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Brain Serotonin Transporter Binding, Plasma Arachidonic Acid and Depression Severity: a Positron Emission Tomography Study of Major Depression

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Abstract

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Contributors:

MG did the initial data analysis and wrote the first draft. FZ performed the final PET modeling and wrote the PET methodology. SZ and RTO performed the statistical mediation modeling and wrote the statistical sections. HRF curated the imaging data and prepared the Table. JMM and GS contributed to the data interpretation. JJM and MAO designed and implemented research protocols under which the clinical and PET data were collected. TBC performed the PUFA assays and wrote the PUFA purification methodology section. MES secured NARSAD funding, designed and implemented the portion of the study relating to PUFA data collection, and wrote the final manuscript. All authors contributed to manuscript preparation and data interpretation.

Conflicts of Interest:

Dr. Mann receives royalties for the commercial use of the C-SSRS from the Research Foundation for Mental Hygiene. Dr. Oquendo receives royalties for the commercial use of the Columbia Suicide Severity Rating Scale; her family owns stock in Bristol Myers Squibb. Dr. Sullivan is currently an employee of Tonix Pharmaceuticals Inc. All other authors have no conflicts of interest to declare.

BACKGROUND.—Serotonin transporter (5-HTT) binding and polyunsaturated fatty acids (PUFAs) are implicated in major depressive disorder (MDD). Links between the two systems in animal models have not been investigated in humans.

METHODS.—Using positron emission tomography (PET) and [^{11}C]DASB, we studied relationships between 5-HTT binding potential and plasma levels of PUFAs docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA) in medication-free MDD patients (n=21). PUFAs were quantified using transesterification and gas chromatography. Binding potential BP_P, and alternative outcome measures BP_F and BP_{ND}, were determined for [^{11}C]DASB in six *a priori* brain regions of interest (ROIs) using likelihood estimation in graphical analysis (LEGA) to calculate radioligand total distribution volume (V_T), and a validated hybrid deconvolution approach (HYDECA) that estimates radioligand non-displaceable distribution volume (V_{ND}) without a reference region. Linear mixed models used PUFA levels as predictors and binding potential measures as outcomes across the specified ROIs; age and sex as fixed effects; and subject as random effect to account for across-region binding correlations. As nonlinear relationships were observed, a quadratic term was added to final models.

RESULTS.—AA predicted both 5-HTT BP_P and depression severity nonlinearly, described by an inverted U-shaped curve. 5-HTT binding potential mediated the relationship between AA and depression severity.

LIMITATIONS.—Given the small sample and multiple comparisons, results require replication.

CONCLUSIONS.—Our findings suggest that AA status may impact depression pathophysiology through effects on serotonin transport. Future studies should examine whether these relationships explain therapeutic effects of PUFAs in the treatment of MDD.

Keywords

Depression; polyunsaturated fatty acids; arachidonic acid; PET; [^{11}C]DASB; serotonin transporter

Introduction

Major depressive disorder (MDD) is a leading cause of disability worldwide, affecting 300 million people (World Health Organization, 2016). The exact pathogenesis of MDD is unknown, but both altered levels of polyunsaturated fatty acids (PUFAs) (Lin et al., 2010) and serotonergic system abnormalities (Selvaraj et al., 2011) are potential contributors.

PUFAs are long-chain lipids that are ubiquitous in the phospholipid bilayers of human cell membranes (Benatti et al., 2004), where they are important both structurally and functionally, affecting physicochemical cell membrane properties, serving as second messengers, and generating metabolites with key roles in inflammatory cascades (Liu et al., 2015). PUFAs are essential dietary components that play a critical role in brain development and health (Spector, 1999). Major PUFA species include n-3 (omega-3) PUFAs docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), and the n-6 PUFA arachidonic acid (AA, 20:4n-6). DHA and EPA occupy somewhat different functional niches in many physiological contexts (Gorjao et al., 2009). In the brain, DHA and AA are found in large quantities (Lin et al., 2010) and are important for brain

development and function. EPA's brain functions are less well understood; EPA is much less abundant in the brain, but exhibits rapid turnover and metabolism through beta-oxidation (Chen and Bazinet, 2015). The relative peripheral levels of PUFA species are associated with neuropsychiatric illnesses, with elevated AA in proportion to DHA and EPA in MDD (Lin et al., 2010) and suicide risk (Huan et al., 2004, Lewis et al., 2011, Sublette et al., 2006). AA is particularly implicated in bipolar disorder, both postmortem (Kim et al., 2009) and in translational studies showing that multiple mood stabilizing medications downregulate AA metabolism (Rapoport, 2014).

We have previously reported lower 5-HTT binding (BP_P), using positron emission tomography (PET) and [^{11}C]McN5652, in unmedicated MDD (Parsey et al., 2006a) and bipolar depressed (Oquendo et al., 2007a) groups compared with healthy controls. [^{11}C]McN5652, however, has high non-specific binding, dampening the signal-to-noise ratio, and lacks a measurable plasma free fraction (f_P). The PET radioligand [^{11}C]DASB does not have these shortcomings and thus is a superior alternative for imaging 5-HTT (Frankle et al., 2004). Using [^{11}C]DASB, Selvaraj et al. (Selvaraj et al., 2011) also found lower 5-HTT binding (BP_P) in MDD patients. We have reported lower [^{11}C]DASB binding (V_T/f_P) in MDD patients with a history of suicide attempt (Miller et al., 2013), and lower binding potentials (BP_F, BP_P, and BP_{ND}, using a suboptimal reference region) in bipolar depression (Miller et al., 2016), considering *a priori* regions of interest (ROIs), although V_T/f_P did not differ in that study. (For further discussion about methodological advances in quantification and optimal outcome measures for [^{11}C]DASB see the Methods section.) In studies by other groups, compared with 5-HTT binding in healthy controls, 5-HTT binding in depression has been variously reported as lower (Selvaraj et al., 2011, Parsey et al., 2006a, Newberg et al., 2005, Malison et al., 1998, Joensuu et al., 2007, Oquendo et al., 2007a, Reimold et al., 2008, Staley et al., 2006, Willeit et al., 2000, Lehto et al., 2006, Nye et al., 2013), higher (Boileau et al., 2008, Cannon et al., 2007, Cannon et al., 2006, Ichimiya et al., 2002, Reivich et al., 2004, Dahlstrom et al., 2000), or not different (Meyer et al., 2001, Meyer et al., 2004, Miller et al., 2013). However, the present study did not have a control group so is not directly comparable to these case-control studies.

