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Examining the Underpinnings of Loudness Dependence of Auditory Evoked Potentials with Positron Emission Tomography

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Abstract

Loudness dependence of auditory evoked potentials (LDAEP) has long been considered to reflect central basal serotonin transmission. However, the relationship between LDAEP and individual serotonin receptors and transporters has not been fully explored in humans and may involve other neurotransmitter systems. To examine LDAEP's relationship with the serotonin system, we performed PET using serotonin-1A (5-HT_{1A}) imaging via [^{11}C]CUMI-101 and serotonin transporter (5-HTT) imaging via [^{11}C]DASB on a mixed sample of healthy controls (n=4: 4 females, 0 males), patients with unipolar (MDD, n=11: 4 females, 7 males) and bipolar depression (BD, n=8: 4 females, 4 males). On these same participants, we also performed electroencephalography (EEG) within a week of PET scanning, using 1000 Hz tones of varying intensity to evoke LDAEP. We then evaluated the relationship between LDAEP and 5-HT_{1A} or 5-HTT binding in both the raphe (5-HT_{1A})/midbrain (5-HTT) areas and in the temporal cortex. We found that LDAEP was significantly correlated with 5-HT_{1A} positively and with 5-HTT negatively

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in the temporal cortex ($p<0.05$), but not correlated with either in midbrain or raphe. In males only, exploratory analysis showed multiple regions in which LDAEP significantly correlated with 5-HT_{1A} throughout the brain; we did not find this with 5-HTT. This multimodal study partially validates preclinical models of a serotonergic influence on LDAEP. Replication in larger samples is necessary to further clarify our understanding of the role of serotonin in perception of auditory tones.

Keywords

LDAEP; electroencephalography; auditory evoked potential; serotonin 1A; serotonin transporter; positron emission tomography

1. Introduction

Psychiatric disorders, including mood disorders such as major depressive disorder (MDD) and bipolar disorder (BD), cause immense global burden. In 2013, MDD and BD were the 2nd and 17th leading causes of years lost to disability, respectively (Collaborators G.B.o.D.S., 2015). A physiological understanding of these disorders is critical to providing effective care.

One of the principal leads into mood disorder pathophysiology is the serotonin system (Belmaker and Agam, 2008; Sobczak et al., 2002). Serotonin is understood to be involved in auditory perception—layer IV of the primary auditory cortex is richly innervated with both serotonergic fibers from the raphe nucleus (Lewis et al., 1986; Morrison and Foote, 1986) and ascending projections from the auditory pathway (Winer, 1984). Therefore, examining this region may provide a window into the serotonergic system. For example, hearing a tone results in an auditory evoked potential as measured by electroencephalography (EEG). If the volume of a tone is increased, it results in a proportional increase in evoked potential, called the loudness dependence of the auditory evoked potential (LDAEP). LDAEP is hypothesized to be linked to basal levels of central serotonergic transmission in the auditory cortex due to serotonin's modulatory effects on the cortex (Hegerl and Juckel, 1993). Dipole source analyses have supported involvement of auditory cortex in generation of LDAEP (Hegerl et al., 1994).

This hypothesis has received some support using various methods. Notably, in cats, when serotonin transmission was blocked via serotonergic modulators applied directly to the brainstem, a larger LDAEP was measured, and when serotonin transmission was increased, a smaller LDAEP was measured (Juckel et al., 1999; Juckel et al., 1997). In humans, a reduced LDAEP with alcohol administration (which has serotonergic agonist activity) (Hegerl et al., 1996) and an inverse correlation of LDAEP with a serotonin syndrome scale have been observed (Hegerl et al., 1998). Moreover, LDAEP has been found to correlate with depressive symptoms in people with a history of childhood trauma, which is suggested to reduce central serotonin transmission (Lee and Park, 2016) and has also been found to vary with mood states in BD (Lee et al., 2012), suggesting that LDAEP may be connected to serotonergic process that cause SSRIs to carry a risk for mania. In addition, studies with single photon emission computed tomography (SPECT) suggest a correlation between

serotonin transporter (5-HTT) binding in the midbrain and LDAEP, though findings have been mixed across radiotracers; two studies showed a negative correlation (Lee et al., 2011; Pogarell et al., 2008) while one showed a positive correlation (Pogarell et al., 2004). Pretreatment LDAEP has also been shown to be related to selective serotonin reuptake inhibitor (SSRI) treatment response in individuals with depression (Gallinat et al., 2000; Hegerl et al., 2001; Jaworska et al., 2013; Lee et al., 2015; Park et al., 2012) and in other disorders with agents such as lithium (Hegerl et al., 1988; Hegerl et al., 1992). These studies have consistently found an association between a large LDAEP, hypothesized to reflect low central serotonin, and successful response to treatment.

However, there has been evidence in humans that does not support the relationship between LDAEP and central serotonin transmission such as the lack of change in LDAEP with acute tryptophan depletion, which decreases central serotonin (O'Neill et al., 2008b) and the fact that LDAEP appears not to change with SSRI treatment (Gallinat et al., 2000; Guille et al., 2008). In addition, there may be other neurotransmitter systems involved in the LDAEP signal (O'Neill et al., 2007; O'Neill et al., 2008a); LDAEP has been found to correlate with illness duration in schizophrenia, which may involve dopamine (Park et al., 2015). Interestingly, LDAEP has also been positively associated with serum brain derived neurotrophic factor (BDNF) which has been posited to be altered in depression, and negatively associated with serum triglycerides which has been thought to be related to suicide risk (Park et al., 2014a; Park et al., 2014b).

Positron emission tomography (PET) imaging may provide a useful means to directly assess LDAEP's relationship with the serotonergic system. PET has several advantages over SPECT imaging including increased sensitivity (Rahmim and Zaidi, 2008) and the ability to utilize ^{11}C and ^{18}F for radiolabeling, which are more chemically similar to endogenous compounds than the heavier nuclides required for SPECT imaging. Comparing quantitative PET signals with LDAEP can help clarify the relationship of LDAEP with serotonin receptor and transporter levels in humans. While this does not directly answer whether LDAEP reflects serotonin transmission, it allows exploration of which receptors or transporters are associated with the signal, which can provide valuable information about the underpinnings of this EEG signal.

In the present study, we performed both EEG and PET imaging with two radiotracers—one that binds to serotonin-1A receptors (5-HT_{1A}) and one that binds to serotonin transporters (5-HTT)—to fully explore LDAEP's relationship with the serotonergic system. We included healthy control participant data, as well as data from both patients with MDD and BD before and after lithium treatment, as these populations combined with the effect of treatment would be expected to yield a wide range of serotonin system states that could help assess the validity and generalizability of a relationship between 5-HT_{1A}, transporter and LDAEP.

