Seasonal Changes in Brain Serotonin Transporter Binding in Short Serotonin Transporter Linked Polymorphic Region-Allele Carriers but Not in Long-Allele Homozygotes

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Background: A polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) has been associated with seasonality both in patients with seasonal affective disorder and in the general population.

Method: We used in vivo molecular imaging to measure cerebral serotonin transporter (5-HTT) binding in 57 healthy Scandinavians and related the outcome to season of the year and to the 5-HTTLPR carrier status.

Results: We found that the number of daylight minutes at the time of scanning correlated negatively with 5-HTT binding in the putamen and the caudate, with a similar tendency in the thalamus, whereas this association was not observed for the midbrain. Furthermore, in the putamen, an anatomic region with relatively dense serotonin innervation, we found a significant gene × daylight effect, such that there was a negative correlation between 5-HTT binding and daylight minutes in carriers of the short 5-HTTLPR allele but not in homozygote carriers of the long allele.

Conclusions: Our findings are in line with S-carriers having an increased response in neural circuits involved in emotional processing to stressful environmental stimuli but here demonstrated as a endophenotype with dynamic changes in serotonin reuptake.

Key Words: 5-HTTLPR, DASB, endophenotype, s-allele, seasonality, serotonin transporter

Light deprivation is a known environmental stressor in Scandinavia and other regions of high latitude; in a large fraction of the northern population, behavioral changes such as reduced physical activity, higher food intake, and increased urge to sleep (1) are frequently noted during months with few or no daylight minutes in the depths of winter. In vulnerable people, this environmental stress can provoke a particular form of mood disorder, termed seasonal affective disorder (SAD), which is characterized by the occurrence of depressive symptoms in winter. This observation led to the successful development of rational and efficient treatment with bright light therapy (2).

Several findings suggest that SAD is mediated through the serotonin (5-HT) system. For example, 5-HT concentrations are lowest in post-mortem brain samples from people dying in the winter (3). Also, the concentration of the serotonin metabolite 5-hydroxyindoleacetic acid is lower in jugular blood samples collected in the winter (4). These biomarkers may be associated with seasonal changes in the activity of plasma membrane serotonin transporter (5-HTT), a molecular entity that serves a central role in cerebral serotonin transmission by regulating interstitial 5-HT levels (5,6). A few studies have investigated seasonal variation in vivo in cerebral 5-HTT binding as measured with molecular imaging techniques. One published study reported lower binding (7), whereas another study reported higher binding (8) in winter compared with summer. However, both of these studies suffered from small sample sizes and questionable criteria for the cutoff between summer and winter and employed radioligands of lesser specificity (and sensitivity) for 5-HTT imaging than those currently available. In a large Canadian sample of 88 healthy participants investigated with [^{11}C]DASB and positron emission tomography (PET), a seasonal fluctuation in cerebral 5-HTT binding, lowest in summer and highest in winter, was observed (9). This effect was also observed in a single photon emission computed tomography (SPECT) study that included 49 depressed patients and 49 control subjects (10).

A link between 5-HTT and SAD has been identified in that carriers of a 44-base pair deletion in the 5-HTT-linked polymorphic region (*short* 5 − HTTLPR allele = *S-allele*) generally are more vulnerable to SAD than carriers of the insertion (*long* 5 − HTTLPR allele = *L-allele*) (11). This polymorphism influences 5-HTT expression, with lower expression occurring in carriers of the *S-allele* (5). In comparison with *L-allele* homozygotes, *S-allele* carriers have increased propensity to develop mood disorders in response to stressful environmental cues (12,13), in particular, in response to the transition to winter (11,14). Furthermore, results of functional magnetic resonance imaging (fMRI) studies revealed that exposure to stressful and fearful environmental cues evoke greater activation of limbic brain structures in *S-allele* carriers (15,16). However, there was no in vivo difference in cerebral 5-HTT binding between carriers of the *S-allele* and *L-allele* homozygotes within a group of 96 healthy participants

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(17) investigated by SPECT, nor was there any difference discernible in a group of 42 healthy participants (18) investigated with PET. Only one SPECT study reported lower 5-HTT binding in eight healthy control subjects (19). Two subsequent imaging studies did demonstrate an association between in vivo 5-HTT binding and 5-HTTLPR genotype (20,21) but only when the participants were stratified according to an additional single nucleotide polymorphism (SNP) in the area of the *L-allele*; this triallelic stratification had not been employed in most fMRI activation studies (6,16).

To attempt to resolve the discrepant findings, we investigated in a group of healthy Scandinavians whether there were any seasonal effects on cerebral 5-HTT binding in vivo as measured with [^{11}C]DASB-PET. To accommodate a potential time delay in any seasonal effect, given that 5-HTT expression might not respond immediately to seasonal changes, we applied a harmonic statistical model. In the next step we tested for a gene X daylight effect, hypothesizing that the seasonal effect is more pronounced in carriers of the *S-allele*.