Of note, while previous work has focused on either PUFAs or the serotonin system as being key factors in MDD, these systems are not independent of one another. Rodent studies indicate a relationship between AA and the serotonin system, including specific interactions with 5-HTT. One study reported that a high omega-6 PUFA diet reduces 5-HTT binding significantly in hippocampus and at a trend level in amygdala and dorsomedial and ventromedial hypothalamic nuclei, compared with high omega-3, low-fat, and saturated fat diets (du Bois et al., 2006). Higher AA brain uptake is also seen in 5-HTT knockout mouse models (Basselin et al., 2009). Together, these studies suggest a bidirectional, inverse AA – 5-HTT relationship. Yet, while we have previously demonstrated associations of plasma phospholipid DHA with the cerebrospinal (CSF) dopaminergic metabolite homovanillic acid in MDD, we did not identify a relationship of DHA with CSF serotonergic metabolite 5-hydroxyindoleacetic acid (Sublette et al., 2014). Nonetheless, relationships of PUFAs with human cerebral 5-HTT binding have never been studied. We therefore sought to investigate relationships between plasma PUFA levels and 5-HTT binding potential in six *a priori* brain

ROIs associated with depression pathophysiology in MDD patients, and possible relationships with depression severity.

Methods and Materials

Sample

Adult MDD patients (n=21) were recruited through advertisement and clinician referrals. Each participant was evaluated by a Masters'-level psychologist and a Board-certified, licensed psychiatrist, and found to have capacity to give written informed consent to participate in the relevant New York State Psychiatric Institute IRB-approved studies, in conformity with US Federal Policy for the Protection of Human Subjects. This study was performed in a subset of MDD patients enrolled in [^{11}C]DASB neuroimaging studies who also gave informed consent to have blood PUFA levels assayed. Other articles based on data from the larger [^{11}C]DASB dataset have been published answering different research questions (Miller et al., 2013, Parsey et al., 2006a, Oquendo et al., 2016, Schneck et al., 2016).

All patients were evaluated diagnostically with the Structured Clinical Interview for DSM-IV (SCID) (First et al., 1994) and underwent a comprehensive psychiatric and medical assessment, physical examination, and screening laboratory tests including urine toxicology. Depression severity was assessed using the 17-item Hamilton Depression Rating Scale (HDRS-17) (Hamilton, 1960).

Inclusion criteria for participants included: 1) current DSM-IV major depressive episode; 2) HDRS-17 ≥16 at screening; 3) age 18 to 65 years; 4) off all drugs or medications that interact with the arachidonic acid pathway or affect the 5-HT system for ≥2 weeks prior to scan, and ≥ 6 weeks for fluoxetine or antipsychotic medications; and 5) no active inflammatory or neurologic illness.

Plasma polyunsaturated fatty acid analysis

Most participants had non-fasting blood drawn for PUFA levels within one (n=13) or two (n=4) weeks preceding their PET scans; the remaining four participants' blood samples were drawn at 22, 29, 38 and 50 days prior to the scan. Blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA), placed on ice, and centrifuged for ten minutes. Samples were kept in a freezer at −80°C until analyzed. The plasma PUFAs EPA, DHA, and AA were separated and quantified as previously described (Lepage et al., 1989). Briefly, plasma PUFAs were quantified using direct transesterification to produce fatty acid methyl esters (FAMEs). The FAMEs were then separated using gas chromatography. Peaks were identified using standardized retention times and quantified using an internal standard.

Radiochemistry and input function measurement

Preparation of [^{11}C]DASB and measurement of the radioligand's arterial input function, metabolites, and f_P were performed as previously outlined (Belanger et al., 2004, Ogden et al., 2007). The chemical purity of [^{11}C]DASB was ≥95%.

PET protocol

The PET scanning protocol has previously been described (Ogden et al., 2007). Briefly, each participant had a venous and an arterial catheter placed. Following a short transmission scan, the [^{11}C]DASB radioligand was injected intravenously over 30 seconds, and 3-dimensional emission data were collected for 100 minutes with 19 frames of increasing duration using the ECAT HR+ scanner (Siemens/CTI, Knoxville, TN).

Magnetic resonance imaging (MRI)

As previously described (Parsey et al., 2000), MRI images were acquired on either a 1.5T Signa Advantage or a 3T Signa HDx system (General Electric Medical Systems, Milwaukee, WI) for co-registration with PET images and extraction of ROIs.

Image analysis

PET frames were motion-corrected using the FMRIB Software Library (FSL) linear image registration tool (FLIRT; FMRIB Image Analysis Group, Oxford, UK). A mean PET image was determined for each participant, and this was aligned to its corresponding MRI using FLIRT (DeLorenzo et al., 2009). An automated algorithm was used to create masks for each ROI; ROI masks were then used to extract regional time activity curves (TACs) for the PET analysis (DeLorenzo et al., 2011).

