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Inverse changes in raphe and cortical 5-HT_{1B} receptor availability after acute tryptophan depletion in healthy human subjects

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Abstract

Serotonergic neurotransmission plays a key role in the pathophysiology and treatment of various neuropsychiatric diseases. The purpose of this study was to investigate changes in serotonergic neurotransmission after acute tryptophan depletion (ATD) using positron emission tomography (PET) with [¹¹C]P943, a 5-HT_{1B} receptor radioligand previously shown to be sensitive to changes in 5-HT. Five healthy subjects were scanned on a high resolution PET scanner twice on the same day, before and approximately 5 hours after ingesting capsules containing an amino acid mixture that lacks tryptophan. For each scan, emission data were acquired for 120 minutes after intravenous bolus injection of [¹¹C]P943. Binding potential (BP_{ND}) values were estimated from parametric images using the second version of the multilinear reference tissue model (MRTM2, $t^* = 20$ min) with cerebellar grey matter used as a reference region. The change in [¹¹C]P943 binding (BP_{ND} , %) was calculated as $(BP_{ND,post} - BP_{ND,pre}) / (BP_{ND,pre}) \times 100$, and correlation analysis was performed to measure linear associations of BP_{ND} between raphe and other regions of interest (ROIs). BP_{ND} ranged from -6% to 45% in the raphe, with positive values indicating reduced competition from 5-HT. In cortical regions, BP_{ND} ranged from -28% to 7%. While these changes did not reach significance, there were significant negative correlations of BP_{ND} of the raphe with those of cerebral cortical regions and the thalamus (e.g., $r = -0.96$, $p = 0.011$ for average cortex). These findings support the hypothesis that raphe serotonin is a critical modulator of cortical serotonin release via projecting neurons in healthy human subjects.

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Keywords

acute tryptophan depletion; binding potential; [^{11}C]P943; positron emission tomography; serotonin; 5-HT_{1B} receptor

Introduction

The neurotransmitter serotonin is involved in an extensive range of neurochemical, physiological, and behavioral functions. Recently there has been increasing interest in the role of the serotonin 1B (5-HT_{1B}) receptor in the pathophysiology of major depressive disorder (MDD) amongst other types of serotonin receptors [1]. 5-HT_{1B} receptors are located at the nerve terminals of serotonergic neurons where they act as autoreceptors and also in non-serotonergic neurons where they act as heteroreceptors. They are involved in the regulation of release of various neurotransmitters, including serotonin itself, glutamate, gamma-aminobutyric acid (GABA), acetylcholine, and dopamine [2-5].

Studies in animals and humans suggest that the 5-HT_{1B} receptor is a key factor in mood dysregulation [6]. Previously, we developed a 5-HT_{1B} receptor antagonist positron emission tomography (PET) radioligand, [^{11}C]P943, and validated it for in vivo imaging of the 5-HT_{1B} receptor in humans [7]. P943 (R-1-[4-(2-methoxy-isopropyl)-phenyl]-3-[2-(4-methyl-piperazin-1-yl)benzyl]-pyrrolidin-2-one) has high affinity to the human 5-HT_{1B} receptor ($K_i = 0.77$ nM) and 50- to 1,000-fold selectivity for the 5-HT_{1B} receptor than for most of the other types of serotonin receptors [8-11]. P943 has only 10 times higher affinity for the 5-HT_{1B} receptor than for the 5-HT_{1D} receptor, which has the most similar structural and pharmacological properties to the 5-HT_{1B} receptor amongst others [12]. However, the density of the 5-HT_{1D} receptor is low in the human brain and expressed mainly in the ventral pallidum [13]. Thus, [^{11}C]P943 signal attributed from the 5-HT_{1D} receptor binding should be negligible. The test-retest reproducibility of [^{11}C]P943 binding is very good with mean and standard deviation of test-retest variability ranging from 0.2% to 5.4% and from 5.1% to 19.1%, respectively, across brain regions [14]. [^{11}C]P943 can thus be used to reliably quantify the 5-HT_{1B} receptor availability in the human brain.

