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Lack of Association Between the Serotonin Transporter and Serotonin 1A Receptor: an *in vivo* PET Imaging Study in Healthy Adults

Michael Strupp-Levitsky[a], **Jeffrey M. Miller**[a,b,*], **Harry Rubin-Falcone**[a,b], **Francesca Zanderigo**[a,b], **Matthew S. Milak**[a,b], **Gregory Sullivan**[a,b], **R. Todd Ogden**[a,c], **Maria A. Oquendo**[a,b], **Christine DeLorenzo**[d], **Norman Simpson**[a,b], **Ramin V. Parsey**[d], and **J. John Mann**[a,b]

[a]Department of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, 1051 Riverside Drive #42, New York, NY 10032, U.S.A

[b]Department of Psychiatry, Columbia University, 1051 Riverside Drive #42, New York, NY 10032, U.S.A

[c]Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY, U.S.A

[d]Now at Department of Psychiatry, Department of Radiology, Stony Brook Medicine, Stony Brook, NY, U.S.A

Abstract

The serotonin neurotransmitter system is modulated in part by the uptake of synaptically released serotonin (5-HT) by the serotonin transporter (5-HTT), and by specific serotonin autoreceptors such as the somatodendritic 5-HT_{1A} receptor, which can limit serotonin neuron depolarization. However, little is known about how 5-HTT and 5-HT_{1A} are related *in vivo*. To study this question, we reanalyzed positron emission tomography (PET) data obtained earlier in 40 healthy participants (21 females) using [^{11}C]WAY-100635 for quantification of 5-HT_{1A} binding and [^{11}C](+)-McN-5652 for quantification of 5-HTT binding. We hypothesized negative correlations between 5-HT_{1A} binding in the raphe nuclei (RN) and 5-HTT binding in RN terminal field regions. Controlling for sex, no significant correlations were found (all $p>0.05$). Similarly, an exploratory analysis correlating whole-brain voxel-wise 5-HTT binding with 5-HT_{1A} binding in RN identified no significant clusters meeting our *a priori* statistical threshold. The lack of correlation between 5-HT_{1A} and 5-HTT binding observed in the current study may be due to the different temporal responsiveness of regulatory processes controlling the somatodendritic 5-HT_{1A} receptor and 5-HTT in response to changing availability of intrasynaptic serotonin.

*Corresponding Author: jm2233@cumc.columbia.edu.

Keywords

serotonin; serotonin transporter; serotonin 1A receptor; PET

1. Introduction

Serotonin neurons project throughout the brain from the brainstem raphe nuclei (RN), modulating a diverse array of functions including mood, responses to stress, sex, appetite, and memory (Hensler, 2012). Serotonin neurotransmission is modulated by the serotonin transporter (5-HTT) through uptake of synaptically released serotonin (5-HT), and by specific serotonin autoreceptors such as the somatodendritic 5-HT_{1A} receptor, which can limit serotonin neuron depolarization (Hensler, 2012). 5-HTT is located on nerve terminals and transports serotonin from the synaptic cleft and extracellular space into the nerve terminal (Hensler, 2012). 5-HTT protein levels are regulated by intra-synaptic levels of serotonin, because the rate of internalization of the transporter is slowed when it is bound by intra-synaptic serotonin (Hensler, 2012).

Serotonin cell bodies reside in the raphe nuclei in periaqueductal brainstem and have major projections to a broad array of cortical and subcortical structures (Hensler, 2012; Hornung, 2003; Hoyer et al., 2002; Vertes and Linley, 2007). The 5-HT_{1A} receptor subtype is widely distributed in the mammalian brain (Hensler, 2012; Hornung, 2003), serving as a somatodendritic autoreceptor on the serotonergic cell bodies and dendrites in the brainstem raphe nuclei, and as a post-synaptic heteroreceptor in the terminal field on non-serotonergic neurons as a target of 5-HT neuron projections (Garcia-Garcia et al., 2014).

The 5-HT_{1A} receptor and 5-HTT play important roles in the serotonin signaling, the 5-HT_{1A} autoreceptor regulating serotonin neuron resting potential (and hence propensity to generate action potentials and release serotonin at the terminal fields), and 5-HTT mediating serotonin clearance (Hensler, 2012). We therefore sought to examine the relationship between the somatodendritic 5-HT_{1A} autoreceptor and terminal field 5-HTT *in vivo* in healthy volunteers. We expected that greater density of 5-HT_{1A} autoreceptors would result in reduced firing rates of serotonin neurons, and thereby decreased 5-HT release, which in turn would reduce mean 5-HTT availability for binding due to increased 5-HTT internalization. This model would suggest an inverse relationship between 5-HT_{1A} autoreceptor binding in raphe nuclei and 5-HTT binding in the terminal fields.

