Serotonin transporter gene promoter methylation status correlates with in vivo prefrontal 5-HTT availability and reward function in human obesity

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A polymorphism in the promoter region of the human serotonin transporter (5-HTT)-coding SLC6A4 gene (5-HTTLPR) has been implicated in moderating susceptibility to stress-related psychopathology and to possess regulatory functions on human in vivo 5-HTT availability. However, data on a direct relation between 5-HTTLPR and in vivo 5-HTT availability have been inconsistent. Additional factors such as epigenetic modifications of 5-HTTLPR might contribute to this association. This is of particular interest in the context of obesity, as an association with 5-HTTLPR hypermethylation has previously been reported. Here, we tested the hypothesis that methylation rates of 14 cytosine–phosphate–guanine (CpG) 5-HTTLPR loci, in vivo central 5-HTT availability as measured with [11C]DASB positron emission tomography (PET) and body mass index (BMI) are related in a group of 30 obese (age: 36 ± 10 years, BMI > 35 kg/m²) and 14 normal-weight controls (age 36 ± 7 years, BMI < 25 kg/m²). No significant association between 5-HTTLPR methylation and BMI overall was found. However, site-specific elevations in 5-HTTLPR methylation rates were significantly associated with lower 5-HTT availability in regions of the prefrontal cortex (PFC) specifically within the obese group when analyzed in isolation. This association was independent of functional 5-HTTLPR allelic variation. In addition, negative correlative data showed that CpG10-associated 5-HTT availability determines levels of reward sensitivity in obesity. Together, our findings suggest that epigenetic mechanisms rather than 5-HTTLPR alone influence in vivo 5-HTT availability, predominantly in regions having a critical role in reward processing, and this might have an impact on the progression of the obese phenotype.

INTRODUCTION
The serotonin (5-hydroxytryptamine, 5-HT) transporter (5-HTT) has a crucial role in regulating emotional and reward processing. Changes in 5-HTT availability and thus synaptic serotonin levels have been associated with stress-related diseases such as mental and metabolic disorders including obesity. Specifically, obesity has been associated with reduced subcortical (caudate–putamen–thalamus) 5-HTT availability and increased 5-HT4 receptor availability in the nucleus accumbens and ventral pallidum, regions that are intimately involved in reward processing. These changes are possibly compensatory in nature because of reduced brain serotonin levels, which is well known to promote a negative energy balance through suppression of appetite. The precise mechanisms however that lead to changes in 5-HTT availability in vivo remain unclear. At the genetic level, a 43-bp insertion/deletion polymorphism in the promoter region upstream of the 5-HTT-coding sequence (5-HTTLPR) of the SLC6A4 gene has been shown to result in a short (S) and a long (L) allelic variant that alters SLC6A4 transcription and the magnitude of 5-HTT function. As a result, carriers of the homozygous L-allele variant exhibit elevated concentrations of 5-HTT mRNA and express nearly twofold greater 5-HT re-uptake in contrast to S-allele carriers. Consequently, the existence of S-allele lowers the transcriptional activity of 5-HTTLPR, leading to diminished 5-HTT expression and 5-HT reuptake. However, positron emission tomography (PET) studies using the highly 5-HTT-selective radiotracer [11C]-3-amino-4-(2-dimethylamino-methylphenylsulfanyl)-benzonitrile (DASB) have produced inconclusive results on 5-HTTLPR genotype and in vivo 5-HTT availability in the human brain.

To further strengthen our understanding of how DNA sequence-based genetic variation shapes individual differences in human brain 5-HTT function, behavioral traits and related risk for psychopathology, non-sequence-based epigenetic variation, that is, DNA methylation of 5-HTTLPR, need to be considered, but as yet remain relatively unexplored. DNA methylation mainly occurs at DNA sections where cytosine is succeeded by guanine (cytosine–phosphate–guanine (CpG) dinucleotides). Site-specific changes typically involve DNA methyltransferases transferring a methyl group to position five of the cytosine ring of most CpG dinucleotides in the mammalian genome without affecting the
DNA sequence. 19,20 Recent data have shown a potential link between epigenetic variation, for example, methylation in CpG-rich regions of 5-HTTLPR and behavior. 21–24 Explorative studies also revealed that 5-HTTLPR methylation patterns of different CpG loci are a functionally relevant biomarker of reduced in vivo 5-HTT expression (assessed from peripheral blood cells) 25–27 associated with behavioral outcomes. 24,28 Specifically, increased 5-HTTLPR methylation predicted an increased threat-related amygdala reactivity in vivo and decreased mRNA expression in postmortem amygdala tissue from a third independent cohort (to gain further mechanistic insight into the in vivo findings) 29 functionally coupled with key nodes of the salience network. 30