Acute tryptophan depletion (ATD) is a well-established strategy to investigate serotonergic neurotransmission in humans [17]. ATD lowers extracellular serotonin level safely and transiently by depleting its precursor tryptophan (TRP) in the body through intake of a TRP-free mixture of large neutral amino acids (LNAAs) in capsules or liquid [18]. LNAAs compete with TRP to bind to the same carrier and to be transported across the blood-brain barrier (BBB). The liver also consumes available TRP to manufacture proteins stimulated by an increased load of amino acids in the bloodstream. Thus, ATD reduces TRP in the brain, which in turn reduces serotonin synthesis and function [19]. ATD is known to deplete plasma TRP consistently and robustly [4] and does not cause serious side effects or long-term sequelae to the subjects [19]. Though not previously studied following ATD, [^{11}C]P943 has been previously shown to be sensitive to endogenous serotonin release following the response to fenfluramine (a 5-HT releaser) in non-human primates [15, 16]. Values of 25-29% and 42% 5-HT_{1B} receptor occupancy by serotonin were found following

low and high dose fenfluramine, respectively, indicating endogenous 5-HT changes consistent with the competition model.

The purpose of this study was to investigate the impact of the ATD procedure on the brain 5-HT_{1B} receptor availability using [¹¹C]P943 PET imaging in healthy subjects. We hypothesized that ATD would lead to an increase in non-displaceable binding potential (BP_{ND}) of [¹¹C]P943 in raphe due to reduced synaptic serotonin. We expected most prominent changes in raphe, as it is known to express presynaptic 5HT_{1B} autoreceptors, while other projection areas have mixed expression of autoreceptors and heteroreceptors.

Materials and methods

Subjects

Five healthy subjects (3 men and 2 women, mean age 35 years, range 19-61 years) participated in the study. They were examined by a physician to exclude major medical or neurologic illness. Electrocardiography, complete blood counts, serum chemistries, thyroid function test, liver function test, urinalysis and urine toxicology screening were performed. Urine pregnancy test was performed for women. The Structured Clinical Interview for DSM-IV axis I Disorders (SCID-CV) was administered [20] by a trained clinician to determine the presence of any major mental disorders.

Exclusion criteria were: diagnosis of lifetime or current Axis I or II disorders as judged both by the SCID-CV and a semi-structured clinical interview with an experienced clinician; current smoking; current or past serious medical or neurological illness which would preclude participation; diagnosis of lifetime or current Axis I disorders in first degree relatives; current pregnancy (as documented by pregnancy testing at screening and on the day of the PET imaging study) or breast feeding; general MRI exclusion criteria; and significant alcohol or illicit substance abuse or dependence in the past 3 months. All subjects were medication-free for a minimum of 6 weeks at the time of the scans.

The study was performed under a protocol approved by the Yale Human Investigation Committee and the Yale-New Haven Hospital Radioactive Drug Research Committee. Subjects were recruited from New Haven and surrounding areas by advertisement. Written informed consent was obtained from all participants after a complete explanation of study procedures.

Magnetic Resonance Imaging

Brain magnetic resonance (MR) imaging was done to exclude individuals with abnormal brain lesions and to register PET and MR for image analysis. MR scans were performed within 2 months of the PET scans. MR images were collected on a 3T scanner (Trio, Siemens Medical Systems, Erlangen, Germany) with a circularly polarized head coil. A 3D T1-weighted gradient-echo (MPRAGE) sequence with 1 mm³ isometric resolution (FA=7°, TE=3.34 ms, TI=1100 ms, and TR=2500 ms) was used to acquire images.

Radiochemistry

[¹¹C]P943 was prepared by *N*-methylation of the precursor with [¹¹C]methyl triflate, as previously described [12]. Both the P943 standard and the *N*-desmethyl precursor were provided by Pfizer (Groton, CT, USA).

ATD Procedure

ATD was achieved by having subjects ingest about seventy capsules of amino acid mixture lacking TRP. The capsules contained *L*-isoleucine (4.2 g), *L*-leucine (6.6 g), *L*-lysine (4.8 g), *L*-methionine (1.5 g) *L*-phenylalanine (6.6 g), *L*-threonine (3.0 g), *L*-valine (4.8 g), and histidine (1.6 g). The composition of the mixture of amino acids was determined based on past experiments that had demonstrated a meaningful effect of the mixture on TRP levels [21]. Subjects were observed for any physiological or behavioral changes including cognition, mood and sleep up to 5 hours after ATD procedures. Mood changes from baseline were assessed using the Profile of Mood States Questionnaire (POMS) [22], State Trait Anxiety Inventory (STAI-S) [23], and the Visual Analog Mood Scale (VAMS) [24].