Empirical studies investigating the relationship between 5-HT_{1A} and 5-HTT report divergent findings. A postmortem study of brain samples from suicide victims found a negative correlation between 5-HT_{1A} and 5-HTT binding within prefrontal cortex (Arango et al., 1995), consistent with the theoretical mechanism described above. A PET imaging genetics study found no relationship between a functional polymorphism in the 5-HT_{1A} receptor gene promoter region [(−1018) C>G] (shown to be involved in regulating the transcription of 5-HT_{1A}) and 5-HT_{1A} binding, but observed lower 5-HT_{1A} binding potential in all regions examined in short allele carriers of the a 5-HTT gene polymorphism (5-HTTLPR) (David et al., 2005), suggesting complex interactions between the genetic variation and protein expression across these two loci.

Animal studies have investigated 5-HTT/5-HT_{1A} relationships using transgenic approaches. Monoamine oxidase-A (MAO-A) knockout mice, which have increased levels of extracellular 5-HT, exhibit downregulation of 5-HTT and a concurrent desensitization of 5-HT_{1A} autoreceptors (Evrard et al., 2002). Studies of 5-HTT knockout mice have variably observed decreased 5-HT_{1A} receptor mRNA levels and protein density in the dorsal raphe nuclei and hypothalamus; increased levels in the hippocampus, and no differences in other high-density projection areas (Alexandre et al., 2006; Fabre et al., 2000; Li et al., 1999). These studies suggest that the absence of 5-HTT may cause a complex pattern of differential responses in different subpopulations of 5-HT_{1A} receptors.

In vivo human brain imaging studies examining the relationship between 5-HTT and 5-HT_{1A} are also inconclusive. One study (Lundberg et al., 2007) used [^{11}C]WAY-100635 for quantification of the 5-HT_{1A} receptor and [^{11}C]MADAM for quantification of 5-HTT and found a positive correlation between 5-HT_{1A} and 5-HTT BP_{ND} in the raphe nuclei and the hippocampus but not in frontal cortical areas. Another study (Jovanovic et al., 2008) found 5-HT_{1A} and 5-HTT BP_{ND} to be positively correlated only in hippocampus and only in women using [^{11}C]WAY-100635 and [^{11}C]MADAM. In contrast, a study of seventeen healthy male subjects found negative correlations between 5-HTT and 5-HT_{1A} BP_{ND} within multiple brain regions (cingulate, insula, frontal, temporal and parietal cortices) using [^{11}C]WAY-100635 and [^{11}C]DASB (Takano et al., 2011). Another study (Bose et al., 2011) used [^{11}C]WAY-100635 and [^{11}C]DASB and found no intra-regional correlations after controlling for relevant covariates, but a positive correlation between [^{11}C]WAY-100635 BP_{ND} in the raphe and [^{11}C]DASB BP_{ND} in the superior temporal gyrus and insula. In summary, these studies observed positive, negative, and no correlations between *in vivo* 5-HTT and 5-HT_{1A} binding using PET. Our model predicts a negative correlation.

In this manuscript, we have examined the relationship between 5-HT_{1A} and 5-HTT binding in a large sample of forty healthy volunteers who underwent PET using [^{11}C]WAY-100635 and [^{11}C](+)-McN-5652 in a secondary analysis of previously published data (Parsey et al., 2006a; Parsey et al., 2006c). Based on our previous post-mortem findings and the theoretical model described above, we expected to find negative correlations between 5-HT_{1A} in the raphe nuclei (RN) and 5-HTT in serotonergic projection areas: thalamus, hippocampus, caudate, amygdala, temporal lobe and insula. Prefrontal cortical regions were not included in this analysis, despite being serotonin projection areas, because quantification of transporter binding in these regions with [^{11}C](+)-McN-5652 is not reliable due to inadequate signal-to-noise ratio.”. We also performed an exploratory voxel-wise whole-brain analysis to examine the relationship between 5-HT_{1A} autoreceptor binding in the RN and 5-HTT binding throughout the brain.

