior cingulate cortex and this cognitive control network links with

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prefrontal-cingulate integrity. These findings have implications for underlying the serotonergic basis of physiologies in aversion.

Keywords

Behavioral inhibition system; Serotonin system; Cingulate cortex; Positron emission tomography; resting state functional magnetic resonance image

Introduction

The fundamental features of complex behavior have long been discussed as being categorizable into the approach to rewards and the avoidance of punishments. These two systems can be applied to account for personality and motivation (Davidson, 1994; Gray, 1982; Higgins, Roney, Crowe, & Hymes, 1994), positing that there are independent sensitivity in the respective systems. Gray provided a powerful theoretical framework that was rooted in behavioral psychology and neuroscience, called Reinforcement Sensitivity Theory (RST) (Gray, 1982). Gray proposes two systems together with an additional one: Behavioral Approach System (BAS), Behavioral Inhibition System (BIS), and Fight-Flight System (FFS) (see revised version of RST(Gray & McNaughton, 2000)). BAS corresponds to impulsivity, drug addiction, and attention deficit hyperactivity disorder, BIS is related to anxiety, depression, and pain, and FFS is fear at the psychological and psychiatric level (Bijttebier, Beck, Claes, & Vandereycken, 2009; P.J. Corr, 2002; P. J. Corr, 2004; Jensen, Ehde, & Day, 2016).

In contrast to BAS, however, only a handful of studies have investigated the neural basis of BIS. The trait sensitivity to aversive events was associated with increased gray matter volume in amygdala and hippocampus (Barros-Loscertales et al., 2006; Cherbuin et al., 2008) and decreased volume in orbitofrontal cortex (OFC) and precuneus (Fuentes et al., 2012). BIS variability was also associated with individual differences in the neural activities of dorsal anterior cingulate cortex (ACC), OFC, striatum, amygdala, and hippocampus during anticipation of aversive events, such as monetary loss, measured by functional magnetic resonance imaging (fMRI) (Beaver, Lawrence, Passamonti, & Calder, 2008; Kim, Yoon, Kim, & Hamann, 2015; Simon et al., 2010). A resting-state fMRI (rs-fMRI) study similarly found that BIS correlated negatively with regional homogeneity in amygdala and hippocampus (Hahn, Dresler, Pyka, Notebaert, & Fallgatter, 2013).

Meanwhile, a number of neuroimaging studies have investigated the neural responses to aversive stimuli such as signal pain, punishment and monetary loss. The core regions of these aversive anticipations are found in ACC, anterior insula, OFC, and amygdala (De Martino, Camerer, & Adolphs, 2010; Eisenberger, 2012; Hayes & Northoff, 2011; Kringelbach & Rolls, 2004; Nitschke, Sarinopoulos, Mackiewicz, Schaefer, & Davidson, 2006; Wrase et al., 2007). Congruent brain regions (ACC, OFC, amygdala) between aversive anticipation and individual variations in the sensitivity to aversive events leads to the notion that these regions are the hub for understanding the neural mechanisms of BIS.

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The serotonergic system is supposed to be associated with behavioral inhibition in the human brain (Cloninger, 1987). Notably, serotonin 2A ($5\text{-}HT_{2A}$) receptor has been reported to be intimately involved in the modulation of negative emotions, such as anxiety, depression, and pain (Baldwin & Rudge, 1995; Sommer, 2009). According to Gray's concept of BIS, harm avoidance is characterized as excessive anxiety and fear, but the evidences for the role of serotonergic system in harm avoidance, so far remains inconclusive. For instance, harm avoidance and $5\text{-}HT_{2A}$ receptor availability showed a negative correlation in prefrontal cortex and left parietal cortex (Moresco et al., 2002), a positive correlation in dorsal prefrontal cortex (Baeken, Bossuyt, & De Raedt, 2014), or no significant regional correlation (Soloff, Price, Mason, Becker, & Meltzer, 2010). Although human positron emission tomography (PET) studies have shown that the $5\text{-}HT_{2A}$ receptors are highly and widely distributed in cortical regions (Savli et al., 2012), it remains unclear whether the individual variations in $5\text{-}HT_{2A}$ receptor availability are involved in the trait sensitivity to aversive events, i.e., BIS.