Outcome measure estimation

[^{11}C]DASB binding potentials BP_P, BP_F, and BP_{ND} were calculated as $BP_P = (V_T - V_{ND})$, $BP_F = (V_T - V_{ND})/f_P$, and $BP_{ND} = (V_T - V_{ND})/V_{ND}$, respectively, where V_T is the radioligand total volume of distribution in the ROI, and V_{ND} is the radioligand non-displaceable distribution volume, common to all ROIs within a subject (Innis et al., 2007). V_T was obtained using likelihood estimation in graphical analysis (LEGA) (Ogden, 2003, Parsey et al., 2003), considered the quantification approach of choice for [^{11}C]DASB, given its test-retest repeatability (Ogden et al., 2007). For LEGA, the initial time for the linear phase, t*, was set to 25 minutes post injection (Ogden et al., 2007). V_{ND} was estimated using a hybrid deconvolution approach (HYDECA) (Zanderigo et al., 2017) that combines model-free deconvolution (Zanderigo et al., 2015) and simultaneous search across regions (Todd Ogden et al., 2015) to estimate V_{ND} without relying on any reference region, which is appropriate for [^{11}C]DASB, given the ubiquitous distribution of 5-HTT in the brain. HYDECA tuning parameters β and γ were set to 3.5 and 10, respectively, which are optimal values for [^{11}C]DASB as determined via blocking studies (Zanderigo et al., 2017). Brain tissue TACs were corrected for the contribution of plasma activity, assuming a 5% blood volume in the ROIs (Mintun et al., 1984), before applying LEGA and HYDECA.

As a check on the consistency of our HYDECA results with historically used approaches, we performed regional scatterplots comparing HYDECA with the simplified reference tissue model (SRTM) (Lammertsma and Hume, 1996). Correlating BP_{ND} (the only metric possible with SRTM) estimated with the two methods yielded the following Pearson's correlation coefficients: r=0.404 (amygdala, midbrain), r=0.476 (anterior cingulate), r=0.526 (hippocampus), r=0.710 (putamen), and r=0.806 (thalamus). This is consistent with our previous work comparing HYDECA and SRTM applied to [^{11}C]DASB, in which BP_{ND}

correlates fairly well but SRTM underestimates the BP_{ND} values with respect to those generated by HYDECA (Zanderigo et al., 2018). When the main statistical models were run with SRTM, however, the relationships seen with HYDECA were not detectable with SRTM (data not shown).

Prior to the development and validation of our HYDECA method, in some previous studies we had quantified [^{11}C]DASB binding using V_T/fp (Miller et al., 2016, Miller et al., 2013) due to uncertainty about the validity of the cerebellum as a reference region (Parsey et al., 2006b). Now that binding potentials are available as a preferable outcome measure, among the possible *in vivo* binding potentials, BP_F theoretically would be the outcome measure of choice, since it is the closest to B_{avail}/K_d and thus to the density of available target. However, f_P sometimes can add more noise than the variance it explains (Innis et al., 2007). To assess the reliability of f_P for [^{11}C]DASB, we performed assessments of this measure in a test-retest healthy volunteer sample (n=11) in which the characteristics of other measures have previously been studied (Ogden et al., 2007). This test-retest analysis returned an intraclass correlation coefficient of only 0.54 (mean percent difference=13.17%; SD=14.81%; CV=112.43%; range=[0 43]%). Therefore our primary outcome measure of choice was BP_P; we also report here the analyses using both BP_F and BP_{ND}, for the interested reader.

Regions of Interest

Consistent with previous studies by our group examining regional 5-HTT binding in depression (Miller et al., 2013, Parsey et al., 2006a), we selected six *a priori* brain ROIs that have measurable 5-HTT and are associated with depression pathophysiology: amygdala, anterior cingulate cortex, hippocampus, midbrain, putamen, and thalamus.

Statistical Analyses

All analyses were performed in R, version 3.3.0 (http://cran.r-project.org). To mitigate skew, to stabilize variance levels, and to allow for proportionality of fixed effects variables across brain regions, plasma PUFA levels and measures of binding potential were logarithmically (*ln*)-transformed before modeling the data (Emerson and Stoto, 1983, Tukey, 1957). A bootstrap algorithm was used to calculate standard errors for each binding value while controlling for errors in metabolite, plasma and brain data; the errors were then used to weight the data (Ogden and Tarpey, 2006). One subject was found to have an unusually low BP_P value in the hippocampal region; thus all subsequent analyses excluded this data point from this subject.

To test relationships between PUFAs and 5-HTT binding potential, linear mixed models were used initially. We examined in separate models each plasma PUFA species (DHA, EPA, and AA) as a predictor variable and BP_P across the aggregated 6 specified ROIs as outcome. Region, PUFA levels, age, and sex were treated as fixed effects; random effect of subject was included to account for correlations in binding across regions for each subject. The same analysis procedure was applied to both BP_{ND} and BP_F data. After finding that among PUFAs, only the model containing AA had a significant effect on 5-HTT binding potential, we also tested for a nonlinear relationship between AA levels and binding

potential by adding a quadratic term, given that visual inspection of correlation plots suggested nonlinearity was present.

Since our ultimate interest lies in the clinical significance of relationships between PUFAs and 5-HTT, we next tested whether 5-HTT binding potential mediated a relationship between AA levels and depression severity, measured by the HDRS-17, using mediation analysis based on Baron and Kenny (Baron and Kenny, 1986). Our situation is somewhat different from the straightforward mediation model based on ordinary linear regression models, because we considered binding potential measures in six different ROIs and because a nonlinear relationship was potentially the best fit between the AA levels and binding potential. Therefore, we used a measure of binding potential aggregated across all considered ROIs for each subject as a univariate potential mediator. Specifically, this is the predicted subject-level random effect from the linear mixed model that contains both a linear and a quadratic term of AA level. Apart from these differences, our mediation analysis is the same as that used in (Baron and Kenny, 1986). To rule out the possibility that AA levels obtained more than two weeks distant from the PET scanning had a differential effect on the results, we repeated the significant AA - HDRS-17 and AA - binding potential correlations after removing those four data points. Similarly, we performed *post-hoc* testing of possible medication effects by removing the four patients who had been most recently taking medications (2 to 6 weeks prior to scanning) and repeating the analyses of AA effects on BP_P and on HDRS-17.