2. Materials and methods

2.1. Subjects

Forty healthy volunteers (21 females; 19 males) who had no history of an Axis I or Axis II psychiatric disorder (including absence of current or past alcohol or substance abuse or dependence), no family history of a mood disorder or schizophrenia, no significant medical illness, free of all medications that may affect the brain or specifically the serotonin system,

and aged 18–69 years (mean = 39 +/− 15) were included in this analysis. The Structured Clinical Interview for DSM-IV Axis I Disorders (non-patient version- SCID NP) (First et al., 2012), physical examination, routine blood tests, pregnancy test, urine toxicology, and electrocardiogram were used to evaluate study eligibility. Other exclusion criteria included lack of capacity to provide informed consent and pregnancy. The Institutional Review Board of the New York State Psychiatric Institute approved the protocol, and all subjects provided informed consent after an explanation of the study protocol and associated risks.

2.2. Radiochemistry and input function measurement

All subjects were scanned with both [^{11}C]WAY-100635 (for quantification of 5-HT$_{1A}$) and [^{11}C](+)-McN-5652 (for quantification of 5-HTT) on the same day. For details of radiotracer preparation see (Parsey et al., 2000b) for [^{11}C]WAY-100635 and (Frankle et al., 2004) for [^{11}C](+)-McN-5652. A metabolite corrected arterial input function was obtained for both tracers (Parsey et al., 2000b). Plasma free fraction (f_P) of [^{11}C]-WAY-100635 was assayed in triplicate (Parsey et al., 2006b).

2.3. Image acquisition and analysis

All subjects underwent a T1-weighted magnetic resonance image (MRI) scan for identification of regions of interest (ROIs) and tissue segmentation into gray and white matter. T1 images were acquired with either a 1.5-T Signa Advantage or a 3-T Signa HDx (General Electric Medical Systems, Milwaukee, Wisconsin), at a resolution of 1.5 × .9 × 1.0 mm or 1.0 mm × 1.0 mm × 1.0 mm, respectively. PET images were acquired from an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, Tennessee) as previously described (Parsey et al., 2000b). A 15-minute transmission scan was obtained, and injection of [^{11}C]-WAY-100635 was followed by an emission scan of 110 minutes with 20 frames of increasing duration (3 × 20 s, 3 × 1 min, 3 × 2 min, 2 × 5 min, 9 × 10 min). After a break (mean break duration = 67 minutes, range = 28 to 129 minutes), subjects returned to the PET scanner and received a bolus injection of [^{11}C](+)-McN-5652 followed by a 130-minute emission scan also using frames of increasing duration (3 × 20 s, 3 × 1 min, 3 × 2 min, 2 × 5 min, 11 × 10 min). One participant underwent [^{11}C](+)-McN-5652 scanning prior [^{11}C]-WAY-100635 scanning for logistical reasons, with a break of 91 minutes between scans. To correct for subject motion, PET frames were registered to the eighth frame using the FMRIB linear image registration tool (FLIRT), version 5.0 (FMRIB Image Analysis Group, Oxford, UK). An automated algorithm identified ROIs (amygdala, dorsal caudate, hippocampus, insula, thalamus, temporal cortex) as well as reference regions of cerebellar gray matter (CGM) for [^{11}C](+)-McN-5652 and cerebellar white matter (CWM) for [^{11}C]-WAY-100635 on individuals' T1-weighted MRIs (Milak et al., 2010). The raphe nuclei ROI cannot be reliably identified on MRI images, so this region was labeled using a mask of the average location of the RN in 52 healthy subjects, which was created using [^{11}C]-WAY-100635 voxel binding maps warped into standard space (Delorenzo et al., 2013). Each subject's MRI was transformed into standard space using Advanced Normalization Tools (Avants et al., 2014), and the reverse transform was applied to the RN mask to bring it into MRI space. RN masks were then applied to the PET through the PET-to-MRI coregistration.

Each subject's mean PET image was co-registered to their MRI using FLIRT, optimized as previously described (DeLorenzo et al., 2009). ROI maps superimposed on MRI and PET images for a sample subject are shown in supplemental figure 1. Time activity curves were generated as the average activity measured across the voxels within each ROI over the time course of the acquisition.