We examined two regions of interest (ROIs) per PET radiotracer: the raphe nucleus (5-HT_{1A}) or midbrain (5-HTT), and the temporal cortex (both 5-HT_{1A} and 5-HTT - which includes superficial structures (e.g. the primary auditory cortex), but excludes deep structures (e.g. the hippocampus and amygdala)—see Figure 1), based on evidence from previous studies in humans and cats of that indicate the temporal cortex as the likely source

of LDAEP (Hegerl et al., 1994). Our inclusion of the raphe nucleus and midbrain stems from aforementioned studies in which serotonergic agents applied directly to the brainstem of cats caused changes in LDAEP (Juckel et al., 1999; Juckel et al., 1997). In addition, we have found in our own work greater raphe nucleus 5-HT_{1A} density in individuals with MDD relative to controls (Miller et al., 2009; Parsey et al., 2010; Parsey et al., 2006b) and BD (Lan et al., 2013; Sullivan et al., 2009) (though we have not replicated a difference in BD in our most recent study (Ananth et al., Submitted), which could indicate an overall deficit in central serotonin transmission, as 5-HT_{1A} autoreceptors reduce overall serotonin output from the raphe nucleus (Blier et al., 1998) (though other neurotransmitter systems could also be affected, such as the noradrenergic system (Szabo and Blier, 2001)). This may have a direct effect on LDAEP. For 5-HTT, we examined the midbrain, encompassing the superior and inferior colliculi, rather than just the raphe, as 5-HTT does not have the same somatodendritic distribution as 5-HT_{1A} (Miller et al., 2013). Moreover, prior SPECT studies found a correlation of 5-HTT with LDAEP in the midbrain (Lee et al., 2011; Pogarell et al., 2008; Pogarell et al., 2004).

We hypothesized that LDAEP would correlate positively with 5-HT_{1A} in the raphe nucleus and temporal cortex, as greater 5-HT_{1A} in the raphe nucleus will halt serotonin transmission, which would be reflected by post-synaptic 5-HT_{1A} upregulation and a larger LDAEP in the temporal cortex. We also hypothesized that LDAEP would correlate negatively with 5-HTT in the midbrain and temporal cortex, as a greater LDAEP should correspond with reduced serotonin in the synaptic cleft and hence, less serotonin transporters for reuptake.

Given the known sex differences in MDD and BD (Arnold, 2003; Diflorio and Jones, 2010; Karanti et al., 2014), as well as in LDAEP (Oliva et al., 2011) it is also important to determine whether sex can influence the PET/EEG relationship. Further, sex differences have been seen in both 5-HTT and 5-HT_{1A} binding (Cannon et al., 2013; Jovanovic et al., 2008), although conflicting evidence exists for sex effects in 5-HTT especially when considering PET outcome measure, radiotracer used, and population studied (Martinez et al., 2013; Miller et al., 2013). Further, a previous study examining the correlation between SPECT-derived 5-HTT binding and LDAEP found that the correlation was much stronger in females than in males (Pogarell et al., 2008), though no explanation was given for this finding. Similar lines of conflicting evidence exist for age effects in both 5-HTT/5-HT1A (Dillon et al., 1991; Karrer et al., 2019; Parsey et al., 2002; van Dyck et al., 2000). Finally, the temporal lobe has connections across the brain, including such areas as the cingulate cortex, frontal cortex, and occipital complex (Bajada et al., 2017)—while it is likely that LDAEP originates in temporal cortex, it is possible that serotonin receptor and transporter distribution elsewhere is associated with the signal due to these connections. Therefore, as an exploratory measure, we analyzed sex and age differences in the relationship between LDAEP and both 5-HT_{1A} and 5-HTT across all regions of the brain.

2. Methods

2.1 Participants

The participants in this study are a subset of those acquired under a protocol aimed at elucidating lithium's mechanism of action in BD (R01MH090276; PI: Ramin Parsey).

Importantly, this study also included healthy control participants and participants with MDD. Therefore, this study utilizes a mixed-population sample with PET imaging pre- and post-treatment for MDD and BD participants.

The Institutional Review Boards of Columbia University Medical Center, the New York State Psychiatric Institute, Yale University, and Stony Brook Medicine approved the study. All participants provided informed written consent. Though 27 individuals received both PET and EEG (one participant was removed due to not receiving a PET scan), four were removed due to noisy EEG data (see section 1.2.5), in which evoked potential peaks could not be discerned. The resulting sample comprised 23 adults (see Table 1). Of these, 11 met Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for MDD, 8 met DSM-IV criteria for BD, and 4 did not meet criteria for any axis I disorders. Diagnosis was made based on a structured clinical interview for DSM-IV Axis I Disorders (SCID IV), the 17-item Hamilton Depression Rating Scale (HDRS-17), and the Beck Depression Inventory (BDI). Inclusion criteria for participants with BD and MDD included: age 18–70 years, in a current depressive episode as defined by DSM IV criteria for MDD or BD, a score of at least 15 on the HDRS-17, able to tolerate a 3-week washout of current antidepressant treatment prior to the start of the study, able to stop all psychotropic and other drugs likely to interact with 5-HTT or 5-HT_{1A}, and be medication free for 3 weeks (most medications, neuroleptics), 6 weeks (fluoxetine), or 3 months (serotonin depleting drugs such as reserpine) prior to the start of the study, and able to be off anti-coagulant treatment, with the exception of aspirin, for 10 days before PET imaging (short-acting benzodiazepines for distressing anxiety/insomnia allowed up to 24 hours prior to each PET scan). Exclusion criteria included: lack of capacity to consent, already on an effective medication for their depression, other major psychiatric disorders such as schizophrenia, drug or alcohol dependence within six months, anorexia or bulimia nervosa within a year (except for bulimia nervosa, non-purging type plus a normal BMI, which was not exclusionary), intravenous drug use within 5 years, or MDMA (ecstasy) use more than 15 times in the past 10 years or any MDMA use in the past month, a first degree relative with schizophrenia if the participant was less than 33 years old (mean age of onset of schizophrenia plus two standard deviations), significant physical illness that may affect the brain or serotonergic system, significant suicidal ideation with intent before or during medication washout, electroconvulsive therapy (ECT) within six months, pregnancy, currently lactating, or abortion within past two months, metal implants, pacemaker, shrapnel, metal prostheses, medicinal patch that cannot be removed (contraindicated for MRI, which is needed for regional delineation), neurological disease or loss of consciousness for more than a few minutes (asked of patients to screen for neurological damage), and hearing loss greater than 20 decibels (dB) or inter-aural hearing difference of 10 dB as measured by screening audiometry. Demographic data can be found in Table 1. Of the 23 participants with usable PET and EEG data, 2 participants with BD and 5 with MDD received post-treatment (lithium) PET and EEG, in addition to pre-treatment scans. These comprised 3 females and four males. These 7 extra scans were included, bringing the total number of PET/EEG scans to 30. Pre/post treatment status was accounted for in statistical analyses to include a larger range of serotonergic states, which would yield more information on the PET-LDAEP relationship.