The aim of this study was to elucidate the neural and molecular mechanisms associated with individual variations in BIS. In this regard, the relationships among BIS, the $5\text{-}HT_{2A}$ receptor availability using PET and the brain functional connectivity measured by rs-fMRI were investigated. We first conducted a PET imaging study to explore which brain regions of $5\text{-}HT_{2A}$ receptor availability correlated with BIS in healthy volunteers. Then, we analyzed rs-fMRI data to detect functional connectivity showing correlation with local $5\text{-}HT_{2A}$ receptor availability and BIS. Finally, mediation analysis was conducted to elucidate the relationships among BIS, functional connectivity and the $5\text{-}HT_{2A}$ receptor availability.

Materials and methods

Participants

Sixteen healthy right-handed male subjects (age: 23.3 ± 2.9 years, mean $\pm$ standard deviation) were recruited. Two subjects were excluded due to incomplete data collection, and the data of fourteen participants (23.4 ± 2.9 years) were analyzed. All participants were free of current and past psychiatric or somatic disorders, and had no history of drug abuse. Each participant completed psychological testing and underwent both rs-fMRI and PET scans. All participants provided written informed consent before participating in the study, which was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences in accordance with the ethical standards laid down in the 1964

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Declaration of Helsinki and its later amendments.

Psychological measurement

To test Gray's original theory, Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ) was developed by Torrubia (Torrubia, Ávila b, Moltó, & Caseras, 2001). This scale indicates good reliability and validity, and accurately expresses the essence of Gray's theory (e.g., extraversion and neuroticism in expected directions). All participants completed the Japanese version of SPSRQ (Takahashi & Shigemasu, 2008). SPSRQ is a 48-item self-report measure that consists of two subscales, representing sensitivity to reward (SR) to measure impulsivity, i.e., BAS, and sensitivity to punishment (SP) to measure anxiety, i.e., BIS. Each item is scored on a 4-point Likert scale (1 = disagree, 4 = agree). SR and SP scores with higher scores indicating greater impulsivity and sensitivity to punishment, respectively. Participants also completed the Beck Hopelessness Scale (BHS(Beck, Weissman, Lester, & Trexler, 1974)) and State-Trait Anxiety Inventory (STAI (Spielberger, Gorsuch, Lushene, Vagg, & Jacob, 1983)) to measure the levels of depressive hopelessness and anxiety, respectively.

PET acquisition and analysis

All subjects underwent a PET scan to measure regional 5-HT$_{2A}$ receptor availability. A 90-min dynamic PET acquisition was performed after an injection of [^{18}F]altanserin (190 ± 5.4 MBq with molar activity of 167 ± 77 GBq/μmol). The scan protocol consisted of 33 frames (10 s x 6, 20 s x 3, 1 min x 6, 3 min x 4, and 5 min x 14 frames). All of the PET scans were performed on an Eminence SET-3000 GCT/X PET scanner (Shimadzu; Kyoto, Japan) with a head fixation device to minimize head movement. Each PET scan was preceded by a transmission scan for attenuation correction using a ^{137}Cs source. All PET images were reconstructed with the filtered back-projection method (Gaussian filter, kernel 5 mm; reconstructed in-plane resolution was 7.5 mm in full width at half maximum; voxel size: 2 x 2 x 2.6 mm) corrected for attenuation, randoms and scatter.

During the scans, arterial blood samples were obtained manually 33 times after radioligand injection to obtain arterial input function (Ishii et al., 2017). Each blood sample was centrifuged to obtain plasma and blood cell fractions, and the concentrations of radioactivity in whole blood and plasma were measured (Ishii et al., 2017). The fractions of the parent compound and its radiometabolites in plasma were

determined using high-performance liquid chromatography from 6 samples of each subject (Ishii et al., 2017).