2.3.1 Outcome measure estimation—Distribution volumes (V_T) of [^{11}C]WAY-100635 were estimated for each ROI using kinetic analysis with an arterial input function and a two tissue compartment constrained (2TCC) model (for more details, see (Parsey et al., 2000b)). Time activity curves were fit with a 2TCC model in which the K_1/k_2 ratio was constrained to that of the reference region (CWM). For [^{11}C]WAY-100635, BP_F was calculated as $(V_{T(ROI)} - V_{T(REF)})/f_P$. Derivation of [^{11}C](+)-McN-5652 regional V_T was performed using likelihood estimation in graphical approach (LEGA) (Ogden, 2003; Ogden et al., 2002; Parsey et al., 2003). As f_P cannot be reliably measured for [^{11}C](+)-McN-5652, the outcome measure BP_P was estimated as $(V_{T(ROI)} - V_{T(REF)})$, with CGM as the reference region. For both tracers, for comparison with other published studies, we also calculated BP_{ND} as $(V_{T(ROI)} - V_{T(REF)})/ V_{T(REF)}$.

2.3.2 Voxel-level image generation—For [^{11}C](+)-McN-5652, BP_P was also estimated at the voxel-level for each participant, to generate parametric maps of 5-HTT binding. Briefly, V_T parametric images were obtained by applying empirical Bayesian estimation in graphical analysis (EBEGA) (Zanderigo et al., 2010), a fully automatic approach that incorporates LEGA (Ogden, 2003) estimation of V_T in a Bayesian framework. Once the V_T parametric images were obtained for each subject using EBEGA, the corresponding BP_P images were generated by subtracting the mean reference region voxel value ($V_{T(REF)}$) from each target voxel (Parsey et al., 2000a).

2.4. Statistics

The associations between 5-HT_{1A} binding measures in the raphe and 5-HTT binding in the regions of interest (thalamus, hippocampus, caudate, amygdala, temporal lobe, and insula; selected because of high 5-HTT binding) were examined using a linear mixed effects model with subject as the random effect and region and sex as fixed effects. To correct for some slight skewness in the data, to stabilize the variance across regions, and to allow for a proportional effect across regions, the analysis was performed on log-transformed estimates of 5-HTT binding. The observations were weighted according to standard errors that take into account variation in PET data, plasma data, and metabolite data (Ogden and Tarpey, 2006).

[^{11}C](+)-McN-5652 BP_P voxel images were analyzed using Statistical Parametric Mapping software (SPM8; Institute of Neurology, University College of London, London, England) implemented in Matlab2009b (The Mathworks Inc, Natick, Mass). Voxel images were transformed into standard space as described under image acquisition and analysis. A standard space gray matter mask from the FSL toolbox was applied to consider only gray matter voxels, and the images were smoothed using a Gaussian filter with a kernel of 5 mm. A two-tailed voxel-by-voxel t-test was performed using [^{11}C]WAY-100635 BP_F within the

RN as a regressor and sex as a covariate, looking for correlations with 5-HTT BP_P. A statistical threshold was set to $p < 0.01$ a priori, and extent threshold was set to $p < 0.05$ after correction for multiple comparisons.

3. Results

3.1. Correlations between [^{11}C]WAY-100635 BP_F and [^{11}C](+)-McN-5652 BP_P

No significant effect of [^{11}C]-WAY-100635 was found on [^{11}C](+)-McN-5652 binding across the *a priori* ROIs after accounting for sex (F=0.00; df=1, 37; p=0.99). Figure one shows the relationship between [^{11}C]-WAY-100635 BP_F in raphe nuclei and [^{11}C](+)-McN-5652 BP_P in a representative region (thalamus).

To test whether there was a heterogenous relationship between WAY raphe binding and McN binding across the *a priori* regions, we considered an interaction term with region, which was also not significant (F = 0.44; df = 7, 226; p = 0.88). Similarly, post-hoc testing for relationships between WAY raphe binding and McN binding in each individual ROI were also not significant (all p>0.54).

3.2 Voxel-level Results

At the voxel level, there were no clusters of 5-HTT BP_P that correlated with raphe 5-HT_{1A} BP_F at our a priori statistical threshold (p>.01 for all clusters, uncorrected).

