Seasonal changes in brain serotonin transporter binding in short 5-HTTLPR-allele carriers but not in long-allele homozygotes

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Light deprivation is a known environmental stressor in Scandinavia and other regions of high latitude; in a large fraction of the population, behavioral changes such as reduced activity, higher food-intake, and increased urge to sleep take place during months with few or even no daylight hours[1]. In some 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 successfully develop the rational and efficient treatment strategy of bright light therapy[2].

Several findings suggest that SAD is mediated through the serotonin (5-HT) system. In post mortem human brain samples, 5-HT concentrations are lowest in people dying in the winter[3]. Also, the concentration of the serotonin metabolite 5-HIAA is lower in jugular blood samples collected in winter[4]. These biomarkers may be associated with seasonal changes in the activity of plasma-membrane serotonin transporter (5-HTT) that serves a central role in neural serotonin transmission by regulating synaptic interstitial 5-HT levels[5, 6]. However, previously there has been no clear evidence for seasonal variation in the 5-HTT binding in brain *in vivo*. Two contradicting brain molecular imaging studies, one reporting lower binding[7] and one higher[8] binding in winter compared to summer, suffered from small sample sizes, questionable criteria for the cut-offs between summer and winter, and radioligands less specific than those available today.

However, the link between 5-HTT and SAD is also pointed out by the observation 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 the disorder than carriers of the insertion (*long 5-HTTLPR allele = L-allele*)[9]. This polymorphism influences 5-HTT expression, with lower expression in carriers of the *S-allele*[5]. In comparison to *L-allele* homozygotes, *S-allele* carriers have increased propensity to develop mood disorders in response to stressful environmental cues[10, 11], in particular in response to seasonal changes[9, 12] . Further, in functional magnetic resonance (fMRI) studies exposure to stressful and fearful environmental cues evoke greater limbic activation in *S-allele* carriers[13]. However, there was no difference in cerebral 5-HTT binding between carriers

of the *S-allele* and *L-allele* homozygotes in a two large samples of 96 healthy volunteers[14] measured *in vivo* with single photon emission computed tomography (SPECT) and of 42 healthy volunteers[15] measured with positron emission tomography (PET). Only when subjects were stratified according to an additional single nucleotide polymorphism (SNP) on the *L-allele*, which has been taken into account by activation studies, were differences in *in vivo* 5-HTT binding identified[16, 17]. Furthermore, phasic 5-HTT changes, which would be the molecular equivalent to the endophenotype identified by activation studies, in terms of a different molecular response to environmental cues in *S-allele* carriers compared to *L-allele* homozygotes, have – to our knowledge - not yet been studied with PET.

We sought evidence for seasonal variation in the 5-HTT binding data from a sample of 54 healthy subjects studied with the highly specific radioligand [^{11}C]DASB[18]. To avoid arbitrary classification, we predicted [^{11}C]DASB binding with the number of daylight minutes at the latitude of Copenhagen (http://aa.usno.navy.mil/data/docs/Dur_OneYear.php/), and incorporated adjustment for age and gender in a general linear model. We found that length of daylight time in minutes correlates negatively with BP_{ND} for [^{11}C]DASB in the putamen and the caudate (putamen: -0.0438 BP_{ND}/(100 minutes) [-0.0689; -0.0186], p=0.001, caudate: -0.0363 [-0.0702; -0.0023] BP_{ND}/(100 minutes), p = .037), with a similar tendency in the thalamus (-0.0299 BP_{ND}/(100 minutes) [-0.0656; 0.00581, p=0.099), but found no such association in the midbrain (0.0192 BP_{ND}/(100 minutes) [-0.0858; 0.1242], p=0.715). The findings were also reproduced when data were modeled to a harmonic function, that allows for a time delay in the seasonal effect. Correction for age and gender revealed a marked gender effect (higher binding in men) in the caudate (p = 0.0002), and decreasing [^{11}C]DASB BP_{ND} with age in the putamen (p = 0.034) and the thalamus (p = 0.043). It could be argued that seasonal changes in availability of food selection and seasonal changes in food preferences might affect neural serotonin levels through variations in tryptophan-intake. However, in our sample there was no correlation between [^{11}C]DASB BP_{ND} and plasma tryptophan, neither in terms of absolute concentration nor relative to large neutral

amino acids with which tryptophan is competing at the blood-brain barrier[19]. Also, there was no seasonal variation of absolute or relative tryptophan concentrations in our sample.