Significance was defined as a p-value < 0.05 and all tests were two-sided. Results are presented throughout with no adjustment for multiple comparisons.

Results

Sample

Demographic and clinical characteristics are detailed in Table 1. With regard to history of medication use, all twenty-one patients were medication-free at the time of the scan. Nine patients were medication-naïve; six had no medication exposure within 1 year prior to the PET scan; and two had no medication exposure within 3 months. The remaining four patients had not taken antidepressants within 2 to 3 weeks of their scans: of these, two patients had previously been on selective serotonin reuptake inhibitors (escitalopram and sertraline, respectively) and two had been on other antidepressants (duloxetine plus bupropion, and venlafaxine, respectively).

Relationships between PUFA levels and 5-HTT binding potential

Separate models testing effects of DHA, EPA, and AA revealed no effects of DHA nor EPA on [^{11}C]DASB binding potential (data not shown). However, AA had an effect on BP_P (F=9.14; df=1,19; p=0.006) across regions, and given graphical evidence of a nonlinear relationship between AA level and BP_P (Figure 1A), we found that an added quadratic term was significant (F=9.62; df=1,19; p=0.006; also see Figure 4, path a). The resulting inverted U-shaped relationship demonstrates that for most of the concentration range of AA, higher BP_P correlated with lower AA levels. Exploratory analyses of PUFA effects on BP_{ND} and

BP_F (see Figures 1B and 1C) similarly found that AA, but not DHA or EPA, demonstrated a comparable effect on BP_{ND} (F=7.40; df=1, 19; p=0.014) while controlling for region, but had no statistically significant effect on BP_F (F=0.51; df=1,19; p=0.484). Neither age nor sex showed a statistically significant effect on any measure of binding potential, so these covariates were removed from the models. When we removed four participants whose PUFA levels were obtained more than 2 weeks prior to scanning, the significance of correlations between AA and HDRS-17, and between AA and binding potential were essentially unchanged (data not shown).

Mediation analysis: 5-HTT binding potential as a mediator of AA effects on depression

In a simple model for effects of AA and the corresponding quadratic term (AA^2) on HDRS-17 severity (Figure 2 and Figure 4, path c), both the linear (F=5.25; df=1,18; p=0.034) and quadratic (F=5.35; df=1,18; p=0.033) term effects were significant. In a simple correlation between 5-HTT binding potential (aggregated across ROIs as described in Materials and Methods) and HDRS-17 severity, 5-HTT positively correlated with depression severity (F=19.16, df=1,19 p=0.0003; Figure 3 and Figure 4, path b). However, when AA and the corresponding quadratic term were included in the model as predictors of HDRS-17 severity, along with the aggregate measure of 5-HTT binding potential as another predictor (random effect), 5-HTT binding was significant as a positive predictor of HDRS-17 score (F=6.16; df=1,17; p=0.024; Figure 4, path c'), while the effects of AA and AA^2 were no longer significant (linear: F=0.37; df=1,17; p=0.553; quadratic: F=0.37; df=1,17; p=0.552). These results are consistent with the classic definition of mediation, in which the inclusion of the mediator (in this case, 5-HTT binding potential) in the model partially or completely attenuates the effects of the predictor (AA) on the outcome (HDRS-17). Therefore, our results suggest that 5-HTT binding potential may mediate the effect of AA levels on severity of depression symptoms.

Effects of medications

When we removed the four participants who had taken medications within 2 to 3 weeks prior to the PET scanning, the quadratic relationship between AA and BP_P remained significant (AA p=0.019; AA^2 p=0.018), as did the linear relationship between BP_P and HDRS-17 (p=0.0004), while the relationship between AA and HDRS-17 remained significant in a linear model (p=0.028), but the quadratic model lost significance to a trend level (AA p=0.105; AA^2 p=0.100).

Discussion

This is the first report that AA, but not DHA or EPA, levels are associated with 5-HTT BP_P. Dietary intake is the primary determinant of AA levels, either directly or through intake of precursor shorter-chain molecules such as linoleic acid (18:2) that are converted at variable, generally low rates to AA (reviewed in (Liu et al., 2015)). Based on the mediation results, it appears that AA affects depression severity through effects on 5-HTT binding potential. For this to be true, AA would have to have a biological effect that involves 5-HTT. Rodent (Mathews et al., 2004, Qu et al., 2003, Qu et al., 2005, Dubois et al., 2006, Strosznajder et al., 1996, Basselin et al., 2009, Bambico et al., 2007, Gobbi et al., 2005, McLaughlin et al.,

2012) and cell culture (Garcia and Kim, 1997, Berg et al., 1998, Felder et al., 1990) studies suggest possible mechanisms for AA - 5-HTT interactions. These putative pathways are illustrated schematically in Figure 5, and include the following observations: low 5-HTT expression elevates intrasynaptic 5-HT (Mathews et al., 2004), which binds to postsynaptic 5-HT receptors. The $5\text{-HT}_{2A/2C}$ and 5-HT_{1A} receptors have opposite effects on the balance between membrane-bound and unesterified (free) AA, presumably subserving a homeostatic function. Specifically, $5\text{-HT}_{2A/2C}$ stimulates activation of cytosolic phospholipase A2 (cPLA2) (Berg et al., 1998, Qu et al., 2005, Garcia and Kim, 1997, Felder et al., 1990), triggering the release of unesterified AA from membrane phospholipids. Some of the released AA is recycled back into the membrane, a process that is stimulated by 5-HT_{1A} receptors (Strosznajder et al., 1996). Remaining unesterified AA can decrease 5-HTT in certain brain regions (du Bois et al., 2006). AA influences on 5-HTT and depression also may be a function of AA cascade products, anandamide (N-arachidonylethanolamide) and 2-arachidonoylglycerol, binding to cannabinoid (CB_1) receptors. CB_1 agonists dose-dependently stimulate dorsal raphe serotonergic neurons (Bambico et al., 2007). Serotonergic neurons in dorsal raphe nucleus likewise increase firing in response to inhibition of anandamide breakdown (McLaughlin et al., 2012), which is associated with antidepressant-like effects in rodents and is blocked by CB_1 antagonism (Gobbi et al., 2005). Furthermore, CB_1 knockout mice exhibit decreased brain 5-HTT binding site density (Burokas et al., 2014), and both 5-HTT (Kalueff et al., 2010) and CB_1 (Aso et al., 2008) rodent knockouts exhibit a depressive-like phenotype. Other possible factors regulating 5-HTT binding levels not assessed in this study include the rate of 5-HTT internalization (Rahbek-Clemmensen et al., 2014), governed partly by intrasynaptic 5-HT concentrations (Jorgensen et al., 2014), DNA methylation (Drabe et al., 2017), and gene promoter variants such as HTTLPR (Heils et al., 1996).