3.3. Other analytic approaches

In order to accurately replicate approaches from past literature and because of controversy in the field regarding optimal outcome measures for these radiotracers (Parsey et al., 2010), data was re-analyzed comparing 5-HT_{1A} BP_{ND} vs. 5-HTT BP_{ND} and then again comparing 5-HT_{1A} BP_P vs. 5-HTT BP_P. Consistent with our primary findings, no significant effect of raphe 5-HT_{1A} binding on 5-HTT binding in the selected regions was observed (F=1.50; df=1, 37; p=0.23 for BP_P and F=0.33; df=1, 37; p=0.57 for BP_{ND}).

Although we have not previously found an effect of age on [^{11}C]WAY-100635 or [^{11}C](+)-McN-5652 binding (Parsey et al., 2002), other groups have found such an effect (Tauscher et al., 2001). Including age as a co-variate in the current analyses did not affect the significance of the results reported across all outcome measures.

4. Discussion

In this reanalysis of this large dataset of 40 healthy volunteers who underwent quantification of 5-HT_{1A} and 5-HTT binding, we did not observe an association between regional 5-HT_{1A} in RN and 5-HTT binding in *a priori* terminal field ROIs. We also did not find an association between 5-HT_{1A} binding in the RN and 5-HTT binding in the rest of the brain in an exploratory voxel-wise whole-brain analysis. These data do not support our hypothesis of inverse intra- and inter-regional 5-HT_{1A} – 5-HTT binding relationships. These findings were not affected by removing variance attributable to sex and age differences or by the use of alternative PET outcome measures.

In contrast to our results, several studies have found correlations between 5-HT_{1A} and 5-HTT, some positive (Bose et al., 2011; Jovanovic et al., 2008), and others negative (Takano et al., 2011). In contrast to our previously described model of responses positing a negative correlation, a positive correlation between 5-HTT and 5-HT_{1A} binding might be expected if 5-HTT genetic variation is primary: for example, lower 5-HTT expression and protein and would lead to increased intrasynaptic 5-HT, with resultant downregulation of the 5-HT_{1A} autoreceptor.

Lundberg et al., who also found significant positive correlations within the RN in an all male sample, built on the explanation of Bose et al. in saying that subtle, temporally synchronized covariation in expression of 5-HTT and 5-HT_{1A} autoreceptors on neurons in the raphe is feasible because both proteins exist on the same neuron in this region (Bose et al., 2011; Hensler, 2012; Lundberg et al., 2007). However, the 5-HT_{1A} receptors and 5-HTT proteins are expressed by different neuron types in the prefrontal cortex and other target brain regions, with the 5-HT_{1A} in these regions existing as post-synaptic heteroreceptors on and around the axon hillock of glutamatergic pyramidal neurons or the dendrites of GABAergic interneurons and the 5-HTT located pre-synaptically on axon terminals of serotonin neurons whose cell bodies reside in RN.

One possible interpretation for the lack of an observed correlation between *in vivo* 5-HT_{1A} and 5-HTT binding is that regulatory responses of these proteins may occur according to different time scales. 5-HTT appears to be rapidly regulated to appropriately match extracellular levels of serotonin, and up-or down-regulates within hours as a function of available 5-HT, as demonstrated by a tryptophan depletion experiment (Milak et al., 2005). In that study, baboons underwent [^{11}C]DASB PET scans at baseline and after acute tryptophan depletion. 5-HTT binding decreased as a function of tryptophan and subsequent serotonin depletion (which occurred within 6 hours of scanning), demonstrating both the high sensitivity of SERT to serotonin levels and the rapidity of internalization. This is consistent with *in vitro* work demonstrating that 5-HT stabilizes 5-HTT at the cell membrane, preventing phosphorylation and internalization (Ramamoorthy and Blakely, 1999; Whitworth et al., 2002). Some conflicting evidence does exist from human imaging studies of tryptophan depletion, however (Praschak-Rieder et al., 2005). In contrast to the animal and *in vitro* work suggesting rapid responsiveness of 5-HTT to 5-HT, 5-HT_{1A} receptors appear to have a slower time-course of response to serotonin levels as evidenced by the delayed action of SSRI antidepressants, which take weeks to produce desensitization of the autoreceptors (Blier and de Montigny, 1999; Gardier et al., 1996). Consistent with those animal findings, human PET studies have demonstrated that 5-HT_{1A} binding is down-regulated by several weeks of SSRI treatment (Gray et al., 2013), but is not affected by acute SSRI administration (Pinborg et al., 2012). The lack of correlation between 5-HTT and 5-HT_{1A} binding at both the ROI and voxel-level in the current analysis may therefore reflect different time courses of response to changes in 5-HT levels, leading to a de-coupling of 5-HTT and 5-HT_{1A} protein levels.