2.2 Experimental Protocol

Participants with MDD or BD who were on ineffective medication underwent a medication free period, as described above, following a washout from any antidepressant treatment. Following the medication free period, participants received a structural magnetic resonance imaging (MRI) scan, PET scans with both radiotracers on the same day, and EEG, all within a week of each other. Participants were then started on treatment with lithium and titrated to a therapeutic plasma level of 0.8 – 1.2 mEp/l. After 6–10 weeks of lithium therapy, participants received repeat MRI, PET, and EEG. As described above, seven PET/EEG studies in our sample were post-treatment scans.

2.3 Radiochemistry and Input Function Measures

23 participants (11 MDD, 8 BD, 4 control) received [^{11}C]CUMI-101 scans, and 17 (7 MDD, 6 BD, 4 control) received [^{11}C]DASB scans (see Figure 1). [^{11}C]CUMI-101 and [^{11}C]DASB were synthesized as previously described (Belanger et al., 2004; Kumar et al., 2007). For both radiotracers, arterial samples were collected automatically for the first 6 minutes and manually thereafter throughout the PET scans. Samples were measured for radioactivity using a 1480 Wizard 3M automatic gamma counter (Wallac, Turku, Finland). Six arterial samples were also analyzed using high-pressure liquid chromatography to assess the percentage of parent compound—these were fit to a Hill function for [^{11}C]CUMI-101 or a bi-exponential function for [^{11}C]DASB (Wu et al., 2007). Plasma radioactivity counts were multiplied by the percentage of parent compound to obtain the metabolite-corrected arterial input function, which was fit with a line before the peak and a sum of 3 exponentials after the peak (Milak et al., 2010; Ogden et al., 2007).

For 11 scans (5 MDD, 3 BD, 3 control), a less-invasive quantification method known as simultaneous estimation (SIME) was used. Briefly, this method works by estimating input function fit parameters, assumed to be common to all brain regions, simultaneously with kinetic parameters from several ROIs with varying kinetics (Ogden et al., 2010). A single blood sample, either arterial (1) or venous (10), was used to anchor the arterial input function. We initially validated this technique using a single arterial sample (Ogden et al., 2010), and have recently validated the use of a venous sample for both [^{11}C]CUMI-101 and [^{11}C]DASB (Bartlett et al., 2018)

2.4 Image Acquisition and Analysis

MRI was performed on either a Siemens Magnetom Skyra or Prisma scanner at Stony Brook University. The parameters are as follows: for the Skyra, relaxation time 2300 ms, echo time 2.98 ms, T1 900 ms, flip angle 9 degrees, slice thickness 1 mm, resolution 1mm × 1mm × 1mm; for the Prisma, relaxation time 2300 ms, echo time 3.05 ms, T1 900 ms, flip angle 9 degrees, slice thickness 0.87 mm, resolution 0.9mm × 0.9mm × 0.9mm. PET was performed with an ECAT EXACT HR+ (Siemens/CTI, Knoxville, Tennessee). [^{11}C]CUMI-101 emission data were collected for 120 minutes after bolus radiotracer injection as 20 frames of increasing duration (3 × 20 sec, 3 × 1 min, 3 × 2 min, 2 × 5 min, 9 × 10 min). [^{11}C]DASB emission data were collected for 100 minutes after bolus radiotracer injection as 19 frames of increasing duration (3 × 20 sec, 3 × 1 min, 3 × 2 min, 2 × 5 min, 8 × 10 min). Image analysis was performed using in-house Matlab 2012b software (The Mathworks, Natick,

Massachusetts), with extensions to Functional Magnetic Resonance Imaging of the Brain's (FMRIB's) Linear Image Registration Tool (FLIRT v.5.2 (Jenkinson and Smith, 2001)), Brain Extraction Tool v1.2 (Smith, 2002), and Statistical Parametric Mapping (SPM5, (Ashburner and Friston, 2005)). Frame-by-frame rigid body registration was performed to a reference frame to correct for subject motion using FLIRT, followed by co-registration of the average PET image to the subject's MRI, also using FLIRT, to generate eight possible coregistrations. The eight possible coregistrations were estimated as previously validated using different combinations of the PET frames (e.g. the mean over the early frames vs the late frames) and MRI image (e.g. skull-stripped vs whole field of view) as the sources and targets, respectively (DeLorenzo et al., 2009). Each option was visually inspected and the optimal coregistration was selected. As previously shown, PET data with limited whole-brain spatial information, resulting from varying biodistributions across PET radiotracers, can be difficult to accurately coregister and therefore, benefits from visual inspection/ selection with a validated procedure (DeLorenzo et al., 2009).

As previously described, the temporal cortex and midbrain ROIs were previously hand drawn on a set of 18 template MRI scans based on brain atlases and published reports (Duvernoy, 1991; Killiany et al., 1997; Talairach and Tournoux, 1988). These templates were warped to each subjects' MRI using the Automatic Registration Toolbox (ART) (Ardekani et al., 2005) to generate probabilistic temporal cortex and midbrain ROIs in subject-space. Because the raphe nucleus is not visible on MRI scans, a separate [^{11}C]WAY100635 (which binds to serotonin-1A) study in 52 healthy controls was used to delineate the raphe nucleus ROI. In that study, voxel binding maps were calculated for these 52 participants and averaged in a standard space. A thresholding technique was used within the brainstem to identify the raphe nucleus ROI, which has higher binding than the background, in the standard space average image. This average image was associated with a high-resolution MRI image in the same space. The raphe nucleus ROI in standard space, which comprised 279 voxels (2232 mm^3), was used as an atlas for the current study; it was warped to the individual subject's PET image using Advanced Normalization Tools (ANTs) (Avants et al., 2008; Avants et al., 2011) through the individual's MRI (Delorenzo et al., 2013). The activity within the raphe nucleus, midbrain, and temporal cortex ROIs were separately averaged within each PET frame to generate time activity curves for each region (see Figure 1 for ROI placement).