All PET images were spatially normalized to the standard anatomic orientation. First, head motion during the scans was corrected on the emission images after correction of attenuation using μ-maps that were realigned to each frame of the emission images (Wardak et al., 2010). Second, T1-weighted MR images were coregistered to the corresponding mean PET images. Third, the MR images were spatially normalized and segmented into gray matter, white matter, and cerebrospinal fluid using SPM8 (Wellcome Institute of Neurology, University College of London, UK). Finally, all PET images were spatially normalized to the standard anatomic orientation (Montreal Neurological Institute (MNI) 152 standard space; Montreal Neurological Institute; Montreal, QC, Canada) based on the transformation of the MR images.

Because the Logan analysis provided a good compromise between validity, sensitivity, and reliability of implementation (Price et al., 2001), the PET data were analyzed by Logan graphical method (Logan et al., 1990), which was applied across the 12- to 90-min integration intervals, and regional total distribution volume (V_T) values were obtained. We used the cerebellum as reference brain region and estimated the nondisplaceable distribution volume (V_{ND}). 5-HT$_{2A}$ receptor availability was determined as binding potential (BP_P) that was derived from the equation: $BP_P = V_T - V_{ND}$ (Innis et al., 2007). All kinetic analyses were performed using PMOD (version 3.6, PMOD Technologies Ltd., Zurich, Switzerland).

Region-of-interest analysis

ACC, OFC and amygdala, which are involved in the sensitivity to aversive events (Barros-Loscertales et al., 2006; Beaver et al., 2008; Cherbuin et al., 2008; Eisenberger, 2012; Fuentes et al., 2012), were applied to ROI analyses. These brain regions were extracted by the Harvard-Oxford atlas using the CONN toolbox (version 17e, http://www.nitrc.org/projects/conn), averaged over right and left. Subsequently, ACC was divided into four segregated subregions, namely, subgenual ACC (sgACC), pregenual ACC (pgACC), anterior midcingulate cortex (aMCC) and posterior midcingulate cortex (pMCC) (Yeung, Botvinick, & Cohen, 2004). Subgenual ACC was substituted by subcallosal cortex in the atlas due to small volume (0.056 mm^3). The volumes of each ACC subregion were 9.2 mm^3 in subcallosal cortex, 6.5 mm^3 in pgACC, 9.2 mm^3 in aMCC, and 6.7 mm^3 in pMCC.

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Resting-state fMRI acquisition and analysis

Each subject underwent a 6.8-minute rs-fMRI scan, performed with a Magnetom Verio 3.0 T MRI scanner (Siemens, Erlangen, Germany) equipped with a 32-channel head coil. During scanning, subjects were instructed to relax with their eyes open while gazing at a fixation cross. A single session acquired 3.8-mm thick, no gap, interleaved axial 33 slices (in-plane resolution: 3.75 x 3.75 mm) with a 30-degree angle relative to the AC-PC axis, using a T2*-sensitive single-shot EPI sequence with the following parameters: TR = 2000 ms, TE = 25 ms, flip angle = 90 degrees, matrix = 64 x 64. A high-resolution T1-weighted anatomical image using a magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (176 sagittal slices, resolution = 0.49 x 0.49 x 1.00 mm, no gap, TR = 2300 ms, TE = 1.95 ms, flip angle = 9 degrees, matrix = 512 x 512) was acquired for anatomical reference.

Data processing was performed using the CONN toolbox and SPM12 (Wellcome Institute of Neurology, University College of London, UK) working on Matlab version 8.4 (MathWorks, MA, USA). The first four volumes were discarded from analysis to account for magnetization saturation effects. Preprocessing comprised: 1) realignment and unwarping, 2) slice timing correction, 3) segmentation and normalization, 4) smoothing with a Gaussian kernel of 4 mm. To eliminate correlations caused by head motion and artifacts, we identified outlier time points in the motion parameters and global signal intensity using Artifact Detection Tools (ART), which includes the CONN toolbox. For each subject, we treated images as outliers if composite movement from a preceding image exceeded 0.2 mm, or if the global mean intensity was over 3 SDs from the mean image intensity for the entire resting scan. Based on the previous report [62], after removing outlier images, one subject whose total scan time of less than 3 minutes was excluded from the subsequent analyses.

