5-HTTLPR status is associated with cerebral [^{11}C]SB207145 binding as measured *in vivo* in humans

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Summary

5-HTTLPR S carriers show lower 5-HT$_4$ binding assessed with [^{11}C]SB207145 PET in healthy volunteers within caudate and neocortex. In light of previous studies indicating [^{11}C]SB207145 binding may reflect chronic 5-HT signaling, our novel findings provide *in vivo* support for 5-HTTLPR status being associated with altered 5-HT neurotransmission in healthy adults.

Introduction

•Serotonin (5-HT) is a neuromodulator affecting myriad aspects of personality and behavior including emotional processing, sleep, eating and learning and memory. Identifying sources of individual variation in aspects of serotonin signaling is critical for further understanding how such variation emerges and may in turn confer risk for affective disorders and moderate sensitivity to various treatment strategies (e.g., selective-serotonin reuptake inhibitors).

•Animal studies have suggested that 5-HT$_4$ receptor binding exhibits a monotonic response to chronic changes in 5-HT signaling [1,2]. Our lab has recently validated [^{11}C]SB207145 as a PET radioligand for *in vivo* quantification of 5-HT$_4$ binding in humans [3]. As such, [^{11}C]SB207145 PET binding in humans may reflect individual variation in 5-HT signaling and thus reflect an integral feature of serotonergic neurotransmission.

•Putative effects of the 5-HTTLPR polymorphism on serotonin transporter (5-HTT) transcription (i.e., "L" allele displays increased 5-HTT transcription relative to "S" allele *in vitro*) suggest it may contribute to individual differences in serotonin neurotransmission. However, the effects of 5-HTTLPR status on the serotonin system in humans, *in vivo*, are not fully understood.

•We sought to evaluate whether 5-HTTLPR status was associated with [^{11}C]SB207145 binding in a cohort of healthy adult volunteers. Considering the putative effects of the 5-HTTLPR on serotonin signaling, we hypothesized that S allele carriers would show reduced [^{11}C]SB207145 binding compared to LL homozygotes.

Methods

Participants

•47 healthy participants (35M) completed an [^{11}C]SB207145 PET scan session (age: 34.9 ± 17.7 (mean ± s.d.); 26.9 (median); 20-86 (range))

•Exclusion criteria included: 1) primary psychiatric disease, 2) substance abuse, 3) severe systemic or neurological disease based on history and physical examination.

[^{11}C]SB207145 PET scan

•120 minute [^{11}C]SB207145 PET scan acquired on either 1) GE Advance PET scanner or 2) HRRT scanner. All scans were acquired in 3D mode with scan time frames: 6x5, 10x15, 4x30, 5x120, 5x300, and 8x600 seconds. A high-resolution MP-RAGE (voxel-size: 1x1x1 mm) was acquired on a 3T Siemens Magnetom Trio MR scanner. Segmentation of the MP-RAGE was performed with SPM5.

•PET images were corrected for motion and co-registered to MR images. Regions of interest were automatically delineated using the previously validated Svarer method [4].

•*In vivo* 5-HT$_4$ receptor binding potential was calculated based on regional time activity curves and the Simplified Reference Tissue Model. The primary outcome measure was regional $\boldsymbol{BP_{ND}}$. This measure is proportional to f_{ND} (non-protein-bound fraction of non-displaceable binding in brain tissue), B_{avail} (number of receptors available for binding) and K_D (the dissociation constant). Kinetic modeling was performed using PMOD.

5-HTTLPR Genotype

•5-HTTLPR genotype was evaluated from blood samples obtained at time of PET scan. Distribution of genotypes were in Hardy-Weinberg equilibrium (L allele frequency = 0.55, S allele frequency = 0.45; X^2 = 0.13, p = 0.72).

•Regional binding measures were log-transformed prior to statistical analysis. This was based on comparison of the cumulative residual processes of the model fit with and without transformed regional binding measures (Cramér-von Mises test).