We have previously determined likelihood estimation in graphical analysis (LEGA) (Ogden, 2003) as an optimal modeling technique for both [^{11}C]CUMI-101 (Milak et al., 2008) and [^{11}C]DASB (Ogden et al., 2007) PET data. Using the time activity curves and arterial input function, either calculated directly or estimated using SIME as described above, we applied LEGA to generate binding estimates. As previously validated, BP_F was the outcome measure used for [^{11}C]CUMI-101, which is calculated as $(V_T - V_{ND})/f_P$, where V_T is the region of interest's total distribution volume, V_{ND} is the reference region's distribution volume (assumed to contain only nonspecific binding; cerebellar grey matter used here), and f_P is the free fraction of radiotracer in the plasma (Innis et al., 2007). For [^{11}C]DASB, we used the outcome measure V_T/f_P, as previously validated, because there is no accepted reference region to calculate V_{ND} for this tracer (Parsey et al., 2006a). A priori ROIs included the temporal cortex and raphe nucleus for [^{11}C]CUMI-101 and the temporal cortex

and midbrain for [^{11}C]DASB. Standard errors for each PET measure were calculated using a bootstrapping technique that incorporates errors in fitting the brain data (Ogden and Tarpey, 2006) and were used for all statistical analyses.

2.5 Electroencephalography and Loudness Dependent Auditory Evoked Potentials

All EEG data were acquired by a single operator. EEG was acquired with a Biosemi ActiveTwo 32 channel system. Participants had their hearing tested and were then seated in a comfortable chair in a sound-attenuated room. Participants listened to 1000 Hz tones of 60, 70, 80, 90, and 100 dB in pseudorandomized order. Each tone lasted for 40 milliseconds (ms), with an interstimulus interval between 1500 and 2100 ms. Participants listened to five blocks of 100 tones each, for a total of 500 tones. EEG was collected using the 10–20 system via ActiView software and processed using BrainVision Analyzer 2. Data was rereferenced offline to the average activity recorded from the left and right mastoid bones, and band-pass filtered from 0.1 to 30 Hz, with a 24 dB/octave rolloff (Widmann et al., 2015). Ocular activity was recorded using electrodes above and below the right eye and at the outer canthi of both eyes—ocular artifacts were corrected using a regression-based approach (Gratton et al., 1983). EEG data were segmented into 1000 ms epochs and were further examined for artifacts using an automated procedure. Voltage greater than 50 μV between sample points, a voltage difference greater than 175 μV within a segment, or a maximum voltage of less than 0.50 μV within 100 ms intervals were counted as artifacts. One participant had 20 segments per sound level removed by artifact rejection in their post-treatment EEG; the rest had three or less segments removed.

EEG data at each sound intensity were averaged and baseline corrected for 200 ms before each epoch, yielding five waveforms for each participant. These waveforms were examined at the Cz position, as previous studies have shown this to be the most effective position for measuring LDAEP (Gallinat et al., 2000; Simmons et al., 2011). The largest negative peak between 50 and 200 ms was detected automatically and defined as N1, while the largest positive peak between 150 and 300 ms was detected automatically and defined as P2. LDAEP was defined as the slope of the linear regression of the difference between P2 and N1 at each sound intensity (see Figure 2). Averaged waveforms were manually inspected and removed if significant noise was still present and local peaks could not be discerned—six EEGs from four participants (including 2 post-treatment scans) were removed based on this criterion., as mentioned in section 1.2.1. Standard errors for LDAEP were used in all statistical analyses and were calculated as the error of the regression slope, i.e.:

$$\frac{\sqrt{\sum (y_i - \hat{y}_i)^2/(n-2)}}{\sqrt{\sum (x_i - \bar{x})^2}}$$

where $y_i - \hat{y}_i$ is the residual of an N1–P2 peak-to-peak amplitude from the regression line, n is the number of data points in the regression (i.e. 5 data points, one for each decibel level), and $x_i - \bar{x}_i$ is the difference of each decibel level at each data point from the mean decibel level ($\bar{x}$ is equal to 80 dB in all cases).

2.6.1 Statistical Analysis: A priori hypothesis testing—Statistical analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC). To determine the relationship between LDAEP and 5-HT_{1A}/5-HTT binding, separate linear mixed models were constructed with either 5-HT_{1A} or 5-HTT in the a priori regions (5-HT_{1A}: raphe and temporal cortex and 5-HTT: midbrain and temporal cortex) as the model outcome and LDAEP and region as fixed factors. The interaction between LDAEP and region was also examined. Standard errors for both PET measures and LDAEP were included. To account for repeated measures (pre- and post-treatment scans in 7 of the participants), the relationship between pre- and post-treatment scans within participant was modeled as a random factor. LDAEP and PET measures were log-transformed to meet model assumptions in all statistics.

2.6.2 Statistical Analysis: Exploratory Analyses—The effects of sex, age, and pre/post treatment status on the relationships between 5-HT_{1A}/5-HTT and LDAEP were examined with linear mixed models as above, but with independent linear mixed models fit for each region across all individual brain regions in our atlas: anterior cingulate cortex, amygdala, cerebellum, cingulate cortex, dorsal caudate, dorsolateral prefrontal cortex, dorsal putamen, entorhinal cortex, hippocampus, inferior anterior cingulate cortex, insula, medial prefrontal cortex, midbrain, occipital cortex, orbitofrontal cortex, parietal cortex, parahippocampal gyrus, raphe nucleus, superior anterior cingulate cortex, temporal cortex, thalamus, uncus, ventral striatum, and cerebellar white matter. Individual linear mixed models were fit for each region, rather than grouping regions together to avoid issues in fitting the variance-covariance structure for each of the 25 regions. Pre/post-treatment status was included as a fixed factor to control for any differences in PET binding pre- and post-treatment. Rather than an interaction with region as in the *a priori* models, an interaction with sex was considered, as well as age. Post-hoc analyses exploring this sex effect were further conducted (LDAEP-PET relationship in males, females, and comparing this relationship in males vs females).. The significance of main effects, interactions, and post-hocs in all models were determined via Type III F-tests based on the fitted linear mixed effect models. Bonferroni correction was performed for the 25 regions examined across both tracers (50 total linear mixed models tested), with a further penalization for the three post-hoc analyses conducted per linear mixed model (LDAEP-PET relationship in males, females, and males vs females), yielding a total correction for 150 exploratory statistical tests (Bonferroni correction threshold: $p<0.0003$).