Furthermore, epigenetic variations in 5-HTTLPR are increasingly being discussed as an important risk factor for obesity. 21 In a monozygotic twin study covering 84 twin pairs, Zhao et al. found SLC6A4 promoter hypermethylation significantly associated with an increased prevalence of obesity. 21 In addition, 5-HTTLPR DNA methylation in the prefrontal cortex (PFC) has been described to be a highly dynamic process modified by genetic variance and regulating gene transcription due to fast changes of DNA methylation patterns during the prenatal period that slows down after birth and continues to slow down with aging. 31 In the PFC, serotonin represents a major modulator of its function. 32 The PFC exhibits a dense expression of serotonergic receptors and represents a region of serotonergic innervation. 32 Here, 5-HT 1A, 5-HT 2A, and 5-HT 3A receptors are selectively expressed in distinct populations of pyramidal neurons and inhibitory interneurons. 32 This is of particular interest, as the PFC has also been linked with obesity as shown by resting-state and task-related functional magnetic resonance imaging studies. 33–35 Thus, a lesser degree of centrality in the left middle frontal gyrus has been demonstrated, suggesting the important role of the middle frontal gyrus in the pathophysiology of obesity. 33

5-HT has also been discussed as a fundamental mediator of emotional, motivational and cognitive aspects of reward representation. 36 For instance, Nakamura et al. demonstrated in non-human primates that, unlike dopamine-containing neurons of the substantia nigra, serotonin-containing neurons of the dorsal raphe nucleus encode the value of a received fruit juice reward. 37 Moreover, the study of Choi et al. revealed interactions between reward and threat processing in regions of human PFC, that is, the middle frontal gyrus, but also in other distinct brain regions. 38 that is, the nucleus accumbens, a key neural substrate in the reward system since its involvement in natural (that is, food) reinforcement. 39

No studies have been conducted so far comparing 5-HTTLPR methylation and in vivo 5-HTT availability in the PFC, in particular, in human obesity in the context of reward processing. The primary aim of the present study was, therefore, to investigate whether 5-HTTLPR methylation has an impact on in vivo 5-HTT availability in obese and normal-weight individuals. As methylation rates are assumed to be associated with low transcription rates, 19,40,41 we hypothesized that elevated methylation rates of 5-HTTLPR result in lower in vivo 5-HTT availability. Second, we investigated whether there is an impact of CpG-associated 5-HTT availability on measures of behavioral control in 5-HTT-dependent regulatory areas of the PFC. 42 To assess reward function, we used the behavioral approach system (BAS) reward scale as our own previous study data indicated that 5-HTT availability is associated with levels of BAS reward determined by questionnaire score scales in the PFC. Finally, to account for covariates, we studied whether body mass index (BMI) and age have an effect on a possible association between CpG and 5-HTT availability. 5-HTTLPR methylation rates were assessed from peripheral blood cells as a proxy for central in vivo 5-HTT. 53–45