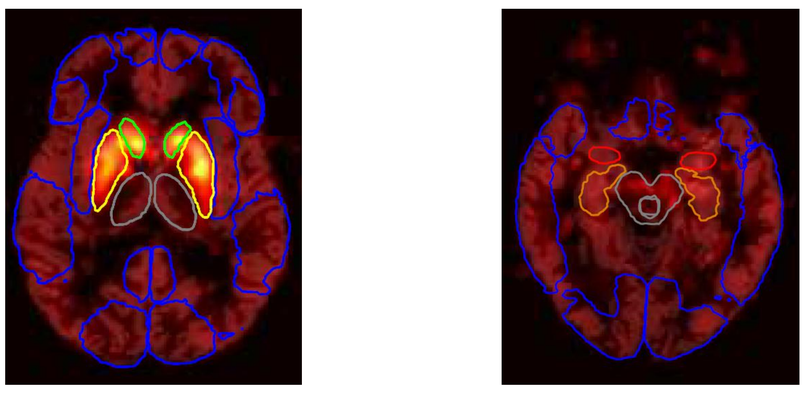

Figure 1. Axial slices of single-subject parametric [^{11}C]SB207145 BP_{ND} PET image with delineated regions (caudate, green; putamen, yellow; amygdala, red; hippocampus, orange; neocortical subregions, blue; delineated but unexamined regions, gray).

Results

Demographics

	L/L	S carriers
N	15	32 (10 SS)
Gender (M/F)	10/5	25/7
Scanner (Adv/HRRT)	9/6	10/22
Age	45.7 ± 21.3	29.9 ± 13.3
BMI	24.4 ± 2.38	23.2 ± 2.26
Inj. Mass µg (range)	2.8 (0.1 - 5.1)	1.9 (0.2 - 5.9)
Inj. Mass µg/kg (range)	0.04 ± 0.02	0.02 ± 0.02

Mean [^{11}C]SB207145 binding

Region	[^{11}C]SB207145 BP_{ND}
Amygdala	0.85 ± 0.18
Caudate	2.97 ± 0.83
Hippocampus	0.95 ± 0.20
Neocortex	0.55 ± 0.19
Putamen	3.03 ± 0.74

Model selection

•Consistent with previous studies, we observed evidence of a correlation between multiple variables and [^{11}C]SB207145 binding including age, gender, BMI, scanner and injected mass. The effect of genotype on regional binding remained consistent when various linear models were evaluated. Results reported here reflect a linear model including each of these as covariates.