In contextualizing our finding that 5-HTT binding potential is positively correlated with depression severity, we are aware of five human studies (Boileau et al., 2008, Cannon et al., 2007, Miller et al., 2013, Meyer et al., 2004, Selvaraj et al., 2011) that assessed the correlation of depression severity with [^{11}C]DASB binding, and nine PET and single photon emission computed tomography (SPECT) studies using other ligands (described below). These studies reported a variety of results that could be related to differences in radioligands, binding potential outcome measures, brain regions studied, and/or sample characteristics. Our findings agreed with only one other study, which reported a positive correlation of depression severity with [^{11}C]DASB BP_{ND} in patients with Parkinson's and depression (Boileau et al., 2008). Other studies in MDD relating depression severity to [^{11}C]DASB found inverse correlations with BP_{ND} (Cannon et al., 2007) or BP_P (Selvaraj et al., 2011), and no correlation with BP_{ND} (Meyer et al., 2004) or V_T/f_P (Miller et al., 2013). In studies with [^{11}C]McN5652, no correlation was seen (Ichimiya et al., 2002, Parsey et al., 2006a). Using SPECT, inverse correlations of binding with depression severity were reported with [123I]-2β-carbomethoxy-3β-(4-iodophenyl)tropane (^{123}Iβ-CIT) at a trend level (p=0.052) (Malison et al., 1998) and significantly (p=0.02) with ^{123}I-ADAM (Newberg et al., 2005) in a very small sample (n=7), but the latter finding was not replicated by the same team in a larger sample (n=20) (Newberg et al., 2012). Other ^{123}I-ADAM (Herold et al., 2006, Catafau

et al., 2006, Ho et al., 2013) and ^{123}Iβ-CIT (Joensuu et al., 2007) SPECT studies likewise found no correlation with depression severity.

Some studies report lower binding specifically in depressed suicide attempters (Meyer et al., 2001, Meyer et al., 2004, Miller et al., 2013); however, only three of our patients had a history of suicide attempt, and their data did not fall at extremes of the depression severity or binding potential distributions.

Our finding of an inverted U-shaped function describing the relationship of plasma AA concentration to depression severity adds complexity to previous meta-analytic findings that depression is associated with low DHA, EPA, and total omega-3 PUFA levels but not with differences in AA or total omega-6 PUFAs (Lin et al., 2010). However, some other groups have observed not only lower omega-3 PUFAs but also lower omega-6 PUFAs, including lower AA levels, in MDD patients compared with healthy controls (Maes et al., 1999, Peet et al., 1998).

That AA plasma levels correlated significantly with BP_P and BP_{ND} but not with BP_F, (in which only a trend is seen) likely relates to measurement error in the free fraction assay required for determining f_P and thus BP_F (Innis et al., 2007).

Prior medication use does not appear to have been a confound in this study, since all patients were medication-free at the time of the scan, and the main findings were only minimally affected when we removed four patients with the most recent medication use (2 to 3 weeks prior to the scan).

Limitations

As this pilot study examined a relatively small sample, testing multiple PUFA species and binding measures, results should be regarded as preliminary. In particular, mediation analyses generally require larger sample sizes. Inadequate numbers of healthy controls with PUFA measurements were available for study; therefore, we cannot comment on whether the AA relationships to [^{11}C]DASB binding potential may be related to the pathogenesis of major depression, although the correlation between binding potential and depression severity suggests pathophysiologic importance. Plasma concentrations of DHA, EPA and AA were assayed because these PUFAs have known clinical associations with brain health and depression. Additional PUFA species, particularly docosapentaenoic acid (DPA), may also have relevance, but we decided *a priori* to limit the number of PUFA species under study. Total AA levels in plasma measured in this study include AA esterified to phospholipids, cholesteryl esters and triglycerides, as well as nonesterified AA. The plasma AA measure is assumed to be a proxy for brain utilization, as AA crosses the blood-brain barrier in both esterified and unesterified forms (reviewed in (Liu et al., 2015)). Moreover, in animal experiments with radiolabeled AA, greater than 90% is taken up from plasma within 2 minutes, and brain phospholipids are virtually completely labeled after 1 minute (DeGeorge et al., 1989, Nariai et al., 1991, Washizaki et al., 1994, Rapoport, 2001). However, it is possible that more specific lipidomic indices (such as the unesterified or plasma phospholipid fractions) might reflect different physiological mechanisms and yield different results from total plasma PUFAs. Blood draws were not conducted at the time of the PET

acquisition, for ethical and technical reasons, but repeated analyses after removing the most chronologically distant four data points did not change results. Blood was not specifically drawn in a fasting state. However, AA plasma levels do not vary greatly over short time periods; e.g., even supplementing with 1076 mg/d of omega-3 PUFA, which is known to decrease levels of AA, results in an average rate of change in plasma AA of only 2% per week (Schuchardt et al., 2016). We have no information concerning seasonal effects that may relate to diet or to 5-HTT. Given the small sample size, we did not include additional potential confounding variables in the model, such as tobacco use, body mass index, comorbid psychiatric conditions, or measures of socio-economic status.