3. Results

3.1 A priori hypothesis testing

LDAEP was not significantly related to 5-HT_{1A} across the *a priori* regions temporal cortex and raphe nucleus ($p=0.22$). The correlation between 5-HT_{1A} and LDAEP was not significantly different between the *a priori* regions ($p=0.38$). However, when examining post-hoc analyses, temporal cortex 5-HT_{1A} binding was significantly *positively* associated with LDAEP ($p=0.02$, $b=0.33$), whereas raphe nucleus 5-HT_{1A} was not significantly associated with LDAEP ($p=0.69$, $b=0.11$). The model accounted for 77.0% of the variance in 5-HT_{1A}.

LDAEP was related to 5-HTT at a trend level across the *a priori* regions temporal cortex and midbrain (p=0.057). The correlation between 5-HTT and LDAEP was not significantly different between the *a priori* regions (p=0.40). However, when examining post-hoc analyses, temporal cortex 5-HTT binding was significantly *negatively* associated with LDAEP (p=0.01, b=−0.72), whereas midbrain 5-HTT was not significantly associated with LDAEP (p=0.18, b=−0.54). The model accounted for 68.7% of the variance in 5-HTT. P-values are reported uncorrected for *a priori* hypothesis testing (see Figure 3).

3.2 Exploratory Analyses

For full results of the exploratory whole-brain analyses examining the sex- and age-specific relationships of LDAEP by 5-HT_{1A}/5-HTT, see Supplemental Tables 1 and 2. In brief, the relationship between LDAEP and 5-HT_{1A}/5-HTT did not significantly differ between males and females (p-values > 0.0003, Bonferroni threshold for the 150 = 25 regions × 2 tracers × 3 post-hoc tests). However, there was a significant relationship between LDAEP and 5-HT_{1A} in *males only* for the brain regions: temporal cortex, anterior cingulate cortex, medial prefrontal cortex, occipital cortex, orbitofrontal cortex, and parietal cortex (p's <= 0.0003; Supplemental Table 1). In females, LDAEP and 5-HT_{1A} were not significantly related in any region (p's>0.0003). These results indicate a global pattern of 5-HT_{1A} and LDAEP correspondence in males (see Figure 4).

With 5-HTT, after Bonferroni correction, the relationship between LDAEP and 5-HTT was not significant in males or females in any region tested.

Across both 5-HT_{1A} and 5-HTT, the main effects of pre/post-treatment status, sex, and age were not significant (p-values>0.0003).

To investigate the effect of diagnosis, the above exploratory models were repeated including a diagnosis (HC, MDD, BD) model factor, whereby the main effect of diagnosis was not significant (p's = 0.09 to 0.96 for the 5-HT_{1A} model and p's = 0.13 to 0.61 for the 5-HTT model).

Because there were only n=4 healthy controls included and all healthy controls were female, we further repeated all *a priori* and exploratory analyses with only the MDD and BD participants. The *a priori* results were replicated where only post-hoc LDAEP by 5-HT_{1A}/5-HTT relationships were significant in the temporal cortex (p=0.03 for 5-HT_{1A} and p=0.02 for 5-HTT). As with all participants included, the relationship between 5-HT_{1A} and LDAEP was significant in several regions of the brain in males only (see Supplemental Tables 3 and 4). The null findings for LDAEP by 5-HT_{1A} in females, as well as for LDAEP by 5-HTT in males and females were replicated.

To avoid the additional statistical confounds that may result from repeated measures within participants, and to ensure the results would replicate, we repeated the analyses using only single, pre-treatment measurements for each participant (the n=7 post-treatment scans were excluded). Here, the a priori results were replicated where only post-hoc LDAEP by 5-HTT relationships were significant in the temporal cortex (p=0.02). However, the relationship between LDAEP and 5-HT_{1A} in the temporal cortex was weakened with the removal of

these scans (p=0.27), possibly due to a smaller sample size. The diffuse pattern of LDAEP × 5-HT_{1A} positive correlation was strengthened relative to the initial model that included both pre- and post-treatment scans, and the null findings for LDAEP by 5-HT_{1A} in females, as well as for LDAEP by 5-HTT in males and females were replicated (see Supplemental Tables 5 and 6).

One male participant had notably higher LDAEP and higher 5-HT_{1A} binding than other male participants. Sex-specific results remained unchanged when this participant was excluded; for example, in the temporal lobe in males, the estimated slope between 5-HT_{1A} and LDAEP decreased from 0.064 to 0.055, but the relationship was still significant (see Supplemental Table 7).

4. Discussion

The prospect of loudness dependence of auditory evoked potentials (LDAEP) as a marker of central serotonin transmission has existed for close to 30 years (Hegerl and Juckel, 1993). Evidence supporting the link between LDAEP and serotonin has been well established in animals (Juckel et al., 1999; Juckel et al., 1997). However, human studies have thus far been conflicting regarding LDAEP's relationship with serotonin transmission. For example, acute tryptophan depletion (O'Neill et al., 2008b) and SSRI administration (Gallinat et al., 2000) have not significantly altered LDAEP in humans, whereas LDAEP has been shown to predict response to SSRIs (Gallinat et al., 2000; Hegerl et al., 2001; Jaworska et al., 2013; Park et al., 2012). These conflicting results have thus obfuscated our understanding of LDAEP (O'Neill et al., 2008a). Part of the difficulty in examining the serotonergic basis of LDAEP in humans is that it is impossible to target specific aspects of the serotonin system in the same manner as animals—one cannot, for example, directly apply a serotonin-1A antagonist to a person's brainstem and observe the response. Compounding this difficulty is the sheer complexity of the serotonin system, which involves over 14 different receptors and 7 different subtypes (Pytliak et al., 2011). We cannot examine all receptors at once *in vivo* and thus, can only probe the relationship with individual receptors.

Our findings suggest that there is a correlation between LDAEP and serotonin receptor/transporter binding in the temporal cortex, but not in the midbrain/raphe nucleus. In addition, there is a sexually dimorphic pattern of correlation between LDAEP and serotonin receptor binding across many brain regions. Specifically, when including sex and age in our model, we found a sex dimorphism such that in *males only*, LDAEP showed significantly positive correlation with 5-HT_{1A} binding in various areas of the brain after Bonferroni correcting for 150 tests (adjusted p-value significance threshold: < 0.0003). It is possible that in an *a priori* analysis without such strict multiple comparisons correction more regions would show a significant positive correlation. Of all the regions, the *a priori* regions had the largest model coefficients in males, with 0.67 for raphe nucleus and 0.96 for temporal cortex. This means that for every unit change of 1 for LDAEP, binding potential for 5-HT_{1A} would change by 0.67 or 0.96, respectively. For the serotonin transporter, however, no significant relationships between LDAEP and 5-HTT were found in any brain region. Our findings were largely consistent when post-treatment scans and controls were removed from

the analysis, which suggests that including multiple measures on the same participant did not bias the results.