**MATERIALS AND METHODS**

**Study population**

The study cohort consisted of 44 non-depressed volunteers (Caucasians) covering an age range of 21–59 years with a mean age of 36 ± 9 years and a BMI range of 19–54 kg/m 2. This included 14 study participants with a BMI < 27 kg/m 2 (mean age 36 ± 7 years, 9 female) and 30 obese participants with a BMI > 35 kg/m 2 (mean age 36 ± 10 years, 20 female) as previously described. 46 All participants gave their written informed consent. Study participants were clinically examined by a physician and assessed for the mental illness via structured interview by a psychiatrist. Exclusion criteria comprised present or past alcohol abuse and/or drug abuse, current or past neurological or psychiatric diseases, epilepsy, migraine, epileptic seizures in their previous medical history (or in the family history), neurosurgical interventions in the past, pregnancy or other medical conditions as well as drugs that may alter or influence brain function (for example, antidepressants). Study participants were running an Obesity program in the Ambulance of the IFB AdiposityDiseases in Leipzig, Germany, which includes metabolic status before selecting patients for studies. They were investigated regarding type I or insulin-dependent type II diabetes. Patients with diabetes requiring insulin therapy as well as those with poorly controlled diabetes (HbA 1c > 7%) were excluded from the study. Exclusion criteria also covered contraindications for magnetic resonance imaging (for example, implanted ferromagnetic devices, claustrophobia), which was performed to exclude structural lesions such as traumatic brain injuries and stroke. Magnetic resonance imaging included sequences for anatomical data co-registration (3 T, Siemens, Erlangen, Germany; T1-weighted 3D magnetization prepared rapid gradient echo; time of repetition: 2300 ms, time of echo: 2.98 ms, 176 slices, field of view: 256 x 240 mm, voxel size: 1 x 1 x 1 mm).

On the day of PET imaging, the extent of depressive symptoms was rated using the Beck Depression Inventory. 47 On the same day prior to PET scanning, typical laboratory parameters, for example, glucose (mg dl 1), insulin (mg dl 1), and Hba 1c (mmol 1), were assessed and peripheral blood samples for genotyping were collected and immediately refrigerated at ~80 °C.

The study was conducted in accordance with the Helsinki Declaration and approved by the ethics committee of the Medical Faculty of the University of Leipzig registered under the number 206-10-08032010 and the German Bundesamt für Strahlenschutz/Federal Office for Radiation Protection (number ZS-22461-2-2011-002). The study was registered at the European clinical trial database (EudraCT 2012-000568-32) and the German Clinical Trials Register (DRKS).

The behavioral inhibition system behavioral activation system scales

In addition, the sensitivity to reward and punishment as based on the behavioral inhibition system behavioral activation system (BIS/BAS) scales 48 was measured as a relevant personality factor in the context of BMI. 49 This self-report questionnaire consists of 20 items designed to assess the responsiveness of BAS and BIS personality characteristics. The 7-item BIS scale measures the reactivity of the aversive motivational system, whereas the 13-item BAS scale measures reactivity of the appetitive motivational system. The BAS scale can be divided into three subscales: drive, fun-seeking and reward.

**PET image acquisition**

The 5-HTT selective, carbon-11-labeled radiotracer was applied as previously described. 7,46 After 10 min transmission scan from three 68Ga sources for attenuation correction and intravenous bolus injection (90 s) of 484 ± 10 MBq (11C)DASB a dynamic PET scan was performed to collect emission data by using an ECAT EXACT HR+ scanner (Siemens; intrinsic resolution at the center 4.3 mm (full width at half maximum, FWHM), axial resolution: 5–6 mm, field of view: 15.5 cm, 3–4 mm FWHM) in a 3D mode over 90 min (23 frames: 4 × 0.25, 4 × 1, 5 × 2, 5 × 5, 5 × 10 min). Data were iteratively reconstructed (10 iterations, 16 subsets) in transverse image series (63 slices, 128 × 128 matrix, voxel size 2.6 x 2.6 x 2.4 mm) with a Hann filter (cutoff 4.9 mm).

**Imaging data processing**

Individual magnetic resonance imaging data sets of the participants were spatially reoriented onto a standard brain data set, defined by the anterior
primer sequences were 5’-GAGGGACTGAGCTGGACAAC-3’ and 5’-GCAGCA GACAACCTGAGCTGGACAAC-3’ yielded to a product length of ~ 620 bp for the L-allele, and 583 bp for the S-allele. Used primers were purchased from Invitrogen (Paisley, UK).

DNA methylation analysis by quantitative bisulfite pyrosequencing
Methylation rates were determined including 14 CpG-loci by using the PyroMark Q24 software (version 2.0.6, Qiagen). Each subject sample was independently analyzed by PyroMark Q24 (Qiagen). Pyrogram analyses occurred via PyroMark Q24 protocol. Pyrosequencing was executed using pyrosequencing instrument technology (Qiagen). Pyrogram analyses occurred via PyroMark Q24 software (version 2.0.6, Qiagen). Each subject sample was independently replicated on different plates. At every CpG locus, the mean percentage (%) of methylation was calculated.