A limitation of the [^{11}C]DASB ligand is the lack of a valid reference region, since binding sites are present throughout the brain. Methods that require the presence of a valid reference region (such as SRTM), when employed using an invalid reference region instead, underestimate BP_{ND} binding potentials (Turkheimer et al., 2012); naturally, therefore, results are less accurate (Oquendo et al., 2007b, Parsey et al., 2005, Parsey et al., 2010, Ito et al., 2001). To overcome this limitation, we have employed the HYDECA method for estimating V_{ND}, and thus BP_P and BP_{ND}, without the need for a reference region. Therefore, when the main statistical models were performed with HYDECA, we saw relationships (as reported in Results) that were not detectable with SRTM. Moreover, in contrast to HYDECA, SRTM with this tracer cannot generate valid estimates of BP_F and BP_P, which comprise the main results in this study.

Conclusions

We have demonstrated relationships between plasma AA, 5-HTT binding potential across six brain regions implicated in depression, and depression symptom severity, consistent with a novel model in which 5-HTT binding serves as a mediator for nonlinear AA effects on depression severity. Future studies with more comprehensive lipidomic measurements are needed to replicate and extend these findings.

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HIGHLIGHTS

- [^{11}C]DASB PET quantified serotonin transporter binding in major depression
- Transporter binding (BP_P) associated nonlinearly with plasma arachidonic acid (AA)
- Plasma AA levels also associated nonlinearly with depression severity
- Mediation analysis finds BP_P mediates AA effects on depression severity
- Lipidomic effects on serotonin neurotransmission merit further study in depression

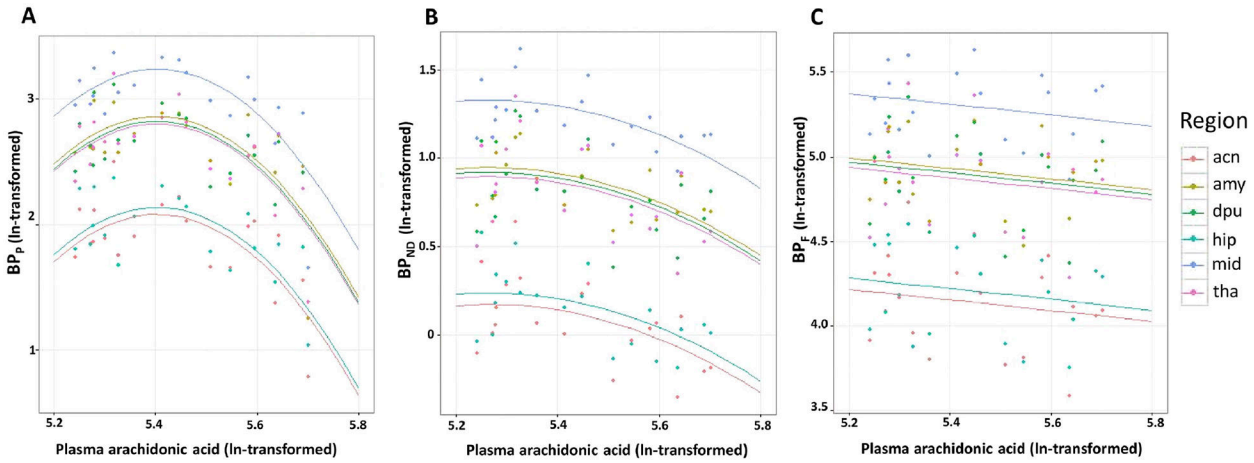


Figure 1. Plasma arachidonic acid levels as a predictor of [^{11}C]DASB binding potentials in six *a priori* regions of interest.

Arachidonic acid and estimated binding potentials are *In*-transformed. Data from separate brain regions and their corresponding regression lines are displayed in different colors as shown in the legend. Independent variables are estimated binding potentials: **A)** $BP_P = (V_T - V_{ND})$ **B)** BP_{ND} $(V_T - V_{ND})/V_{ND}$ **C)** BP_F $(V_T - V_{ND})/f_P$. *Abbreviations*: acn, anterior cingulate; amy, amygdala; dpu, dorsal putamen; hip, hippocampus; mid, midbrain; tha, thalamus.