Another form of interaction between elements in the serotonin neurotransmitter system are gene-protein relationships, eg. between functional polymorphisms in genes within the serotonergic system and protein binding potential of proteins within the serotonin system as

assessed by PET. For example, a recent study demonstrated an interaction effect of the 5-HTTLPR polymorphism in the SERT gene with a polymorphism at the rs6296 locus in the serotonin 1B receptor gene on 5-HT_{1A} BP_{ND} measured by [^{11}C]WAY-100635 (Baldinger et al., 2015). As genotyping at these loci was available in this sample, we performed an exploratory analysis, examining relationships between genotype at these loci and [^{11}C](+)-McN-5652 or [^{11}C]WAY-100635 binding in the *a priori* ROIs in this study; no significant relationships were observed (all $p>0.05$, uncorrected for multiple comparisons). In addition, including genotype at these loci as covariates in the primary analyses of the paper (examining the relationship between [^{11}C](+)-McN-5652 and [11C]WAY-100635 binding) did not affect the significance of reported results.

One potential limitation of the present design relates to the radiotracers used. [^{11}C]WAY-100635 is antagonist tracer that binds to both the high and low affinity states of the 5-HT_{1A} receptor, as opposed to an agonist that binds specifically to the subset of high affinity, active receptors (Kumar et al., 2012). [^{11}C](+)-McN-5652 has suboptimal imaging characteristics, including low specific-to-nonspecific binding ratios and an extended acquisition time required to obtain stable binding estimates (Parsey et al., 2000a), but it was used in this study because it was the tracer for which the largest sample of data for healthy volunteers was available. A second limitation of [^{11}C](+)-McN-5652 is that it has too little specific binding to prefrontal cortices or anterior cingulate to permit reliable quantification of protein density in those brain regions, although specific prefrontal cortical binding is also low with other 5-HTT radiotracers, including [^{11}C]DASB. Alternative methods for quantification of 5-HTT also could have been considered, such as quantification of the ratio of 5-HTT binding in the terminal field region of interest to that in the raphe nuclei (Savli et al., 2012).

A third limitation was the scanner resolution. In this analysis we looked at the RN as one region, but it might have been preferable to look at the dorsal and medial raphe nuclei separately. These two regions have different terminal regions, projecting parallel and overlapping fibers, with their axonal fibers displaying distinct traits (Hornung, 2003). Axons of the dorsal raphe do not form true chemical synapses, but rather release serotonin in a paracrine manner, whereas serotonergic projections from the median raphe form abundant, closely spaced synapses at the terminal fields (Hornung, 2003). Serotonergic neurons originating from the dorsal raphe project primarily to prefrontal, parietal, occipital and temporal cortex, hippocampus, amygdala and striatum (Hensler, 2012; Hornung, 2003). In contrast, median raphe axonal fibers are largely restricted to midline subcortical structures (e.g. dentate gyrus, hippocampus), with only sparse projections to cortex (Hensler, 2012). The small size of these structures in comparison to the spatial resolution of PET, and the associated challenges of partial volume effects and signal-to-noise limitations, makes distinguishing between regions as small as the median and dorsal raphe a technical challenge; we therefore examined binding within a larger ROI encompassing both dorsal and median raphe nuclei. Partial volume effects and signal-to-noise limitations may also have impacted the reliability of our quantification within the entire raphe nuclei ROI employed here, possibly contributing to the lack of observed correlations. Future studies with improved PET resolution may allow dissection of the raphe nuclei into their functionally distinct components.

The large age range in the sample was an additional limitation; however, repeating our analyses with age in the model did not impact any of the reported findings. Finally, with the sample size employed in this study (n=40), we had 80% power to detect a correlation of r=0.43. We were therefore underpowered to detect correlations of smaller magnitudes than this, raising the possibility of Type II error.

5. Conclusions

We do not observe a systematic relationship between *in vivo* 5-HT_{1A} and 5-HTT binding in a large sample of healthy volunteers. The relationship between these components of the serotonin neurotransmitter system is more complex and may not be measureable with the current cross-sectional design. We suspect this complexity is related to the temporal de-coupling of 5-HT_{1A} and 5-HTT due to different regulatory mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Brain serotonin transporter (5-HTT) and serotonin 1A receptor were quantified via PET.
- Serotonin 1A receptor and 5-HTT binding are not correlated in *a priori* regions.
- This suggests different regulatory control for these proteins in the serotonin system.