Positron Emission Tomography Imaging

Each subject was examined by two PET scans performed on the same day. The first scan occurred in the morning before ATD ('pre-ATD scan'), and the second scan occurred in the afternoon after ATD ('post-ATD scan'). The post-ATD scan was acquired approximately 5 hours after ATD to coincide with the known peak pharmacodynamic effect of the mixture. The mean interval between the two scans was 6 hr 56 min ± 0 hr 13 min. All procedures for the two PET scans were identical. Subjects were asked to fast from midnight before the PET scan day until the post-ATD scan was finished. Smoking and caffeine consumption were not allowed. Subject preparation consisted of placement of one intravenous and one radial artery catheter for blood draws and the injection of [¹¹C]P943. Blood samples were taken immediately before ingestion of the amino acid capsules and immediately before the post-ATD scan to assess changes in free TRP levels in serum and to measure f_p .

PET imaging was performed on a high-resolution research tomograph (HRRT) (Siemens/CTI, Knoxville, TN, USA), which acquired 207 slices (1.2 mm slice separation) with a reconstructed image resolution of ~3 mm. A 6-minute transmission scan using an orbiting ¹³⁷Cs point-source was obtained for attenuation correction. Emission data were acquired as list mode for 120 minutes after bolus injection of [¹¹C]P943 over 1 minute using an infusion pump (Harvard PHD 22/2000, Harvard Apparatus, Holliston, MA, USA). Detailed injection parameters are described in Table 1.

Dynamic PET data were reconstructed with all corrections (attenuation, normalization, scatter, randoms, dead time, and motion) using the motion-compensation ordered subset expectation maximization (OSEM) list-mode algorithm for resolution-recovery reconstruction (MOLAR) algorithm [25]. Frame timing was 6 x 30 sec, 3 x 1 min, 2 x 2 min, and 22 x 5 min. Any residual motion was corrected by co-registration to an early summed image (0-10 min after injection) using a six-parameter mutual information algorithm (FLIRT, FSL 3.2, Analysis Group, FMRIB, Oxford, UK).

Determination of Binding Potential

Binding potential (BP_{ND}), the ratio of specifically bound radioligand to that of nondisplaceable radioligand in tissue under equilibrium conditions [26], was estimated from parametric images. BP_{ND} parametric images were computed using the second version of the multilinear reference tissue model (MRTM2, $t^* = 20$ min) analysis. This model was chosen on the basis of our previous experience with quantification of [^{11}C]P943 binding in healthy volunteers [8]. First, the dynamic images were smoothed using a Gaussian filter (FWHM = 3.7 mm). Cerebellar grey matter was used as a reference region. In order to reduce spill-over effects from the occipital lobe, we used a cerebellar region of interest (ROI) whose highest slice was placed 22 mm from the top of the cerebellum [8].

Regions of Interest

Grey matter ROIs were applied using an extended version of the AAL (Anatomical Automatic Labeling for SPM2) template [27], delineated on an MR template [28]. The ROIs and their volumes were: amygdala (3.7 cm³ in template space), calcarine (33.0 cm³), caudate nucleus (15.6 cm³), cerebellar grey matter (55.9 cm³), anterior cingulate cortex (21.7 cm³), posterior cingulate cortex (6.4 cm³), frontal cortex (255.9 cm³), hippocampus (15.0 cm³), hypothalamus (1.4 cm³), insular cortex (29.0 cm³), occipital cortex (80.6 cm³), pallidum (4.6 cm³), parietal cortex (64.6 cm³), putamen (16.6 cm³), temporal cortex (172.4 cm³) and thalamus (17.3 cm³). In addition, average cortex ROI (573.5 cm³) was defined as a combination of frontal, temporal, parietal and occipital ROIs.

Definition of Raphe Nuclei ROI

The raphe nuclei ROI (1.3 cm³) were defined manually on the MR template to include the dorsal raphe nuclei in the dorsal brainstem (Figure 1 and Supplemental Figure 1). This ROI was depicted along the periaqueductal grey on eight transverse slices, starting superiorly at the inferior colliculus and ending inferiorly at the cerebellar peduncle level. Defining the raphe nuclei ROI is challenging because of its small size relative to other anatomic structures in the brain. We created four additional versions of raphe ROIs to test if modification in volume and shape of the ROI had an effect on BP_{ND} estimation in the raphe and subsequent analyses; raphe_v2 (slim and irregular contour; 2.0 cm³), raphe_v3 (wide and rectangular; 3.5 cm³), raphe_v4 (intermediate; 0.8 cm³) and raphe_v5 (slim and rectangular; 0.5 cm³) (Table 4). Different ROI characteristics included a ‘slim’ shape, with longer anterior-posterior diameter than right-left diameter; a ‘wide’ shape, with shorter anterior-posterior diameter than right-left diameter, and an ‘irregular’ shape, being contoured along the periaqueductal grey. All versions were placed in the dorsal part of the brainstem at the same level of eight transaxial slices, and checked visually on each registered BP_{ND} parametric image (see Supplemental Figure 2); all registered well and no modification was needed.