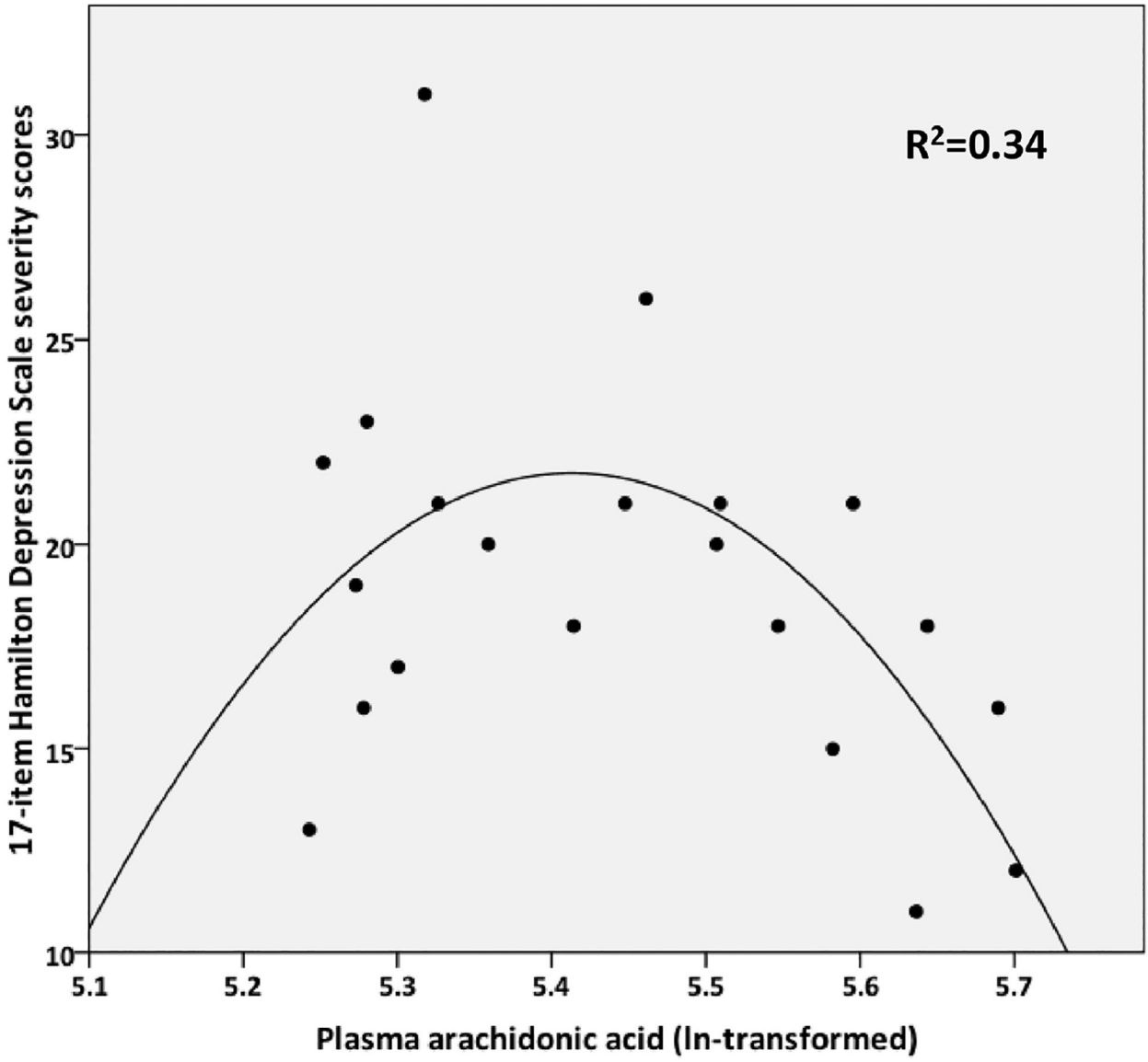


Figure 2.
Correlation between plasma arachidonic acid and depression severity fit to a quadratic function.

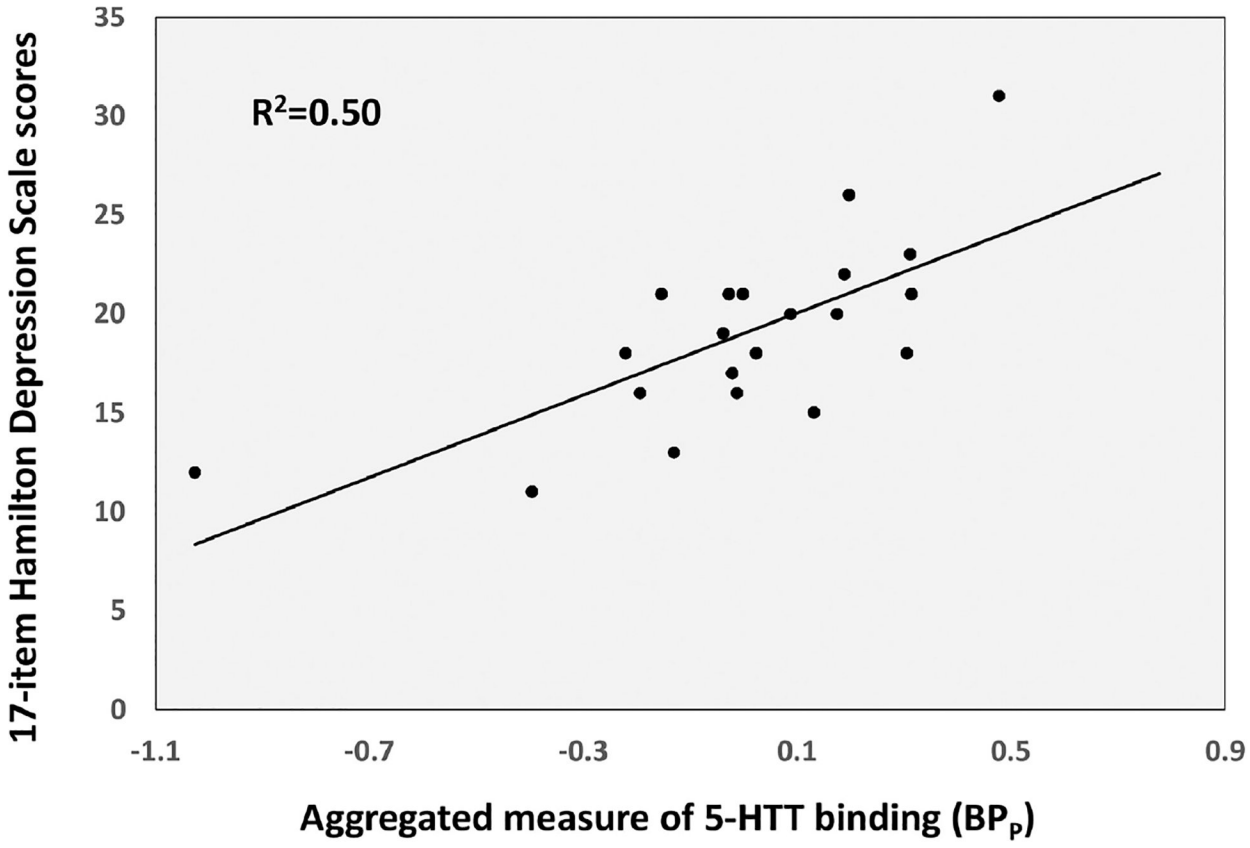
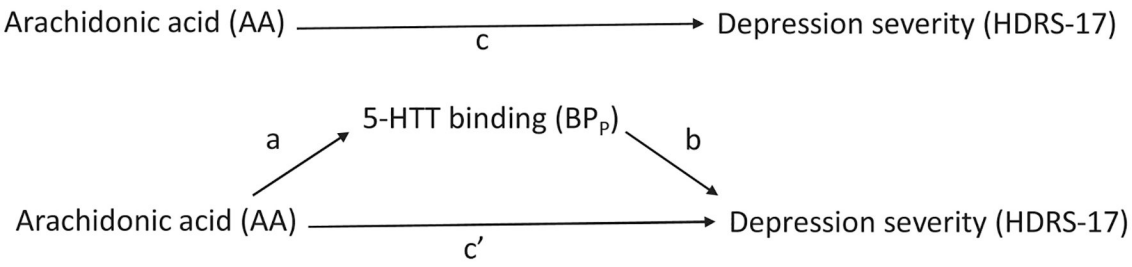