We then asked if the seasonal effect was more pronounced in *S-alleles* carriers, given the evidence from activation and population studies. We stratified our sample according to 5-HTTLPR-allelic status, and searched for a gene-daylight interaction effect; 19 subjects were homozygote *L-allele* carriers and 35 subjects were *S-allele* carriers. Again in putamen, we found a significant gene*daylight effect (p, corrected for age and gender = 0.0448) with a negative correlation between the [^{11}C]DASB BP_{ND} and daylight time in carriers of the *S-allele*, but not in carriers of the *L-allele* (Figures 1 and 2).

Consistent with prior studies in which SAD patients had a higher likelihood of being *S-allele* carriers[9, 12], and having higher 5-HTT binding during depressive episodes[20], we found that the 5-HTT binding in carriers of the *S-allele* is affected by seasonal changes, but not in carriers of the *L-allele*. We found the strongest seasonal effect in the putamen, an anatomical region with a dense serotonin innervation[18], that is implicated in motor functions, but also in processing of aversive stimuli[21, 22]. We did not find any seasonal variation in midbrain, where the serotonergic cell bodies are located[23]. Possibly, [^{11}C]-DASB is merely reflecting the number of neurons than 5-HHT tonus in this region and thus not sensitive to seasonal effects.

By examining the effect of daylight hours on the serotonergic system, we identified a distinct neurobiological endophenotype for the short 5-HTTLPR-allele with dynamic seasonal changes in 5-HTT binding. The neurobiological endophenotype identified here directly links activation studies, showing responses on the neural circuit level, with dynamic changes in transporter expression measured *in vivo*.

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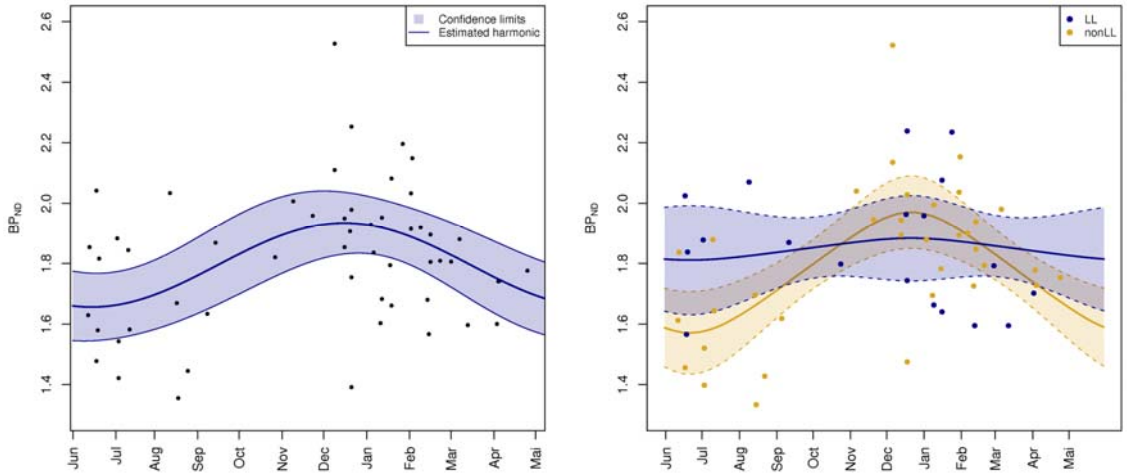

Figure 1. Left figure illustrates the seasonal effect on [^{11}C]DASB BP_{ND} (putamen) with pointwise confidence limits, modeled as a harmonic function with period 1 year (estimated peak in the middle of December, SE = 21 days, in good agreement with the model using daylight minutes as a predictor) adjusting for age and gender. The plotted points are the partial residuals (male of mean age). The functional form was validated by including additional frequency components and by comparison with estimates from an additive model. The right figure displays the interaction between number of daylight minutes and HTTLPR-allelic status adjusting for age and gender. For comparison with the left figure the estimated linear response as a function of daylight minutes was transformed to a function of calendar time.