To apply these ROIs to the PET data, a summed image from 0 to 10 min after injection was created from the motion-corrected data and registered to the subject’s T1-weighted 3T MR image (six-parameter registration based on normalized mutual information), which, in turn, was registered to the MR template by a nonlinear transformation using Bioimagesuite software (version 2.5; <http://www.bioimagesuite.com>).

The change in BP_{ND} (BP_{ND}) of [^{11}C]P943 was calculated as follows:

$$\Delta BP_{ND}(\%) = (BP_{ND,post} - BP_{ND,pre}) / (BP_{ND,pre}) \times 100$$

The change of free TRP level in the serum was calculated as percent change and simple difference as follows.

$$\Delta TRP(\%) = (TRP_{post} - TRP_{pre}) / (TRP_{pre}) \times 100$$

$$\Delta TRP_{diff} = TRP_{post} - TRP_{pre}$$

Statistical Analyses

Paired t-tests were used to compare [^{11}C]P943 injection parameters, plasma free fraction (f_p) and BP_{ND} of [^{11}C]P943, and serum free TRP level between pre- and post-ATD studies. Correlation analysis was performed using Spearman's rho as a measure of linear association of $BP_{ND}(\%)$ between the raphe nuclei and cortical ROIs. False discovery rate (FDR) correction was applied to control type I errors in multiple comparisons. Partial correlation was also performed to account for any effect of age. Partial correlation measures the association between two continuous variables while controlling or adjusting for the effect of one or more other variables. Correlation was also tested between TRP measures (pre- and post-ATD levels, percent change and simple difference) and BP_{ND} measures of each ROI (pre- and post-ATD BP_{ND} values, and $BP_{ND}(\%)$), using Spearman's correlation analysis. A p -value less than 0.05 was considered statistically significant.

Results

[^{11}C]P943 injection parameters were not different between the pre- and post-ATD studies, (Table 1). There was no significant difference in f_p of [^{11}C]P943 between the two studies. ATD significantly decreased free TRP level in the serum by $75\% \pm 11\%$ consistent with previous studies [21].

The highest [^{11}C]P943 uptake was observed in the pallidum and lowest in the cerebellar grey matter among the ROIs examined, consistent with the previously known distribution from human postmortem data [13] and human PET data [8]. Mean BP_{ND} in the pallidum was 2.00 ± 0.52 at the pre-ATD scan. Mean BP_{ND} in the raphe nuclei was 0.50 ± 0.15 and those in the cerebral cortices ranged from 0.43 to 1.3 at the pre-ATD scan (Table 2).

BP_{ND} changed variably across subjects after ATD. BP_{ND} ranged from -6% to 45% in raphe nuclei (coefficient of variation (COV) 114%) and from -28% to 7% in average cortex (COV -255%). The changes did not reach significant level in any ROI. Detailed BP_{ND} and BP_{ND} results of all ROIs are presented in Table 2.

Interestingly, BP_{ND} in the raphe nuclei demonstrated significant inverse correlation with those in the cerebral cortical regions ($\rho = -0.96$, $p = 0.011$) and also with that in the thalamus ($\rho = -0.95$, $p = 0.012$) (Table 3). Graphs displaying the inverse correlation between the raphe and both temporal cortex and thalamus are illustrated in Figure 2. After adjusting for multiple comparisons using FDR, temporal, parietal and occipital cortices and thalamus were found to be significantly associated with the raphe (Table 3). In addition, when the effect of age was considered, the following correlations remained significant (without FDR correction): average cortex ($\rho = -0.98$; $p = 0.017$), frontal ($\rho = -0.95$; $p = 0.050$), temporal ($\rho = -0.99$; $p = 0.010$), parietal ($\rho = -0.99$; $p = 0.008$), occipital ($\rho = -0.98$; $p = 0.017$), and thalamus ($\rho = -0.95$; $p = 0.046$). The rest of the ROIs did not have significant correlations with the raphe BP_{ND} . When the right-most data point with the biggest BP_{ND} change was removed, the inverse correlation of BP_{ND} remained significant between the raphe and temporal, parietal and occipital ROIs. The inverse correlation also held true for the average cortex ($\rho = -0.95$, $p = 0.049$). The correlation was strong but not statistically significant between the raphe/insula ($\rho = -0.93$, $p = 0.066$) and raphe/thalamus ($\rho = -0.93$, $p = 0.073$).