Figure 3.
Correlation between 5-HTT binding potential, aggregated across regions of interest, and depression severity.



Models:

a. BPp~Region+AA+AA²

Coefficient on AA: 100.27, p=0.0063

Coefficient on AA^2: -9.28, p=0.0059

b. HDRS-17~random effects (aggregate BPp)

Coefficient on random effects (BPp): 10.37, p=0.0003

c. HDRS-17~AA+AA²

Coefficient on AA: 1230.70, p=0.034

Coefficient on AA^2: -113.68, p=0.033

c'. HDRS-17~AA+AA²+random effects (aggregate BPp)

Coefficient on AA: 357.54, p=0.5525

Coefficient on AA^2: -32.89, p=0.5521

Coefficient on random effects (BPp): 8.97, p=0.0238

Figure 4. Mediation model for effects of arachidonic acid on depression severity via effects of serotonin transporter (5-HTT) binding potential (BP_P).

Note: BPp, AA and AA^2 are all *ln*-transformed values.

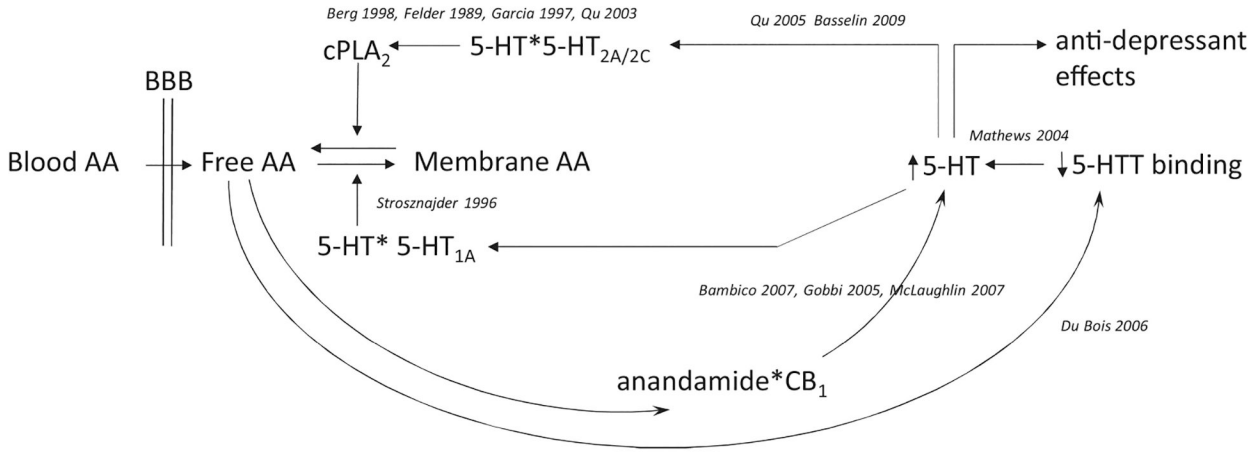


Figure 5. Hypothetical schematic of relationships between arachidonic acid and serotonergic systems.

Abbreviations: AA, arachidonic acid; BBB, blood-brain barrier; CB_1, cannabinoid receptor 1; cPLA2, cytosolic phospholipase A2; 5-HT, 5-hydroxytryptamine, or serotonin; $5\text{-}HT_{2A/2C}$ and $5\text{-}HT_{1A}$, serotonin receptor subtypes; 5-HTT, serotonin transporter. For reasons of space, citations in this figure have omitted "*et al.*"

Table 1.

Demographic and Clinical Characteristics of Study Participants (n=21).

Characteristic	**Number (%)**
Sex (% male)	10 (47.6)
History of suicide attempt	3 (14.3)
Suicidal ideation present	14 (66.7)
Prior exposure to anti-depressants	11 (52.4)
Prior substance use disorder (alcohol or cannabis)	3 (14.3)
Comorbid anxiety disorder	10 (47.6)
Comorbid dysthymic disorder	3 (14.3)
Ethnicity (%Hispanic)	7 (33.3)
Race:	
Asian	1 (4.8)
American Indian or Alaskan Native	1 (4.8)
Black or African American	4 (19.0)
White	13 (61.9)
More than one race	2 (9.5)
	Mean ± SD
Age (yrs)	36.1 ± 11.9
BMI ($kg*m^{-2}$)	26.6 ± 7.0
Education (yrs)	15.3 ± 2.7
Income (US $1000/yr)	31.1 ± 26.1
Illness duration (yrs)	14.6 ± 13.8
Number of depressive episodes	16.4 ± 35.9
Age of onset (yrs)	21.7 ± 9.0
Length of current episode (wks)	125.2 ±236.4
HDRS-17	19.0 ± 4.6
Plasma PUFAs (μg/ml)	
Docosahexaenoic acid (DHA)	51.5 ± 31.3
Eicosapentaenoic acid (EPA)	21.9 ± 22.1
Arachidonic acid (AA)	234.5 ± 36.9

Abbreviations: BMI, body-mass index; HDRS-17, 17-item Hamilton Depression Rating Scale; PUFA, polyunsaturated fatty acids.