After preprocessing, we conducted de-noising as follows: 1) linear regression of noise sources from white matter and cerebrospinal fluid by CompCor (component-based noise correction method) and from outliers by ART and from Friston 24 head motion parameter, 2) band-pass filtering of 0.009 – 0.1 Hz was used to pass the low frequency fluctuations of interest, 3) quadratic trends were removed. Global signal regression was not used to avoid potential false anticorrelations.

Image analyses

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1. **Association between SP and 5-HT$_{2A}$ receptor availability**

     To test for the contribution of 5-HT$_{2A}$ receptor availability to SP, we first conducted Spearman's rank

test between SP and the BP_P value of each ROI using GraphPad Prism (version 7, GraphPad Software,

CA, USA). P-value less than 0.05 with false discovery rate (FDR) correction for multiple comparisons

was considered significant.

2. **Relationship between 5-HT$_{2A}$ receptor availability and functional connectivity**

     Regions of 5HT$_{2A}$ receptor availability that correlated with SP were subsequently investigated by

seed-based functional connectivity analysis modeling the BP_P values in the analogous regions, utilizing

the CONN toolbox. This procedure may explore the functional connectivities that correlate with 5-HT$_{2A}$

receptor availability of SP-related ROIs. The threshold was defined as a cluster-level threshold of p <

0.05, FDR-corrected with voxel-level threshold of p < 0.001, uncorrected for multiple comparisons. All

reported coordinates were of MNI standard space.

3. **Functional connectivity related to SP**

    The correlation coefficient for each specified functional connectivity was extracted. Spearman's rank

test was performed between each extracted correlation coefficient and SP. P-value less than 0.05 was

considered significant.

4. **Mediation analysis**

     Finally, for each functional connectivity that was significantly related to both 5-HT$_{2A}$ receptor

availability and SP, we performed mediation analysis to test whether the functional connectivity might be

involved in the link between SP and 5-HT$_{2A}$ receptor availability. The correlation coefficient of functional

connectivity was included as a mediator. INDECT macro (Preacher & Hayes, 2008) with SPSS (version

24, IBM, NY, USA) were used. Bias-corrected and accelerated 95% confidence intervals based on 10000

bootstrap sampling were used to assess significance.

Results

Behavioral findings

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The median SP and SR scores were 61 (interquartile range, 55 to 67.5) and 51 (interquartile range, 44 to 59), respectively. The median score of BHS was 6 (interquartile range, 4.5 to 10.5) and STAI was 41 (interquartile range, 34 to 54.5). SP scores correlated positively with BHS (r_s = 0.86, p < 0.0005, Spearman's rank test) and at a marginally significant level with STAI (r_s = 0.52, p = 0.07).

Regional 5-HT$_{2A}$ receptor availability measured by [^{18}F]altanserin PET

Figure 1 shows the 5-HT$_{2A}$ receptor availability values (BP_P) in ACC, OFC and amygdala which were selected from the premise that these regions were associated with aversive anticipation. Higher BP_P was measured in the ACC and OFC, while low BP_P was observed in the amygdala. The BP_P values in ACC (1.535 [1.376 to 1.773]), OFC (1.503 [1.41 to 1.633]) and amygdala (0.676 [0.581 to 0.762]) were comparable to that of healthy subjects in previous report (Savli et al., 2012).

Association between behavioral inhibition system and 5-HT$_{2A}$ receptor availability

The SP score was negatively correlated with the BP_P value of ACC (r_s = -0.66, p = 0.016, Spearman's rank test with FDR correction). No correlations were found in OFC or amygdala (r_s = -0.47 and r_s = -0.57, respectively, both p > 0.05 with FDR correction).

We further examined the correlations between SP and the BP_P values in the functional subdivisions of ACC. There was no difference in the BP_P values among subdivisions of ACC (F(3, 48)=1.06, p=0.375, One-way ANOVA). SP scores correlated negatively with the BP_P values in pgACC, aMCC and pMCC but not in subcallosal cortex (pgACC, r_s = -0.59, p = 0.037; aMCC, r_s = -0.60, p = 0.034; pMCC, r_s = -0.66, p = 0.017; subcallosal cortex, r_s = -0.46, p = 0.117, Spearman's rank test with FDR correction; Figure 2).