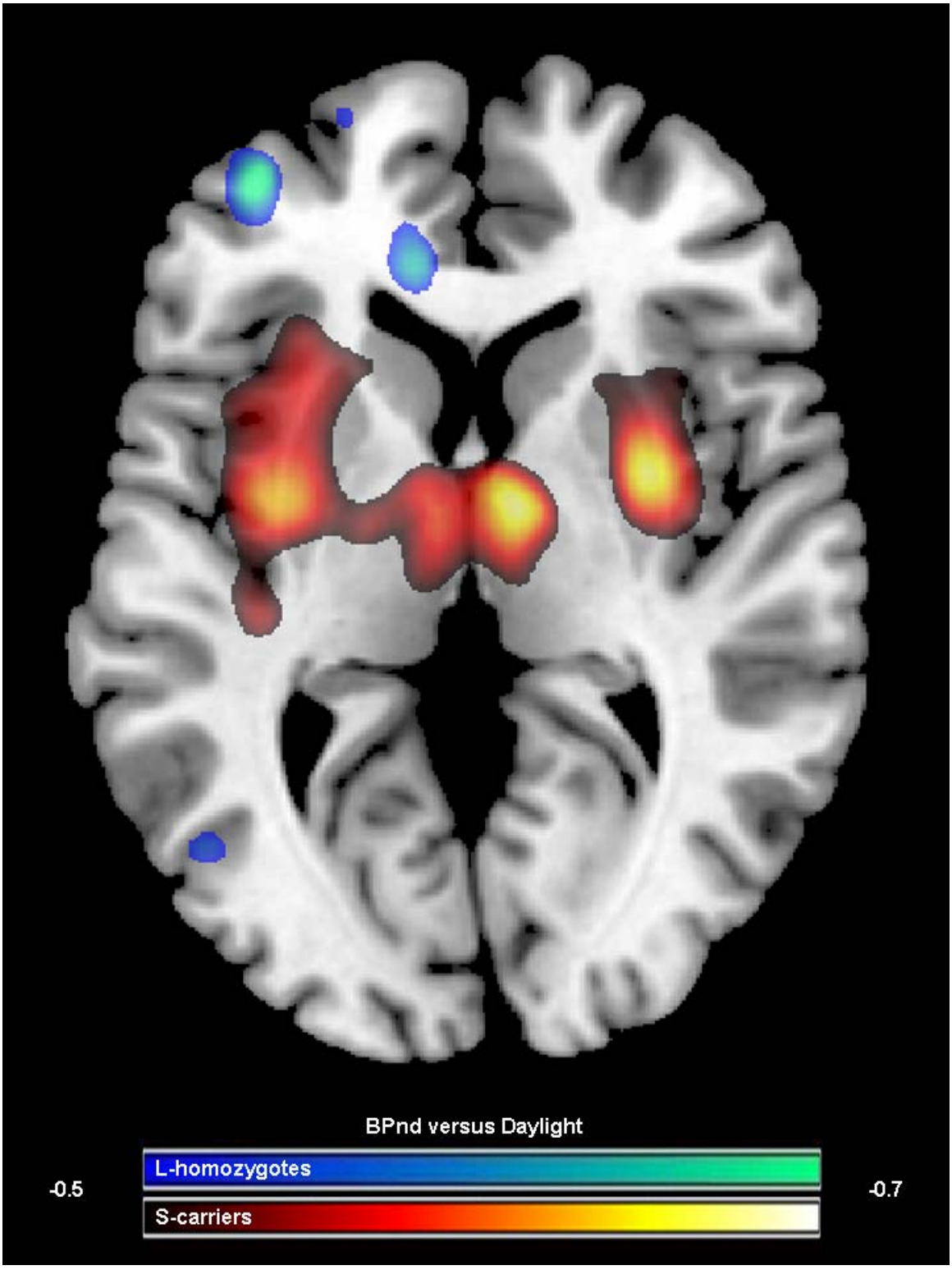

Figure 2. Results of voxel-based analysis using parametric images representing specific 5-HTT binding, all normalized to Montreal Neurological Institute (MNI) space. Correlations between [^{11}C]DASB BP_{ND} (adjusted for age and gender) and amount of daylight on the day of the scan in Copenhagen.