Data analysis
A voxel-wise statistical analysis of BPND maps was performed using SPM8 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurobiology, UK) implemented in Matlab 7.13 (The MathWorks, Natick, MA, USA). Motion correction was performed as implemented in SPM. The mean images (frames 1–13, 0–15 min) were used to determine the spatially normalization matrix on the SPM8-integrated PET template and subsequently applied to the BPND maps. Brain regions were denoted in accordance to the nomenclature by the LONI Probabilistic Brain Atlas (LPBA40). Here, only regions of the PFC by using a prefrontal mask were considered (that is, the left inferior frontal gyrus, the right middle frontal gyrus, the left middle frontal gyrus and the right inferior frontal gyrus (Supplementary Table S1)). Smoothing was performed with an 8 mm FWHM Gaussian filter. Group differences in BPND maps between LL and S+Carriers were tested voxelwise in SPM using a two-sample t-test with methylation rate as nuisance variable and predefined prefrontal mask as explicit mask. A one-sample t-test was used to test for correlation of parametric BPND images and methylation rate throughout the predefined prefrontal mask in SPM. We report effects for clusters of voxels exceeding a peak level threshold of P < 0.001 (uncorrected) and a cluster size threshold of P < 0.05, family-wise error-corrected. For the voxelwise correlation analysis, the first eigenvariate of significant clusters was extracted and correlated with BAS questionnaire scores. The first eigenvariate represents the weighted mean of BPND in clusters defined by their significant correlation to the CpG locus methylation rate.

Statistical analysis
In SPSS (version 21, SPSS, IBM, Chicago, IL, USA), all obtained data were tested for normal distribution through application of Shapiro–Wilk test. Unpaired t-test was performed in the analysis of differences observed between groups (that is, comparison on CpG loci methylation rates between 5-HTTLPR variants, Table 1). To test for correlations between the 5-HTT BPND and other investigated parameters (for example, BAS reward, BAS fun, BAS drive, BIS and BMI), two-sided Pearson’s correlation analysis was applied. Bonferroni correction was carried out to correct for multiple comparisons.

RESULTS
Sample characteristics
Application of Shapiro–Wilk test revealed that all obtained data were normally distributed (P > 0.05).

Effects of 5-HTTLPR genotypes on demographics, neuropsychological scores and methylation rates
Subjective parameters stratified for 5-HTTLPR genotypes of all study participants demonstrated similar data distribution: homoygous LL and S+ carriers did neither significantly differ in demographics, neuropsychological scores nor in their methylation rates in the distinct CpG loci (Table 1). Voxel-wise SPM genotype group comparison (LL versus S+Carriers) with CpG 10 loci as nuisance covariate revealed no significant differences, neither in obese nor in normal-weight individuals (data not shown).

Effects of methylation on BPND in obese versus normal-weight individuals
In SPM analysis, we found 5-HTTLPR methylation rates in locus CpG10 to be significantly negatively correlated with BPND across

Figure 1. Exposure of SLC6A4 gene promoter region for bisulfite sequencing analysis. Analyzed cytosine–phosphate–guanine (CpG) sites are bolded and underlined. Assay design was based on the Origene ID NM_001045. The raphe dorsalis was used as receptor-rich region. The cerebellar cortex (without vermis) referred as reference region. 5-HTTLPR methylation rates in locus CpG10 to be significantly negatively correlated with BPND across 5-HTT binding potential (BPND) maps between LL and S+Carriers were tested voxelwise in SPM using a two-sample t-test with methylation rate as nuisance variable and predefined prefrontal mask as explicit mask. A one-sample t-test was used to test for correlation of parametric BPND images and methylation rate throughout the predefined prefrontal mask in SPM. We report effects for clusters of voxels exceeding a peak level threshold of P < 0.001 (uncorrected) and a cluster size threshold of P < 0.05, family-wise error-corrected. For the voxelwise correlation analysis, the first eigenvariate of significant clusters was extracted and correlated with BAS questionnaire scores. The first eigenvariate represents the weighted mean of BPND in clusters defined by their significant correlation to the CpG locus methylation rate.