Association between 5-HT$_{2A}$ receptor availability and functional connectivity

Seed-based functional connectivity analyses were performed for above three ACC subregions (Figure 3, Table 1) to explore functional connectivity that correlated with the local BP$_P$ value. The BP_P values in pgACC were negatively correlated with the functional connectivity between pgACC and clusters in left lateral occipital cortex and right lingual gyrus (Figure 3a). The BP_P values in aMCC were positively correlated with the functional connectivity between aMCC and left middle frontal gyrus (MFG) (Figure

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3b). The BP_P values in pMCC were positively correlated with the functional connectivity between pMCC and clusters in right inferior frontal gyrus, left precentral gyrus, left supramarginal gyrus and left angular gyrus (Figure 3c).

Functional connectivity related to behavioral inhibition system

Correlation analyses between these specific functional connectivities and SP were carried out. SP was negatively correlated with the functional connectivity between aMCC and left MFG ($r_s = -0.67$, $p = 0.014$, Spearman's rank test; Table 2).

Mediation analysis

Finally, we examined whether functional connectivity between aMCC and left MFC serve as a potential mediator of the link between SP scores and 5-HT_{2A} receptor availability. We tested two possible models: 1) 5-HT_{2A} receptor availability affects functional connectivity, which in turn affects SP; 2) SP affects functional connectivity, which in turn affects 5-HT_{2A} receptor availability. Mediation analyses supported the latter model, indicating that the total indirect effect of SP scores on the BP_P values via functional connectivity was significant (BCa CI: -0.044 to -0.007; $p < 0.05$). In sum, these results imply that the functional connectivity between aMCC and left MFG serves as an important role in linking BIS with the 5-HT_{2A} receptor availability.

Discussion

This study investigated whether the individual variations in 5-HT_{2A} receptor availability contributes to BIS and which brain networks were specifically involved. BIS correlated negatively with 5-HT_{2A} receptor availability in ACC, and the association between BIS and 5-HT_{2A} receptor availability was accounted for by the functional connectivity between aMCC and left MFG.

Our findings indicate the role of serotonergic neurotransmission in ACC, as was previously linked with BIS personality and aversive anticipation. Specifically, high BIS individuals showed reduced levels of serotonin 5-HT_{2A} receptor availability in ACC. The 5-HT_{2A} receptor is known to be related to psychiatric symptoms, such as anxiety and depression, as well as hallucinations in schizophrenia (Quednow, Geyer, & Halberstadt, 2009) and Parkinson's disease (Ballanger et al., 2010). In recent genetic studies,

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polymorphism of the 5-HT$_{2A}$ gene has been frequently reported in depression and schizophrenia (Gu et al., 2013; Tan et al., 2014; Zhao et al., 2014). For example, single nucleotide polymorphism of 5-HT$_{2A}$ gene was associated with pathological gambling and suicide in depressed patients (Arias et al., 2001; Wilson, da Silva Lobo, Tavares, Gentil, & Vallada, 2013). Furthermore, in PET studies, while suicide victims had a high density of 5-HT$_{2A}$ receptors in prefrontal cortex (Du, Faludi, Palkovits, Bakish, & Hrdina, 2001), treatment-resistant depressed patients displayed lower 5-HT$_{2A}$ receptor binding in dorsal prefrontal cortex and ACC (Baeken, De Raedt, & Bossuyt, 2012). These contradictory findings represent the activation and the inhibition of impulsivity (Fineberg et al., 2010), and the current finding supports to the latter. Animal experiments have also shown that blockage of the 5-HT$_{2A}$ receptor in medial prefrontal cortex suppressed impulsive behavior (Fink et al., 2015). Taken together, present result indicates that high BIS individuals showed stronger depressive hopelessness and facilitates lower 5-HT$_{2A}$ receptor availability in ACC. This may reflect the specific involvement of inhibitory control associated with some aspects of depressive symptoms.