There was no correlation observed between any TRP measure (TRP_{pre} , TRP_{post} , TRP (%) and TRP_{diff}) and $BP_{ND,pre}$, $BP_{ND,post}$, or BP_{ND} in any ROI.

Discussion

This is the first PET imaging study to examine the effect of ATD on 5-HT_{1B} receptor availability in healthy human subjects. The key finding was that the change in 5-HT_{1B} receptor availability after ATD showed a strong negative correlation between the raphe and cortical brain regions, i.e., the larger the increase in raphe BP_{ND} consistent with 5-HT depletion, the greater the decrease in cortical BP_{ND} . These differential effects of ATD remained significant even when the subject with the biggest change in BP_{ND} was removed from the analysis, and also when several versions of modified raphe ROIs (0.6 to 2.0 cm³ in volume) were tested. Our findings show the ability to measure reciprocal changes in endogenous serotonin in neural networks of healthy human subjects using ATD and support the existing hypothesis that raphe serotonin is a critical modulator of cortical serotonin release via projecting neurons.

ATD in PET studies of serotonin

The ATD procedure is known to lead to profound decreases in serum total and free TRP levels up to 90% of baseline between five to seven hours after ATD in humans [29]. TRP levels in the cerebrospinal fluid have been shown to be reduced by about 90%, and levels of the serotonin metabolite 5-hydroxyindolacetic acid (5-HIAA) are reduced by about 35% [30–32]. Plasma and brain TRP levels are known to be coupled physiologically [33]. ATD disrupts serotonin neurotransmission and has been reported to affect behavioral and physiological processes including cognition, mood and sleep [34–36], though these effects were not observed clinically in our study subjects. Prior PET studies have investigated the effect of ATD on the binding of radioligands to other serotonin receptors and transporters. For example, [¹⁸F]Setoperone binding to the 5-HT₂ receptor was sensitive to changes in

extracellular serotonin level by ATD procedure in some brain regions [37, 38]. In other studies, ATD did not significantly affect [^{11}C]MDL100907 binding to the 5-HT $_2\text{A}$ receptor [39], [^{18}F]MPPF binding to the 5-HT $_{1\text{A}}$ receptor [40, 41], and [^{11}C]DASB binding to the serotonin transporter [18, 42]. Possible reasons for these discrepant results include the sensitivity of each receptor or transporter tracer to 5-HT concentration as well as the analytic methods employed.

More recent studies used [^{11}C]Cimbi-36, an agonist radiotracer for the 5-HT $_{2\text{A}}$ receptor, to study serotonin transmission in non-human primates and in humans [43, 44]. In non-human primates, [^{11}C]Cimbi-36 binding was reduced after intravenous administration of fenfluramine suggesting feasible application to study human serotonin neurotransmission [44]. In a human study [43], no significant differences in the percent change of neocortical [^{11}C]Cimbi-36 binding from baseline to post-ATD or selective serotonin reuptake inhibitors (SSRI) administration were noted. Of note, larger within-group variation in 5-HT $_{2\text{A}}$ receptor binding was noted after SSRI administration as compared with placebo. There are a few possible explanations for the observed effects. First, the findings may reflect variable effects of raphe autoreceptor regulation. In this scenario, there might be specific subset of individuals who have smaller autoreceptor effects that dilute the overall observed effect. Such individuals might vary clinically from those who have larger autoreceptor effects. Second, it is possible that endogenous baseline serotonin levels were low or had low occupancy at 5-HT $_{2\text{A}}$ receptors. In this case, there may not be a detectable decrease following ATD. Third, the sample size may have been too small with insufficient statistical power to detect clinically significant differences between conditions. Additional imaging studies with larger sample sizes will be helpful to better understand the significance of these findings.