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clusters of PFC: left inferior frontal gyrus, right middle frontal gyrus, left middle frontal gyrus and right inferior frontal gyrus (Figures 2 and 3 and Supplementary Information S1). The results were found only in obese but not in normal-weight individuals. All other investigated CpG loci showed no significant correlations with BPND in clusters of PFC in obese or normal-weight individuals.

Effects of BPND-associated CpG10 methylation rate on reward sensitivity

We investigated whether BPND-associated CpG10 methylation rate correlates with reward as well as covariates, that is, BMI and age by application of two-sided Pearson’s correlation analysis. As presented in Figure 2 and Table 2, BPND in PFC clusters associated to methylation of locus CpG10 correlated negatively with BAS reward score \((r = -0.64, P = 0.001)\) in the obese cohort. However, no significant correlations were found between BPND in PFC clusters associated to methylation of locus CpG10 and other investigated covariates, that is, age, BMI, BAS Fun Seeking, BAS Drive as well as BIS (Table 2).

DISCUSSION

The present study demonstrated that in vivo 5-HTT availability in the PFC represented by the left inferior frontal gyrus, the right middle frontal gyrus, the left middle frontal gyrus and the right inferior frontal gyrus negatively associated with the rate of methylation in locus CpG10 of the 5-HTT gene promoter. These findings were observed in obese but not in normal-weight individuals.

Figure 2. Significant correlations between the mean binding potential (BPND) defined by CpG10 methylation rates (unitless, y axis) and parameters such as: left panel: BAS reward and right panel: methylation rate measured in percent (%) of obese individuals \((n = 30)\) in the prefrontal cortex (PFC). \(r\), Pearson product–moment correlation coefficient; \(P < 0.05\) (significant). BAS, behavioral approach system; CpG, CpG, cytosine–phosphate–guanine.

Figure 3. SPM8 projections superimposed on representative MRI indicating regions of the PFC52 with significant negative correlation between in vivo BPND and methylation of locus CpG10 \((P < 0.001)\) uncorrected on peak level and \(P < 0.05\) FWE on cluster level, \(n = 30\). For corresponding Montreal Neurological Institute coordinates see Supplementary Table 1. BPND, binding potential; CpG, cytosine–phosphate–guanine; FWE, family-wise error; MRI, magnetic resonance imaging; PFC, prefrontal cortex.

Table 1. Characteristics of participants and comparison on CpG loci methylation rates between 5-HTTLPR variants \((n = 44)\)

| Characteristics of participants | \(n\) | LL | S+ | P-value |
|-------------------------------|-----|----|----|---------|
| Age (years)                   | 21  | 35.3±8.8 | 37.4±9.8 | 0.47*  |
| Weight (kg)                   | 23  | 105.1±28.1 | 104.6±32.9 | 0.96*   |
| BMI (kg/m²)                   | 16  | 35.5±8.8 | 35.1±10.9 | 0.88*   |
| BDI                           | 7.4±6.5 | 7.5±7.2 | 7.6±7.1 | 0.96*   |
| Sex (female/male)             | 15/6 | 15/6 | 15/6 | 1* |
| Activity applied (MBq)        | 16/7 | 16/7 | 16/7 | 1* |
| BAS fun                       | 11.9±2.3 | 11.1±1.4 | 11.3±1.5 | 0.19*   |
| BAS reward                    | 17.1±2.2 | 16.1±1.9 | 16.3±2.0 | 0.11*   |
| BIS                           | 12.9±1.8 | 12.6±2.0 | 12.7±2.1 | 0.53*   |
| CpG loci methylation rates    |     |     |     |         |
| CpG1                          | 2.4±0.3 | 2.4±0.3 | 2.4±0.3 | 0.62*   |
| CpG2                          | 3.5±0.5 | 3.5±0.6 | 3.5±0.7 | 0.89*   |
| CpG3                          | 1.6±0.3 | 1.6±0.2 | 1.6±0.3 | 0.84*   |
| CpG4                          | 2.0±0.4 | 1.8±0.3 | 1.9±0.4 | 0.08*   |
| CpG5                          | 3.0±0.6 | 2.7±0.5 | 2.8±0.6 | 0.18*   |
| CpG6                          | 2.9±1.0 | 2.8±0.6 | 2.8±0.7 | 0.81*   |
| CpG7                          | 4.7±0.7 | 4.7±0.4 | 4.7±0.5 | 0.80*   |
| CpG8                          | 4.6±1.0 | 4.7±0.5 | 4.7±0.6 | 0.77*   |
| CpG9                          | 3.3±0.7 | 3.4±0.4 | 3.4±0.5 | 0.57*   |
| CpG10                         | 2.2±0.5 | 2.1±0.4 | 2.1±0.5 | 0.45*   |
| CpG11                         | 2.1±0.5 | 2.0±0.4 | 2.0±0.5 | 0.53*   |
| CpG12                         | 2.7±0.6 | 2.6±0.3 | 2.6±0.4 | 0.81*   |
| CpG13                         | 2.4±0.5 | 2.6±0.4 | 2.6±0.5 | 0.19*   |
| CpG14                         | 7.0±1.5 | 6.8±1.3 | 6.8±1.4 | 0.67*   |