This study newly identified the functional connectivity associated with 5-HT$_{2A}$ receptor availability in ACC subregions (pgACC, aMCC, pMCC), and the mediation test revealed that aMCC-MFG functional connectivity contributed to the link between BIS and 5-HT$_{2A}$ receptor availability. ACC has been consistently linked with cognitive function, emotion processing, and the autonomic nervous system (Bush, Luu, & Posner, 2000; Critchley, Mathias, & Dolan, 2001), which is divided into 4 subregions (Vogt, Berger, & Derbyshire, 2003). The pgACC, the ventral part of ACC, is involved in assessing emotional and motivational information. The pMCC and aMCC are part of the dorsal ACC, mainly involved in cognitive controls. Although the implications of these functional connectivities are still to be examined in the future, the current findings of pgACC functional connectivity with visual areas and pMCC functional connectivity with fronto-parietal attention networks may suggest serotonergic modulation of motivational visual processing and attentional control, respectively.

The aMCC represents a hub where information about punishment and negative feedback, such as pain, is monitored, triggering control signals and/or selective attention generated in dorsolateral prefrontal cortex (DLPFC) (MacDonald, Cohen, Stenger, & Carter, 2000; Miller & Cohen, 2001; Shackman et al., 2011; Walsh, Buonocore, Carter, & Mangun, 2011; Yeung et al., 2004). Other studies have also shown that aMCC is anatomically connected with DLPFC in monkeys (Morecraft & Tanji, 2009), and that the

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functional connectivity between these two regions is correlated with working memory demand according to task-based fMRI (Osaka et al., 2004). Consistent with these previous studies, our finding of the functional connectivity between aMCC and left MFG, a part of DLPFC, possibly reflects the cognitive control associated with negative information processing, and in particular, serves a mechanistic role in linking BIS and serotonergic neurotransmission.

It is puzzling that the direction of the path was found to be from the psychological trait to the molecular system, not vice versa. The mediation test taps on the mathematical linkage rather than on a biological one. Serotonergic stimulation by physiological and acute or chronic pharmacological manner may exhibit differently in the brain function. To further clarify this question, acute and chronic serotonergic intervention may alter both BIS and the functional connectivity between aMCC and left MFG, which shall be left for the future investigations.

There are several limitations to this study. The first is that our sample size is comparatively small. Although we have set a stringent statistical threshold, future study with a larger sample size will be required to replicate the current findings. Second, females were not included in the present study. As estrogen promotes 5-HT synthesis and menstrual cycle, it influences 5-HT$_{2A}$ receptor binding in women (Wihlbäck et al., 2004), thereby we exclusively included male subjects in the current study. Considering that emotional reactions differ between genders, it may be interesting to explore the similarities and differences between male and female subjects in the future. In addition, the enrolled subjects were all Japanese. Previous behavioral studies have indicated that Japanese are motivated more by negative feedbacks than by positive ones (Diener, Oishi, & Lucas, 2003; Heine et al., 2001); thus, our results might be biased in this regard. Lastly, functional connectivity only accounts for a linear association between two brain regions. Whole brain networks and anatomical connectivities were not examined in the present study and should be addressed in the future studies.

Conclusions

In summary, this multimodal neuroimaging study provides novel evidence of the relationship between the behavioral inhibition and the serotonergic function, which is mediated by the functional connectivity between aMCC and left MFG, known as a cognitive control network. The link obtained in the current study may be tested by interventional studies using drugs which modulate serotonergic neurotransmission

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to draw out any biological causal relationships. From the basis from the current findings of healthy subjects, future studies of patients with anxiety, depression, and pain disorder symptoms may shed a light on, further understanding of 5-HT$_{2A}$ receptor function and symptoms associated with behavioral inhibition.

Conflict of interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical Approval

The study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent to Participate

All participants provided written informed consent before participating in the study.

Consent to Publish

All authors provided their consent to publish this manuscreipt.