Importantly, the correlations between LDAEP and the serotonergic system were chiefly present in males and not females. Our lab has previously found that differences in raphe nucleus 5-HT_{1A} binding between people with and without MDD are significantly greater in males than in females (Kaufman et al., 2015; Pillai et al., 2018a). Moreover, we have found a high correlation of 5-HT_{1A} receptor binding across different brain regions in males, which may explain the correlation across many regions here. While this collinearity between brain regions is greater in controls, it is still present in depression (Pillai et al., 2018b). Therefore, it can be inferred that 5-HT_{1A} binding potential may not be directly related to LDAEP in areas outside the temporal cortex and raphe, but rather that LDAEP is associated with global changes in 5-HT_{1A} binding that occurs proportionately in several regions of the brain.

However, our finding that 5-HTT in the midbrain does not significantly correlate with LDAEP contradicts previous SPECT studies (Lee et al., 2011; Pogarell et al., 2008). It is unlikely that this is due to properties of the radiotracer, as a comparative study between 5-HTT tracers, including [^{11}C]DASB and [^{11}C]ADAM found the main difference to be the more rapid kinetics of the former (Huang et al., 2002). A key difference between our studies is that the previous SPECT studies examined healthy controls exclusively (Lee et al., 2011; Pogarell et al., 2008), while our study used a mixed sample of healthy control, MDD, and BD participants. Further investigation with a larger sample size and with pharmacological blocking studies of LDAEP, with agonists or antagonists of various serotonin receptors, would be necessary to further untangle sex-specific and receptor-specific dependencies of LDAEP and the serotonergic system. In addition, previous studies did not explicitly examine temporal cortex, which is the hypothesized source of LDAEP.

The mechanism behind the sexual dimorphism in LDAEP/5-HT_{1A} binding correlation is far from clear. While there have been sex differences documented in auditory evoked potentials (Bakos et al., 2016; Jalaei et al., 2019; Jalaei et al., 2017) and in serotonin receptor binding (Moses-Kolko et al., 2011), if 5-HT_{1A} were solely involved in producing LDAEP, the correlation should hold in spite of these differences. One possible hypothesis is that serotonin receptors are only partly responsible for generating LDAEP; it may be influenced by other neurotransmitter systems, or even different serotonin receptors. In this case, hormonal differences may alter serotonin receptor binding distributions (as has been shown in gonadectomized rats (Zhang et al., 1999)) which can then alter the association between 5-HT_{1A} and LDAEP.

Despite this, our exploratory finding of a significant correlation between 5-HT_{1A} binding and LDAEP in males suggests that there may be a mechanism by which serotonergic activity contributes to auditory potentials. Hegerl and Juckel proposed that LDAEP is related to low-level tonic (that is, slow and sustained) neurotransmission of serotonin to the primary auditory cortex, a hypothesis bolstered by dipole source analysis and prior studies showing decreased LDAEP with administration of serotonin agonists (Hegerl and Juckel, 1993). High levels of tonic activation are thought to decrease the level of cortical response due to lateral inhibition (Grossberg, 1984), i.e. the ability of an activated neuron to lower the activity of

surrounding neurons (Hartline and Ratliff, 1957). Therefore, greater basal serotonergic transmission would result in more lateral inhibition and thus, smaller amplitude changes in evoked potentials as sounds increase in loudness. Recent evidence supporting this has been found in mice: administration of the drug 5-Methoxy-N,N-demethyltryptamine (5-MeO-DMT), a nonselective 5-HT_{1A}/5-HT_{2A} agonist, was found to inhibit serotonin release and reduce low frequency cortical oscillations in the primary auditory, primary visual, and prefrontal cortices (Riga et al., 2016). This suggests that higher 5-HT_{1A} across the brain is related to lower tonic activation of the auditory cortex and a higher LDAEP upon phasic stimulation.

Evidence from rodent studies indicates that both 5-HT_{1A} and 5-HT_{2A} have strong modulatory effects on brain reactivity (Basura et al., 2008; Papesh and Hurley, 2016) and GABAergic transmission (Garcia-Oscos et al., 2015). In fact, while administering 5-meO-DMT in rodents caused a reduction in basal low frequency oscillations in auditory, prefrontal, and primary visual cortex, knocking out the 5-HT_{2A} receptors selectively prevented this reduction in auditory cortex, but not in prefrontal or primary visual cortices. This suggests that 5-HT_{2A} may play a larger role for the auditory cortex and LDAEP in particular. In addition, a polymorphism in the 5-HT_{1B} receptor has been linked to higher LDAEP on the left side of the brain (Juckel et al., 2008). A pharmacological blocking study of LDAEP, with agonists or antagonists of various serotonin receptors, would be necessary to determine if one or the other receptor contributed more to the signal. In addition, our findings do not rule out the influence of other neurotransmitter systems, such as dopamine, on LDAEP.

To consolidate our findings with the serotonergic hypothesis of LDAEP, we hypothesize the following. High levels of serotonin levels result in tonic activation and lower cortical reactivity. This is reflected by high 5-HTT levels, which increase in response to serotonin in the synaptic cleft. Conversely, 5-HT_{1A} will be low, as large amounts of serotonin will cause a homeostatic decrease in post-synaptic receptor levels. Presynaptic 5-HT_{1A} is also expected to be low, as decreased autoreceptor availability will disinhibit the raphe nucleus, resulting in greater serotonin release—however, given the cortical source of LDAEP the correlation is not as strong. Meanwhile, low levels of serotonin, reflected by low 5-HTT, result in a homeostatic increase in 5-HT_{1A} and a corresponding increase in reactivity. It is important to note that, as stated above, there is evidence in preclinical models that 5-HT_{1A} may in fact have a direct effect on tonic 5-HT transmission in addition to being reflective of 5-HT levels, though receptor blocking studies will be necessary to determine whether this is true in humans.