Abbreviations: BAS, behavioral approach system; BDI, Beck Depression Inventory; B, behavioral inhibition system; BMI, body mass index; CpG, cytosine–phosphate–guanine; LL, homozygous L carriers; S+, homoyzogous and heterozygous S carriers. Allele distribution was in Hardy–Weinberg equilibrium (Pearson \(x^2\)-test). The number in brackets in S+ indicates the number of homoyzogous S-carriers. Values represent mean ± s.d. *Student’s \(t\)-test. **Fisher’s exact test. †Did not survive correction for multiple testing.
The present study was intended to detect changes in the in vivo 5-HTT availability in a cohort of highly obese participants and in non-obese cohorts. We tried to avoid covariates in a small sample size as reported in our previous study. Participants were investigated regarding metabolic status in order to exclude those with type I or insulin-dependent type II diabetes or with poorly controlled diabetes in general (HbA1c > 7%). Thus, we could avoid the possibility that the studied relationship between 5-HTTLPR methylation rates may be even higher correlated with the 5-HTT availability in both groups, normal-weight and obese individuals.

The process of DNA methylation is known to result in stable gene repression. Our finding of elevated DNA methylation rates within 5-HTTLPR together with lower in vivo 5-HTT availability would be in line with the specific molecular genetic effects mediated by DNA methylation. DNA methylation is also determined by genetic variation. Accordingly, the epigenetic effects in our study seem to be independent of (and stronger than) the effects of genotypic variation of 5-HTTLPR.

In contrast to the study of Zhao et al. that showed that methylation rate of 5-HTTLPR is positively correlated with BMI, we were not able to demonstrate a significant correlation between 5-HTTLPR methylation and BMI. This could be explained by the fact that this relation follows a nonlinear curve with significant association only in the highly obese. Similar nonlinear or quadratic relationships have been described for other biological markers such as the central dopamine D2 receptor availability or for the central 5-HTT together with 5-HT3A receptors in human obesity. Instead, we found a negative relationship between methylation rates of 5-HTTLPR, in vivo 5-HTT availability in the PFC and the BAS reward subscale as a measure for the sensitivity to reward. This could fit in a model, as it has been proposed for the association between BMI and BAS reward with a quadratic curve indicating high reward sensitivity at moderate-to-high BMI levels.

The findings of our analysis might support a link between the neurobehavioral effects of changes in modifications of the 5-HTTLPR and an enhanced sensitivity to motivationally and/or emotionally relevant environmental stimuli predicting reward. An important modulatory role in reward processing for 5-HTT was found in knockout mice in which disruptions of the development of the reward system and interference with reward-based learning were found. For instance, it has been shown that 5-HTTLPR/rs25531 genotype, an A/G single-nucleotide polymorphism (SNP) within the 5-HTTLPR that have been known to have a differential impact on L allele function, influences reward responsiveness. S-allele carriers showed larger stress-related reduction in reward responsiveness relative to L-allele carriers. Yet, we did not find a significant interaction between genotypic variance of 5-HTTLPR (without investigating rs25531) in the PFC and BAS reward scores. Given the significant negative correlation between CpG methylation and in vivo 5-HTT availability with BAS reward scores, this result furthermore suggests that epigenetic effects of site-specific (that is, locus CpG10) DNA methylation influence behavioral control in regions of PFC. We assume that environmental exposure to rewarding or punishing stimuli and respective behavioral responses will thus have a greater association with methylation of 5-HTTLPR as influenced by genotypic variance.