Drug challenge in PET studies of serotonin

A 2015 review identified 13 studies of serotonin neurotransmission following drug challenge [45] designed to increase serotonin levels. Of particular relevance, drug challenge studies with 5-HT $_{1\text{A}}$ receptor radioligands (i.e. [^{18}F]FPWAY and [^{18}F]MPPF) have primarily shown serotonin-dependent decreases in binding in raphe nuclei and small or absent binding changes in serotonin projection regions suggesting differential regional drug-induced changes in serotonin release. For example, a PET imaging study targeting 5-HT $_{1\text{A}}$ receptors using [^{18}F]FPWAY previously demonstrated differential effects of SSRI on the raphe and the cortex in monkeys [46]. Alternatively, responses to increased synaptic serotonin may vary regionally via receptor internalization or autoreceptor effects [45]. More recent studies have evaluated drug-induced synaptic serotonin release via imaging of 5-HT $_{1\text{A}}$ receptors and 5-HT $_{1\text{B}}$ receptors using the radiotracers [^{11}C]CUMI-101 and [^{11}C]AZ10419369, respectively, in humans and non-human primates (NHP) with mixed results [47-49].

Several studies have successfully used 5-HT $_{1\text{B}}$ receptor radioligands including [^{11}C]P943 and [^{11}C]AZ10419369 to detect serotonin release following amphetamine, MDMA, 5-hydroxy-L-tryptophan (5-HTP), or SSRI administration (predominantly in non-human primates)[15, 16, 48, 50-52], though none has examined the effects of ATD. Our finding of inverse relationship between the raphe and the cortical/thalamic 5-HT $_{1\text{B}}$ receptor

availability is consistent with a prior study that used [^{11}C]AZ10419369 (5-HT_{1B} receptor radioligand) [53], to investigate the effect of the SSRI escitalopram on endogenous serotonin level in the healthy humans. BP_{ND} of [^{11}C]AZ10419369 decreased in the raphe after escitalopram administration, while it significantly increased in some cortical regions. BP_{ND} in the thalamus also demonstrated a trend of increase. Similarly, a human study of [^{11}C]CUMI-101 following SSRI infusion showed significantly increased BP_{ND} at postsynaptic 5-HT_{1A} receptors in several cortical regions, but not in the dorsal raphe [47]. However, a second study involving [^{11}C]CUMI-101 following SSRI infusion in 6 human subjects did not find significant differences in BP_{ND} before or after citalopram infusion [49]. These findings supported the hypothesis that SSRI's decreased raphe cell firing via autoreceptors to reduce 5-HT levels in postsynaptic regions, and that the radiotracer used was sensitive to endogenous 5-HT release in humans. Thus, we suspect the mechanism of the observed decreased binding in the cortex is due to decreased raphe autoreceptor activation and subsequently increased 5-HT release in the cortex. It is not entirely clear why there was a lack of correlation observed between raphe BP_{ND} and subcortical regions. It is well-known that the raphe nuclei project broadly to both cortical and subcortical regions, though the specific nature of its regulatory functions have not been fully uncovered. Perhaps raphe regulatory functions are most pronounced in specific structures that promote arousal, including the cortices and thalamus, both of which showed strong correlations. Other subcortical structures that receive multiple neurotransmitter inputs (e.g., hypothalamus) might be more tightly controlled by other neurotransmitter systems promoting arousal (i.e. norepinephrine, dopamine, acetylcholine, or orexin). Alternatively, the low number of subjects may have reduced the statistical power required to observe differences between certain regions.

Defining the raphe nuclei ROI

We analyzed five different versions of raphe ROIs defined on the MR template to test if modification in volume and shape of the ROI had an effect on BP_{ND} estimation in the raphe and subsequent analyses. Compared with the selected raphe ROI, smaller version with similar slim shape ('raphe_v5' in Table 4) had significantly higher BP_{ND} values by $14 \pm 12\%$ ($p = 0.001$) and the larger version with slim shape ('raphe_v2' in Table 4) had significantly lower BP_{ND} values with a $-5 \pm 7\%$ difference ($p = 0.044$). Inverse changes after ATD were found significant between raphe and cortical 5-HT_{1B} receptor availability in both 'raphe_v2' and 'raphe_v5'. Slim ROIs with any sizes that roughly covered the periaqueductal grey demonstrated significant inverse changes, while wide ROIs that included quite an amount of the surrounding non-periaqueductal grey areas ('raphe_v3' and 'raphe_v4' in Table 4) were not significant.