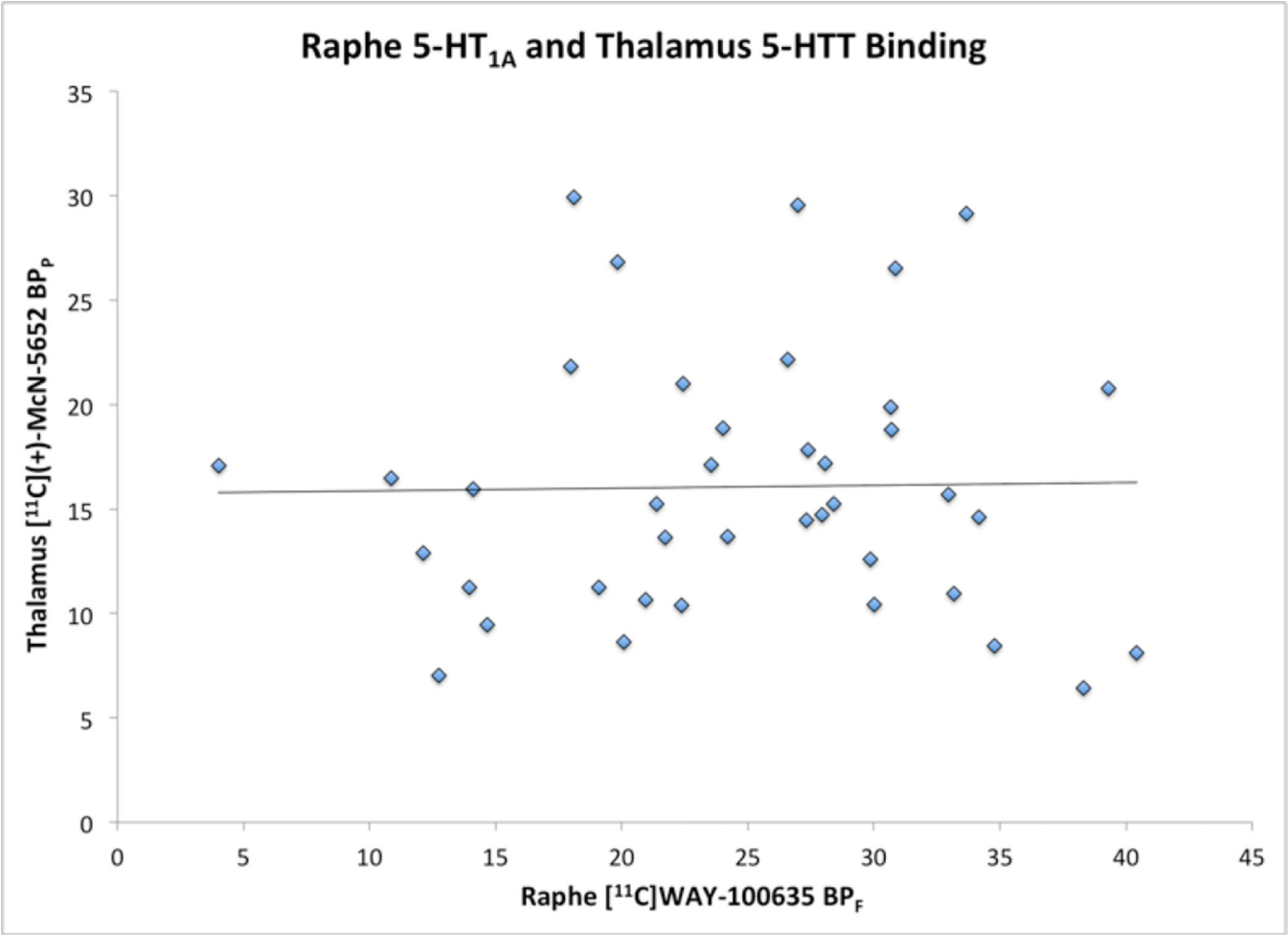

Figure 1.

Scatter plot showing relationship between raphe 5-HT_{1A} binding ([^{11}C]WAY-100635) and 5-HTT binding in a representative terminal field region (thalamus). No significant correlation is observed (see results).