Sample size was the main limitation in this study. While 27 participants received both EEG and PET scans, only 23 participants' data passed our quality control protocols, with 7 of these 23 participants receiving both pre and post-lithium treatment scans, bringing the final sample size for LDAEP/PET data to 30 scan sets. While limited in the number of post-treatment scans (n=7), post-treatment scans were included to represent a range within the serotonin system and provide further information on the nature of the relationship between LDAEP and serotonin receptor/transporter binding. That the relationship between 5-HT_{1A}/5-HTT and LDAEP was preserved even when post-treatment and control participants were

removed further supports its validity. Moreover, main effects of pre/post treatment status were not significant. Importantly, the linear mixed effects models controlled for the repeated measures with fixed and random effects to ensure the models were not biased. Another limitation was the limited number of healthy controls available for analysis with both LDAEP and PET data, and the fact that all of our controls were female. However, the results remained consistent when controls were removed from the analysis. Given our recent finding that the association between raphe nucleus 5-HT_{1A} and post-synaptic 5-HT_{1A} binding is reduced in MDD relative to healthy controls (Pillai et al., 2018b), however, it will be important to assess differences in 5-HT_{1A}-LDAEP relationships across populations in a larger study. While a future study with a large control group would be invaluable, the association of LDAEP with 5-HT_{1A} in a mixed sample such as this is still an important finding that can inform future studies. Finally, the small sample size limited our power to assess sex differences—while our exploratory analysis indicated diffuse correlations between LDAEP and 5-HT_{1A} in males but not females, a larger study would be necessary to definitively affirm this finding.

A possible confound of this study was the cross-reactivity of [^{11}C]CUMI-101 to both 5-HT_{1A} and the noradrenergic a_1 receptor (Shrestha et al., 2014). However, the temporal cortex is not richly innervated with this receptor (Zilles et al., 1993) as it is with 5-HT_{1A} (Varnas et al., 2004), making it unlikely that adrenergic binding accounts the correlation we observed. Another potential limitation is that our thresholding method of delineating the raphe nucleus may have incorporated other regions into the ROI, such as the inferior and superior colliculi, which are known to have serotonin-1A receptors (Butt et al., 2002; Hurley, 2007). Regional heterogeneity, therefore, may have decreased the effect size for the raphe nucleus in this study. However, it should be noted that this is the delineation that we have found to have significantly different 5-HT_{1A} binding between people with and without MDD. In addition, we did not use a dedicated auditory cortex ROI and instead used temporal cortex as a whole; it is possible that using auditory cortex only would yield greater effects. Ideally, PET and EEG data would have been acquired simultaneously to avoid the possibility of changes between the times of PET and EEG, though there are significant technical challenges to such methods including head movement and artifact.

Finally, we used a relatively novel method to recover an arterial input function in the case that only a single blood draw could be performed. It is possible that differences in quantification that resulted from this method influenced our results. However, this is unlikely given that PET binding outcome measures for [^{11}C]CUMI-101 and [^{11}C]DASB were not significantly different across the quantification methods (Bartlett et al., 2018).

5. Conclusions

By comparing PET and EEG, an exploratory analysis suggests that that loudness dependence of auditory evoked potentials (LDAEP) is diffusely correlated with 5-HT_{1A} across the brain, specifically in males. This multimodal, in vivo, human study provides evidence supporting a long-established animal model of serotonin transmission. Should this study be replicated in a larger sample, it would add to our understanding of the role of serotonin transmission in sensory perception.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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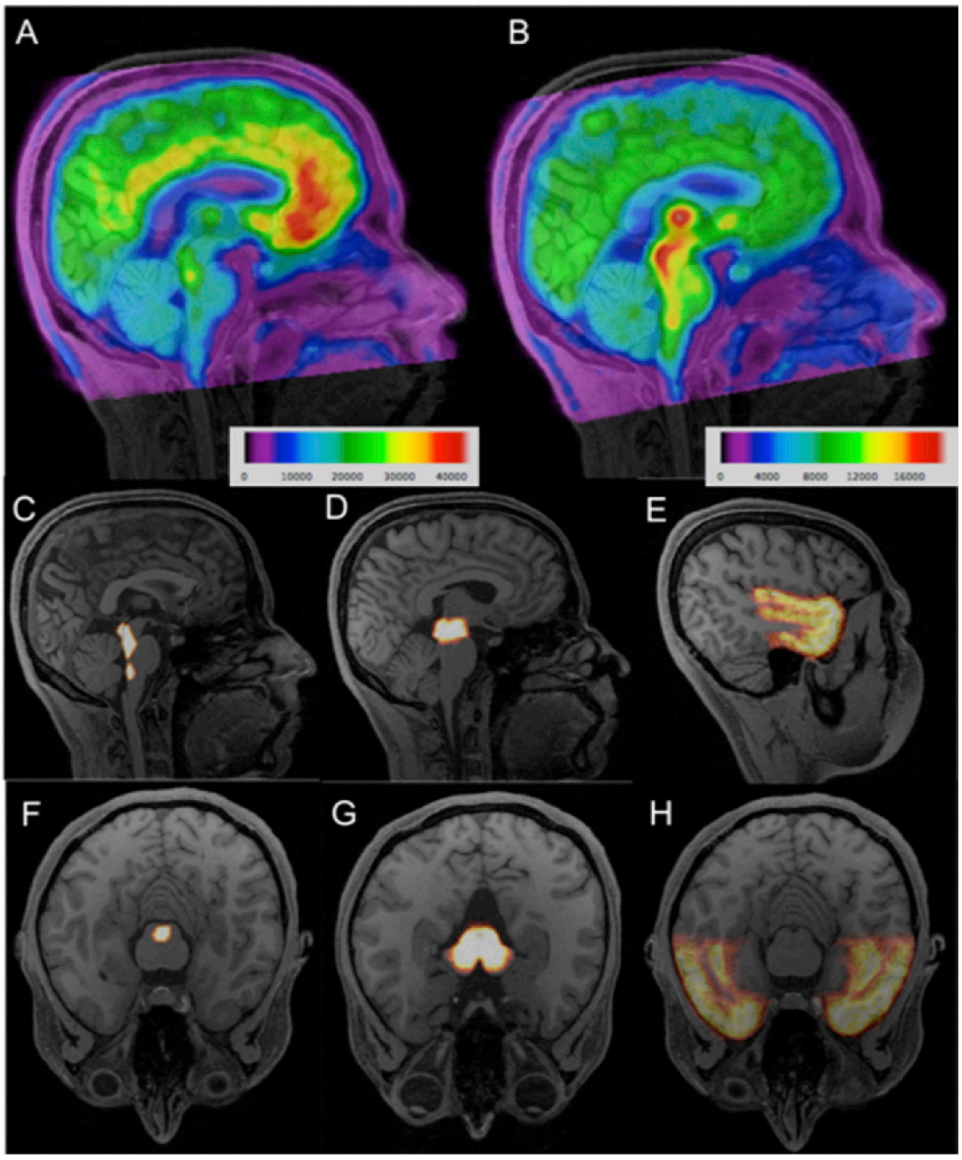

Figure 1:

Top Row (A-B): Representative average [^{11}C]CUMI-101 (A) and [^{11}C]DASB (B) PET images for a single participant, overlaid on the participant's MRI. Colorbar represents activity and is in microCuries. Center and Bottom Rows (C-H): Probabilistic regions of interest labels are shown in sagittal (center row: C-E) and axial (bottom row: F-H) views. For [^{11}C]CUMI-101, the a priori regions were the raphe nucleus (C, F) and temporal cortex (E, H), while a priori regions for [^{11}C]DASB were the midbrain (D, G) and temporal cortex (E, H). Color intensity corresponds to probability of voxel corresponding to region of interest.