Limitations

There were some limitations to this study. There were no statistically significant changes of BP_{ND} observed in any ROI after ATD, despite a mean 75% decrease in serum free TRP levels. With the small number of subjects, statistical power was reduced to detect significant changes in the [^{11}C]P943 binding after ATD. It is possible that a larger sample size would have revealed statistically significant BP_{ND} changes in raphe following ATD. It is therefore important to note that the findings of the study must be interpreted cautiously in the context

of a pilot study. It will be important to confirm our findings using a larger sample size. The study was otherwise limited in that the ratio of TRP to the sum of the other LNAAs was not measured. The ratio is a better predictor of reduced availability of TRP and subsequent synthesis into serotonin than free TRP level itself, as TRP has to compete with the other LNAAs in order to bind to the carrier and be transported into the brain [54]. To avoid additional inconvenience and unnecessary exposure to radiation and intra-arterial cannulation (two extra PET scans), we did not use sham tryptophan depletion procedures to control for the nonspecific effects of ATD, namely, sedation, increased protein synthesis, increased competition across the BBB and nonspecific CNS effects.

Conclusions

In conclusion, we found a strong inverse correlation in the change in 5-HT_{1B} receptor availability in raphe nuclei and cerebral cortex using [¹¹C]P943 PET imaging and the ATD procedure. Our findings show the ability to measure reciprocal changes in endogenous serotonin in neural networks of healthy human subjects using ATD. These data are consistent with the existing hypothesis that raphe serotonin modulates cortical serotonin release via projecting neurons. [¹¹C]P943 is a suitable radioligand to study neural synaptic serotonin neurotransmission and provides mechanistic insight into physiologic functions and psychiatric diseases pertinent to the serotonin system. However, interpretation of such 5-HT intervention studies must account for the autoreceptor effects on cortical binding. Future studies using these methods may enhance our understanding of the role of the 5-HT_{1B} receptor in the pathophysiology of several complex mental processes including MDD. Particular applications of [¹¹C]P943 PET imaging with ATD or drug challenge include studies to more fully understand individual differences in neural serotonin transmission. Discoveries in this area may provide insight into differential human response to treatment, prediction of treatment efficacy, identification of different mood disorder phenotypes, and precision medicine for mood disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:

$[^{11}\text{C}]\text{P943}$ binding distribution and the raphe nuclei region of interest (ROI) placement.

The raphe ROI (green) was defined manually on the (a) MR template in Montreal Neurological Institute space to include the dorsal raphe nuclei in the dorsal brainstem along the periaqueductal grey on eight transverse slices, and then transferred to the registered (b) BP_{ND} parametric image of each subject. The highest binding was observed in the pallidum.

Figure 2:
Correlation of ΔBP_{ND} in the raphe nuclei with: a) temporal cortex ΔBP_{ND} and b) thalamus ΔBP_{ND} .

Table 1.

Study parameters

	pre-ATD	post-ATD	<i>p</i> -value
Specific activity at the end of synthesis (MBq/nmol)	335 ± 80	323 ± 137	0.794
Specific activity at the time of injection (MBq/nmol)	172 ± 41	177 ± 81	0.893
Injection dose (MBq)	641 ± 68	652 ± 42	0.799
Injection mass (µg)	1.7 ± 0.4	1.8 ± 0.7	0.557
Plasma free fraction of [¹¹ C]P943 (%)	6.0 ± 1.1	5.4 ± 1.2	0.176
Serum free TRP level (µg/mL)	3.2 ± 0.7	0.9 ± 0.6	<0.0001