Methods and Materials

Participants

Fifty-seven healthy participants (55 white, 1 half-white/half-Inuit, and 1 half-white/half-Asian, according to the National Institutes of Health race ethnicity criteria) were recruited by advertisement for a research protocol approved by the Ethics Committee of Copenhagen and Frederiksberg, Denmark [(KF) 01-156/04, (KF) 01-124/04, and (KF) 11-283038]. After complete description of the study to the participants, written informed consent was obtained from 37 males with a mean age of 34 years (SD = 18 years) and 20 females with a mean age of 34 years (SD = 20 years). Exclusion criteria for all participants included medical history, drug abuse, or psychiatric disorders. All participants had normal neurologic examinations, and a structural magnetic resonance (MR) imaging of the brain was without pathologic findings.

PET Scans

PET scans were performed with an 18-ring GE-Advance scanner (General Electric, Milwaukee, Wisconsin), operating in three-dimensional acquisition mode and producing 35 image slices with an interslice distance of 4.25 mm. Following a 10-min transmission scan, a dynamic 90-min emission recording was initiated upon intravenous injection during 12 sec of mean 487 (SD 90) MBq (range: 246–601) [^{11}C] DASB with mean specific activity of 32 (SD 16) GBq/μmol (range: 9–82). The emission recording consisted of 36 frames, increasing progressively in duration from 10 sec to 10 min. The attenuation- and decay-corrected recordings were reconstructed by filtered back projection using a 6-mm Hann filter. Daylight minutes on the day of the PET-scan at the latitude of Copenhagen were computed based on data at http://aa.usno.navy.mil/data/docs/Dur-OneYear.php as the time between sunrise and sunset.

Magnetic Resonance Scans

Structural brain scans were acquired on a Siemens Magnetom Trio 3-T MR scanner with an eight-channel head coil (In vivo, Florida). Thirty-six participants underwent a high-resolution 3D T1-weighted sagittal magnetization-prepared rapid gradient echo (MPRAGE) scan of the head (MPRAGE1: echo time (TE)/repetition time (TR)/inversion time (TI) = 3.93/1540/800 msec; slice resolution = 75%; bandwidth = 130 Hz/Px; echo spacing = 9.8 msec), and 21 participants underwent a three-dimensional T1-weighted sagittal MPRAGE (MPRAGE2: TE/TR/TI = 3.04/1550/800 msec; slice resolution = 100%; bandwidth = 170 Hz/Px; echo spacing = 7.7 msec). Common to both MPRAGE acquisitions was a flip angle of 9°, a field of view of 256 mm, a matrix of 256 × 256, 1 × 1 × 1 mm voxels, and 192 slices.

MR and PET Coregistration

All time frames of the attenuation-corrected emission recording were automatically aligned to frame 26 using the Automate Image Registration (AIR) algorithm (http://bishopw.loni.ucla.edu/AIR5/). In the next step, we calculated the mean PET image for frames 10–36 for coregistration to the individual MR image, again using the AIR algorithm. The quality of each coregistration was controlled visually. In three cases, coregistration between PET and MR image was corrected manually. Partial volume correction was performed as described in detail previously (22,23).

Volume of Interest Analysis

The volumes of interest (VOIs) were delineated automatically as described in Svarer *et al.* (24) to identify the brain volumes in a user-independent fashion. A template set of 10 MRIs is automatically coregistered to a new participants' MRI. The identified transformation parameters are used to define VOIs in the new participants' MRI space, and through coregistration, these VOIs are transferred onto the PET images. From the VOI sets, a probability map was created for each participant, and the final VOI set was generated by applying a fixed threshold. These VOI sets were then used for automatic extraction of time activity curves (TAC) for midbrain and volume-weighted (left–right) averages for bilateral thalamus, caudate, putamen, and cerebellum. The midbrain, the caudate, the putamen, and the thalamus were selected as representative brain regions of homogenous (at this resolution), high 5-HTT binding (25), with pooling of left and right, to test for a global effect.

Quantification of Nondisplaceable Tracer Binding

The outcome parameter of [^{11}C]DASB binding within a brain region is the nondisplaceable binding potential, designated BP_{ND}. The BP_{ND} was calculated for the four VOIs using the cerebellum (excluding vermis) input as a reference region. We used a modified reference tissue model designed specifically for quantification of [^{11}C]DASB (Multilinear Reference Tissue Model [MRTM/MRTM2]) as described and evaluated by Ichise *et al.* (26) using the PKIN tool of the software PMOD version 2.9 (www.pmod.com). A fixed washout constant, designated k_2', was calculated for each individual as an average of k_2 in caudate, putamen, and thalamus relative to cerebellum using MRTM. Subsequently, k_2' was used as a constrained input parameter for the calculation of BP_{ND} in the four VOIs relative to cerebellum.

Parametric Images

Parametric images representing BP_{ND} for each voxel were calculated for all participants from the dynamic PET images. Using the PXMOD tool of the PMOD software, we imported the VOIs, generated as described earlier, and recalculated k_2' relative to cerebellum using MRTM. R1 is defined as the relative tracer delivery to a VOI relative to that in cerebellum, the magnitude of which is to some extent related to the regional cerebral blood flow. As described by Ichise *et al.* (26), we applied a threshold of .3 to R1 to exclude voxels with noisy TACs, as was particularly seen in regions with low tracer uptake. Additionally, we constrained the BP_{ND} outcome to a physiologically plausible range