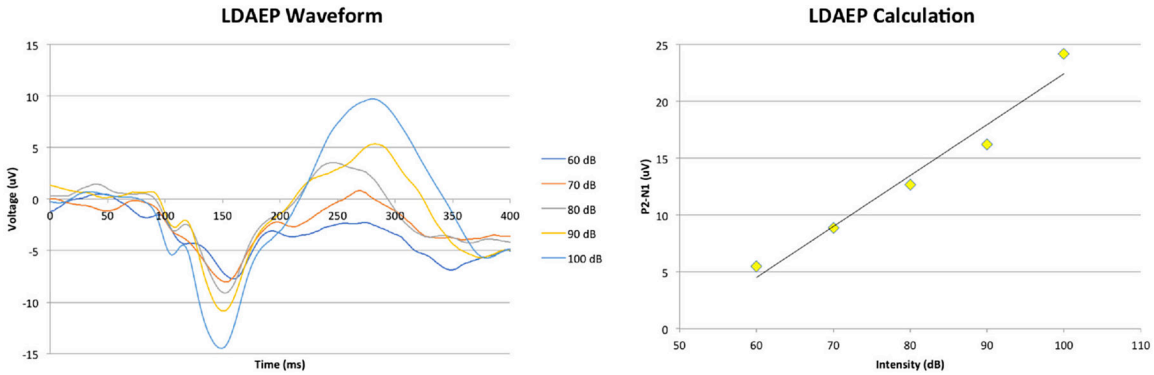

Figure 2:

LDAEP calculation. A: Example EEG waveform in response to tones of varying loudness levels. B: N1–P2 peak-to-peak amplitude by decibel level. LDAEP is defined as the slope of this regression.

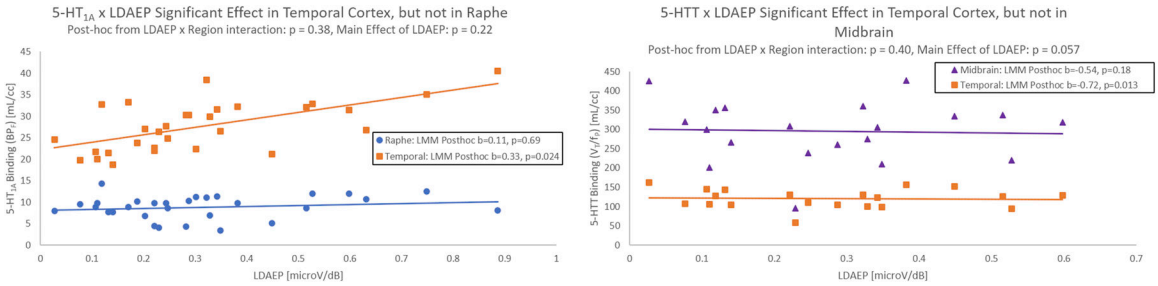

Figure 3:

5-HT_{1A} (LEFT) and 5-HTT (RIGHT) binding shown against LDAEP for all participants in the a priori raphe nucleus (5-HT_{1A}), midbrain (5-HTT), and temporal cortex. Linear regression fits are shown between 5-HT_{1A}/5-HTT and LDAEP for illustration purposes. All beta coefficients (b) and p-values reported are from the linear mixed models (LMM) fit that account for possible repeated measures.

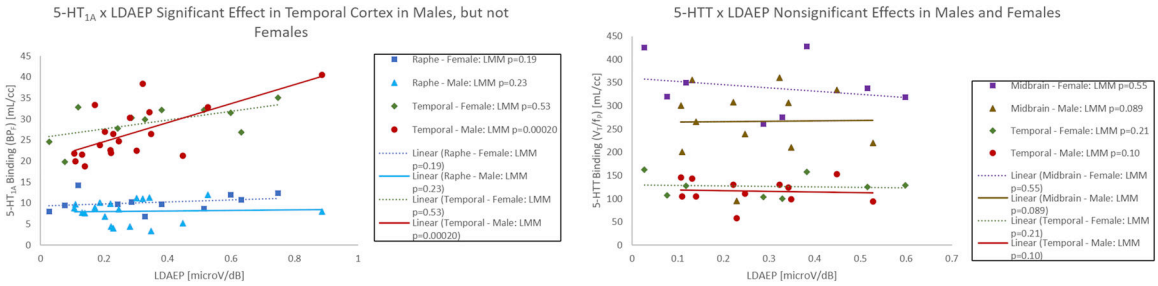

Figure 4:

Results from exploratory analyses illustrated for 5-HT_{1A} (LEFT) and 5-HTT (RIGHT) binding against LDAEP stratified by sex in the *a priori* raphe nucleus (5-HT_{1A}), midbrain (5-HTT), and temporal cortex. Linear regression fits are shown between 5-HT_{1A}/5-HTT and LDAEP for males and females for illustration purposes. All p-values reported are from the exploratory linear mixes models (LMM) fit that account for possible repeated measures and include age, sex, and pre/post treatment as main effects.

Table 1:

Participant Demographics

Diagnosis	MDD	BD	P-value*	Control
N N=23 participants (N=30 total scans**)	11	8	--	4
Age (SD)	34.5 (12.8)	31.3 (14.5)	0.63	22.0 (4.1)
Number Female/Male (% Female/% Male)	4/7 (36%/64%)	4/4 (50%/50%)	0.55	4/0 (100%/0%)
Screening HDRS-17 (SD)	23.6 (4.8)	22.2 (3.7)	0.45	1.5 (0.6)
HDRS-24 Pre-Treatment (SD)	27.4 (6.5)	28.6 (3.9)	0.60	--
HDRS-24 Post-Treatment (SD)	16.6 (12.1)	16.4 (10.5)	0.98	--

MDD: major depressive disorder, BD: bipolar disorder, SD: standard deviation, HDRS: Hamilton Depression Rating Scale, 17 or 24 Item

*Calculated by t-test for continuous variables, chi-square for categorical variables. P-values only computed across MDD and BD groups based on small sample (N=4) and different variables in control group.

**2 participants with BD and 5 with MDD received pre- and post-treatment PET and EEG, making the total sample size 30 even though there were 23 total participants.