n = 5

Values are mean ± standard deviation

ATD: acute tryptophan depletion

TRP: tryptophan

Table 2.*BP*_{ND} and *BP*_{ND} of [¹¹C]P943 before and after ATD

Regions	pre-ATD <i>BP</i> _{ND}	post-ATD <i>BP</i> _{ND}	<i>BP</i> _{ND} (%)	<i>p</i> -value
Average cortex	0.96 ± 0.27	0.89 ± 0.22	-6 ± 14	0.428
Frontal	1.04 ± 0.34	0.93 ± 0.23	-8 ± 16	0.352
Temporal	0.87 ± 0.21	0.82 ± 0.20	-6 ± 14	0.442
Parietal	0.88 ± 0.26	0.83 ± 0.24	-4 ± 13	0.478
Occipital	0.96 ± 0.19	0.97 ± 0.23	+1 ± 11	0.869
Calcarine	1.34 ± 0.30	1.28 ± 0.37	-5 ± 11	0.510
Anterior cingulum	1.07 ± 0.26	0.98 ± 0.21	-7 ± 17	0.397
Posterior cingulum	0.43 ± 0.17	0.45 ± 0.18	+10 ± 23	0.688
Insula	0.92 ± 0.20	0.83 ± 0.17	-9 ± 13	0.219
Hippocampus	0.48 ± 0.13	0.44 ± 0.12	-6 ± 17	0.452
Hypothalamus	0.98 ± 0.14	0.98 ± 0.31	-2 ± 19	0.981
Amygdala	0.87 ± 0.29	0.74 ± 0.14	-10 ± 23	0.372
Pallidum	2.00 ± 0.52	1.83 ± 0.60	-8 ± 23	0.404
Caudate	0.41 ± 0.21	0.40 ± 0.20	0 ± 15	0.857
Putamen	0.98 ± 0.21	0.93 ± 0.23	-5 ± 13	0.406
Thalamus	0.49 ± 0.10	0.45 ± 0.11	-8 ± 9	0.155
Raphe	0.50 ± 0.15	0.57 ± 0.10	+18 ± 20	0.117

n = 5

Values are mean ± standard deviation.

*BP*_{ND}: non-displaceable binding potential, estimated by multilinear reference tissue model 2 (*t** = 20 min) using modified cerebellar region of interest as a reference region

ATD: acute tryptophan depletion

Average cortex is a combination of frontal, temporal, parietal and occipital regions of interest.

Table 3.Correlation of BP_{ND} in the raphe nuclei with the other regions of interest

Regions	ρ	p -value
Average cortex	-0.96	0.011 *
Frontal	-0.90	0.039 *
Temporal	-0.98	0.003 * [†]
Parietal	-0.98	0.004 * [†]
Occipital	-0.98	0.004 * [†]
Calcarine	-0.85	0.070
Anterior cingulum	-0.85	0.066
Posterior cingulum	0.30	0.622
Insula	-0.92	0.028 *
Hippocampus	-0.43	0.469
Hypothalamus	-0.85	0.070
Amygdala	-0.25	0.683
Pallidum	-0.76	0.135
Caudate	-0.49	0.398
Putamen	-0.81	0.094
Thalamus	-0.95	0.012 * [†]

 $n = 5$ ρ Spearman correlation coefficient* $p < 0.05$ (unadjusted for multiple comparisons)[†] $p < 0.05$ (adjusted for multiple comparisons using False Discovery Rate)

Average cortex is a combination of frontal, temporal, parietal and occipital regions of interest and was not included in the FDR correction.

Table 4.Correlation of BP_{ND} between different versions of raphe regions of interest (ROIs) and other selected ROIs

	Raphe		Raphe_v2		Raphe_v3		Raphe_v4		Raphe_v5	
Volume (mL)	1.3		2.0		3.5		0.8		0.5	
Shape	slim, irregular		slim, irregular		wide, rectangular		intermediate		slim, rectangular	
Regions	<i>rho</i>	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>	<i>p</i> -value	<i>rho</i>	<i>p</i> -value
Average cortex	-0.96	0.011 *	-0.96	0.011 *	-0.79	0.113	-0.86	0.061	-0.97	0.008 *
Frontal	-0.90	0.039 *	-0.94	0.016 *	-0.83	0.084	-0.86	0.061	-0.92	0.029 *
Temporal	-0.98	0.003 *	-0.91	0.032 *	-0.68	0.209	-0.81	0.097	-0.99	0.002 *
Parietal	-0.98	0.004 *	-0.97	0.007 *	-0.75	0.145	-0.81	0.096	-0.98	0.002 *
Occipital	-0.98	0.004 *	-0.88	0.050 *	-0.63	0.253	-0.79	0.115	-0.96	0.009 *
Insula	-0.92	0.028 *	-0.98	0.004 *	-0.87	0.055	-0.88	0.046 *	-0.92	0.027 *
Thalamus	-0.95	0.012 *	-0.99	0.002 *	-0.87	0.055	-0.91	0.030 *	-0.93	0.024 *

 $n = 5$

v2 to v5 denotes different versions of the raphe nuclei ROI definition.

rho Spearman correlation coefficient

Slim shape denotes with longer anterior-posterior diameter than right-left diameter.

Wide shape denotes shorter anterior-posterior diameter than right-left diameter.

Irregular shape denotes being contoured along the periaqueductal grey.

* $p < 0.05$ (unadjusted)

Average cortex is a combination of frontal, temporal, parietal and occipital regions of interest.