Authors Contributions

M.Y., Y.K, T.S designed the study; M.Y., Y.K., K.T., T.I., K.Y., H.H., K.Kawamura, M.Z. and H.I. conducted the experiment; K.Kojima., M.Y., Y.K., C.S. and Y.I. analyzed data; K.Kojima, M.Y., S.H., Y.K., S.K., M.H. and T.S. wrote the paper.

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Competing Interests

None of the authors have conflicts of interest to disclose.

Availability of data and materials

Not applicable

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Figure legends

Figure 1. [^{18}F]altanserin binding potentials of the limbic system. Bar graphs represent mean ± standard deviation.

Figure 2. a) Mean parametric image of 5-HT$_{2A}$ receptor binding of [^{18}F]altanserin PET, shown in sagittal view. b) Subdivisions of ACC, overlaid on sagittal T1 MRI template. c) Plot graph of sensitivity to punishment (SP) score and regional 5-HT$_{2A}$ receptor binding potentials (BP_P). SP was negatively correlated with 5-HT$_{2A}$ receptor BP_P in pgACC, aMCC and pMCC, whereas no such association was detected in subcallosal region (*false discovery rate corrected $p < 0.05$). Spearman's rank test was used. pgACC, pregenual anterior cingulate cortex; aMCC, anterior midcingulate cortex; pMCC, posterior midcingulate cortex

Figure 3. Surface rendered images of functional connectivity associated with 5-HT$_{2A}$ receptor binding potentials in each anterior cingulate cortex subregion. a) The functional connectivity of the pregenual anterior cingulate cortex. b) The functional connectivity of the anterior midcingulate cortex. c) The functional connectivity of the posterior midcingulate cortex. Shown clusters remained after a threshold of cluster-level $p < 0.05$ false discovery rate corrected and voxel-level $p < 0.001$ uncorrected for multiple comparisons. Clusters were surface-rendered onto a brain template. Color bar represents T-value; negative correlations as blue-purple, positive correlations as red-yellow.

Tables

Table 1. Coordinates of functional connectivity that correlated with 5-HT$_{2A}$ receptor binding potentials in each anterior cingulate cortex subregion.

Brain Region	Extent	T-value	MNI Coordinates		
			x	y	z
ROI: pregenual anterior cingulate cortex					
Lateral Occipital Cortex	54	-5.70	-22	-80	14
Lingual Gyrus	33	-5.77	14	-72	-2
ROI: anterior midcingulate cortex					
Middle Frontal Gyrus	91	8.35	-36	28	26
ROI: posterior midcingulate cortex					
Precentral Gyrus	119	7.27	-50	2	26
Supramarginal Gyrus	46	7.23	-42	-44	44
Angular Gyrus	67	6.84	-28	-58	34
Inferior Frontal Gyrus	50	5.72	52	18	28

A cluster-level threshold of $p < 0.05$ false discovery rate corrected with voxel-level threshold of $p < 0.001$ uncorrected. ROI: Region-of-interest, MNI: Montreal Neurological Institute.

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Table 2. Correlations between sensitivity to punishment score and functional connectivity of each subregion of anterior cingulate cortex.

	r_s	p value
ROI: pregenual anterior cingulate cortex (pgACC)		
pgACC - left lateral occipital cortex	0.52	0.069
pgACC - right lingual gyrus	0.44	0.135
ROI: anterior midcingulate cortex (aMCC)		
aMCC - left middle frontal gyrus	-0.67	0.014*
ROI: posterior mid cingulate cortex (pMCC)		
pMCC - left precentral gyrus	-0.46	0.112
pMCC - left angular gyrus	-0.39	0.189
pMCC - right inferior frontal gyrus	-0.35	0.243
pMCC - left supramarginal gyrus	-0.41	0.166