5-HTTLPR genotype associated with [^{11}C]SB207145 binding

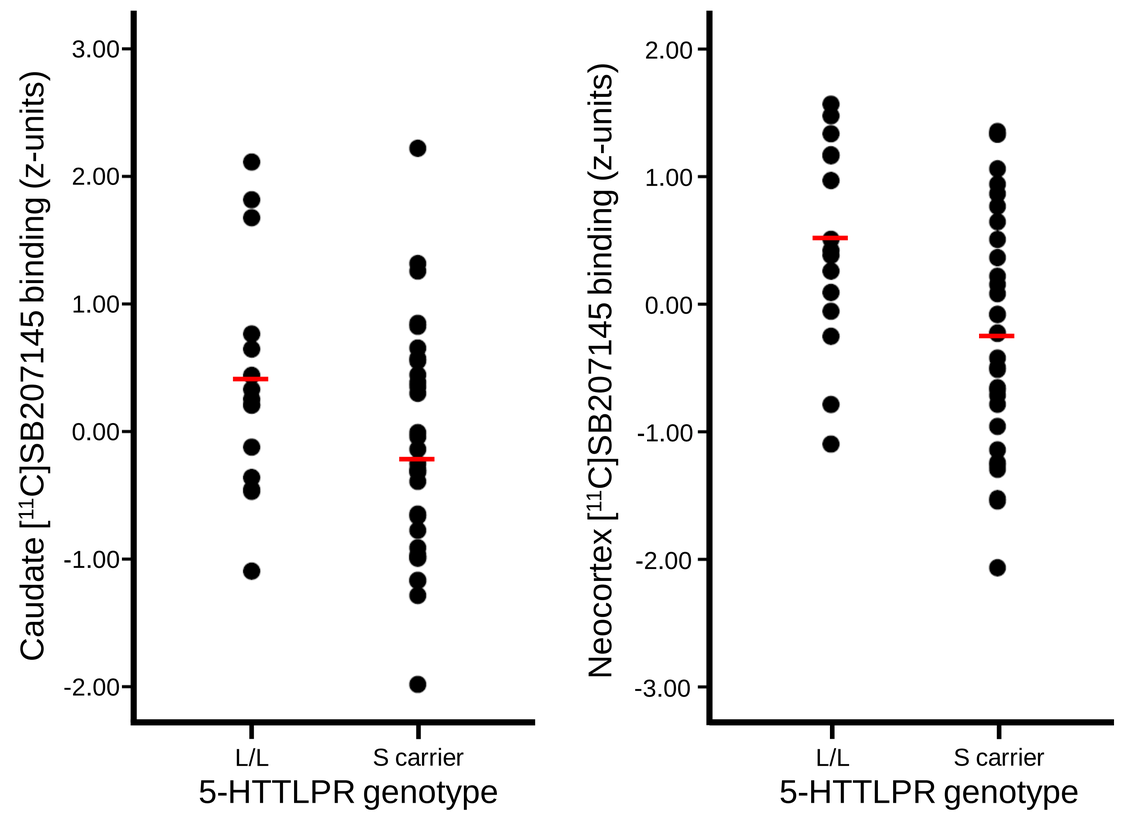

Figure 2. 5-HTTLPR genotype status associated with [^{11}C]SB207145 binding in caudate and neocortex. S carriers show significantly reduced [^{11}C]SB207145 binding relative to L/L homozygotes. To illustrate the observed genotype effect, plots reflect standardized residuals of regional [^{11}C]SB207145 binding after correcting for effects of age, gender, BMI, scanner and injected mass (µg/kg).

Effect of genotype on all regions

	β estimate	β 95% CI	t-statistic	p-value
Amygdala	-0.002	[-0.124;0.120]	-0.03	0.98
Caudate	-0.088	[-0.172;-0.003]	-2.08	**0.044**
Hippocampus	0.016	[-0.078;0.110]	0.35	0.73
Putamen	-0.037	[-0.113;0.039]	-0.97	0.34
Neocortex	-0.100	[-0.176;-0.024]	-2.63	**0.012**

*Parameter estimates reflect a linear model including age, gender, BMI, scanner and injected mass (µg/kg) as covariates

Discussion

•Our results support an association between 5-HTTLPR genotype status and [^{11}C]SB207145 binding. Consistent with our hypothesis we found that S carriers showed reduced binding within caudate and neocortex (8% and 10% reduction in binding, respectively). We did not find support for an effect of 5-HTTLPR on binding within subcortical regions including the amygdala, hippocampus and putamen.

•Previous studies have reported that [^{11}C]SB207145 binding displays a monotonic response to chronic changes in 5-HT signaling. The 5-HTTLPR polymorphism putatively modulates serotonin signaling by impacting efficacy of 5-HTT transcription. Taken together, our findings provide *in vivo* support for an effect of 5-HTTLPR status on serotonin signaling as reflected by [^{11}C]SB207145 binding.

•A limitation of our study is the heterogeneity in age, scanner type and other variables found to be associated with [^{11}C]SB207145 binding. However, our observed genetic effects remained significant when including these variables in our linear model suggesting the effect of genotype is not driven by sample heterogeneity.

•These findings provide novel evidence that the 5-HTTLPR polymorphism biases features of serotonin signaling in humans.

References: [1] Licht CL et al., 2009 *J Neurochem* 109:1363-1374. [2] Jennings K et al., *in press Int J Neuropsychopharm*. [3] Marner L et al., 2009 *J Nucl Med* 50:900-908. [4] Svarer C 2005 *Neuroimage* 24: 969-979.