In search of arguments for the notable outliers in the significant Pearson’s correlations between the mean BPND defined by CpG10 methylation rates and BAS reward, it must be mentioned that the numbers of the included obese individuals were modest, which makes the analysis vulnerable to outliers. Therefore, we cannot exclude that these outliers significantly influence the investigated parameters such as reward.

In the present study we only focused on the 5-HTT availability in the PFC. This does not exclude any correlation between 5-HTTLPR methylation and 5-HTTLPR availability in regions outside the PFC. In that manner, we did not perform an unbiased whole-brain analysis since our own previous study showed that 5-HTT availability determines levels of BAS reward in the PFC such as the orbitofrontal cortex (unpublished). Thus, we are not able to state whether the 5-HTTLPR methylation status varies globally or locally across different brain regions and whether this indicates region-specific functional specialization. It is possible that this region-specific functional specialization involves the distinctive expression of transcription factors that regulate 5-HTT transcription. For instance, mouse midbrain serotoninergic neurons selectively express the transcription factor Pet1, which is required for normal SLC6A4 transcription in this region. It might be speculated that human PFC neurons selectively express a transcription factor that normally increases 5-HTT expression and that is competitively displaced by CpG 10 methylation of the 5-HTT promoter.

Taken together, our finding of higher site-specific methylation of 5-HTTLPR seems to predict decreased 5-HTT availability and, consequently, a reduction of regional 5-HT re-uptake capacity in PFC would be in agreement with previous literature, and may contribute to an altered cognitive and emotional function in obesity. However, we cannot conclude from these results that epigenetic changes associated with molecular changes in the brain and behavior predisposes obesity since we could neither find a specific link of CpG-associated 5-HTT availability with BMI nor have we directly measured gene expression in our study.

LIMITATIONS

Our study has several limitations. First, we investigated peripheral blood leukocytes, which are commonly employed in SLC6A4 methylation and allelic variation studies on the basis that peripheral patterns reflect central processes. Prior work has provided a significant correlation between blood and brain methylyomes, and the 5-HTT promoter methylation level measured in peripheral blood cells has been demonstrated to be associated with central in vivo 5-HT synthesis as detected by
dedicated PET. Moreover, studies investigating a comparison between both platelet and neuronal 5-HTT regulation are rare and inconsistent results exist concerning functional activity of 5-HTT. Second, the sample size of our study, in particular of normal-weight controls (although carefully matched for any variables), was modest: suitable for a PET study, but with limited statistical power for genetic analysis. Furthermore, we did not investigate the 5-HTTLPR/rs25531 genotype, an A/G SNP within the 5-HTTLPR to estimate the in vivo 5-HTT availability.

As this was a pilot study to fuel new hypotheses on an interaction between epigenetics and a distinct molecular target, that is, the 5-HTT availability in vivo, larger sample sizes are warranted to identify the main effects of 5-HTT genotype and its modification on in vivo 5-HTT function in psychiatry and/or metabolic research. Moreover, future studies should be designed to demonstrate the direct evidence that increased methylation of the 5-HTT (SLC6A4) gene in the PFC is related to a lower binding of the radiotracer DASB to the 5-HTT. This could be performed by using human autopsy material to gain additionally further mechanistic insights (that is, by the analysis of 5-HTT mRNA levels in different brain tissues) to confirm the accordant in vivo findings.

CONCLUSION

The present study suggests that epigenetic changes, that is, a DNA methylation of the 5-HTTLPR, contribute even more to in vivo 5-HTT availability than 5-HTTLPR itself does. If confirmed, such an association between distinct (for example, CpG10) loci and prefrontal 5-HTT might explain differences in reward sensitivity, thus leading to changes in eating behavior. However, whether these changes are main drivers of weight gain and obesity and/or reversible has to be proved.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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