r_s: Spearmann's rank correlation coefficient
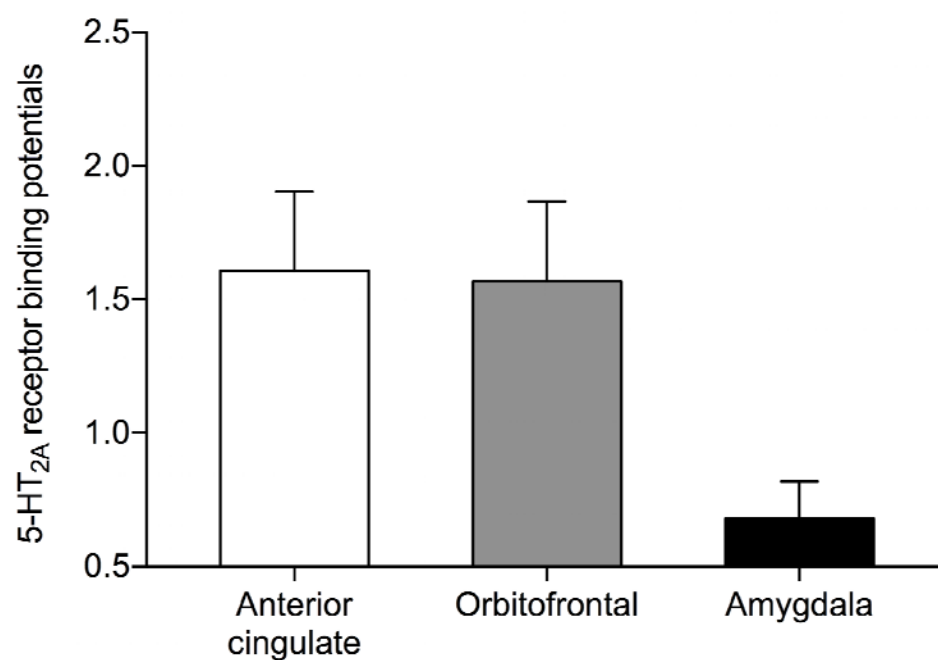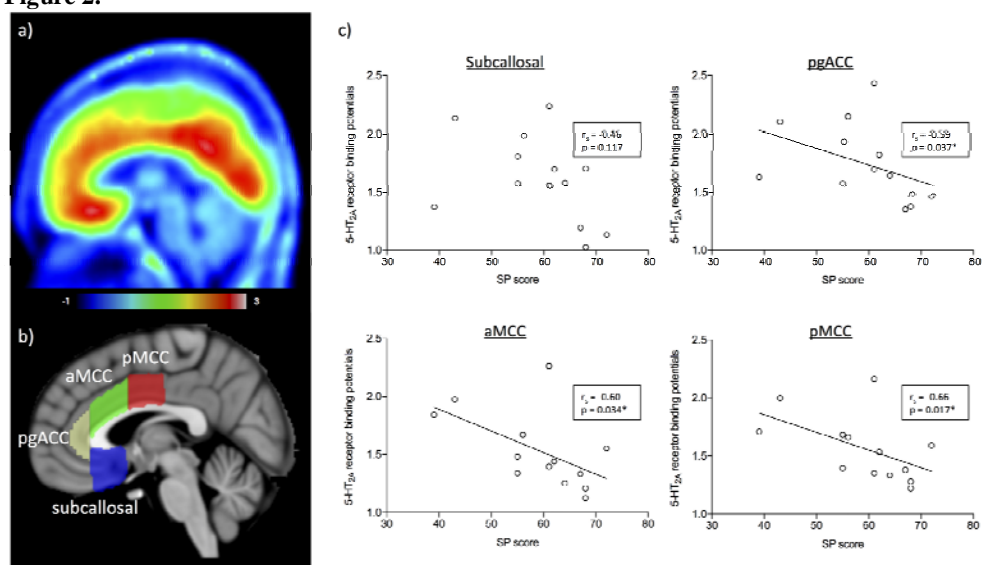
*p < 0.05

ROI: Region-of-interest

Figure 1.

Figure 2.

Figure 3.

a) pregenual anterior cingulate cortex

Lateral occipital cortex
Lingual gyrus

Left lateral hemisphere   Right medial hemisphere

b) anterior midcingulate cortex

Middle frontal gyrus

Left lateral hemisphere   Right lateral hemisphere

c) posterior midcingulate cortex

Supramarginal gyrus
Angular gyrus
Inferior frontal gyrus
Precentral gyrus

Left lateral hemisphere   Right lateral hemisphere