Neurobiological aspects of obesity: dopamine, serotonin, and imaging
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ASSOCIATION OF SEROTONIN TRANSPORTER AVAILABILITY AND BODY MASS INDEX IN HEALTHY EUROPEANS

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ABSTRACT

Objectives: Serotonin-mediated mechanisms, in particular via the serotonin transporter (SERT), are thought to have an effect on food intake and play an important role in the pathophysiology of obesity. However, imaging studies that examined the correlation between body mass index (BMI) and SERT are sparse and provided contradictory results. The aim of this study was to further test the association between SERT and BMI in a large cohort of healthy subjects.

Methods: One hundred twenty-seven healthy subjects of the ENC DAT database of normal $[^{123}]$FP-CIT SPECT scans (58 females, age 52 ± 18 years, range 20-83 years, BMI 25.2 ± 3.8, range 18.2 – 41.1) were analyzed using region-of-interest (ROI) and voxel-based approaches to calculate $[^{123}]$FP-CIT specific binding ratios (SBR) in the diencephalon (hypothalamus/thalamus) and midbrain/brainstem as SERT-specific target regions.

Results: Using voxel-based analysis, SERT availability and BMI were positively associated in the thalamus. Additionally, in the ROI-analysis, the interaction between gender and BMI showed a trend with faster increase per BMI for men in the midbrain (0.033 m$^2$/kg, $p = 0.1$).

Conclusions: The data are in agreement with previous PET findings of an altered central serotonergic tone depending on BMI, as a probable pathophysiologic mechanism in obesity, and should encourage further clinical studies in obesity targeting the serotonergic system.
INTRODUCTION

Obesity rates have reached epidemic proportions worldwide, and might become the number one preventable public health threat for the 21st century (1) with high socio-economic impact due to serious medical sequelae, e.g. an increase in type II diabetes mellitus. Despite rapid progress in identifying the social, environmental and genetic causes of overeating, the mechanisms by which these factors result in obesity are not resolved. Regarding the central mechanism thought to be relevant for obesity, the monoaminergic systems seem to play a pivotal role in reward processing (i.e. the dopaminergic pathways of the ventral tegmental area, nucleus accumbens, and frontal cortex) (2), stress-mediation (in particular norepinephrine; 3), and the homeostatic control of feeding. With respect to this, the modulation by serotonin of eating behaviour integrates homeostatic and hedonic aspects as well as reward regulation at the intersection of the mesolimbic system, hypothalamus and brainstem (4). Changes in serotonergic functioning, as a main factor in the regulation of eating behaviour and energy balance, were shown in a variety of animal and clinical studies. Recently, a study in genetically engineered mice showed that knocking out the serotonin transporter (SERT) leads not only to hypophagia and hyperleptinemia but also to insulin resistance, hepatic steatosis, and obesity independent of food intake (5). Other studies on SERT knock-out mice also showed increased levels of abdominal fat and susceptibility to obesity (6,7). In addition, selectively bred polygenic obese rats had lower SERT binding when compared to polygenic diet-resistant rats (8), whereas no change in SERT was seen in diet-induced obesity in outbred rats. This was not the case in a mouse model: diet resistant mice have lower SERT binding than diet-induced obese mice (9). Also, a recent imaging study showed that obesity is associated with high serotonin-4 receptor availability in the brain reward system (10). Evidence for a serotonergic involvement in the pathophysiology of satiety and overeating also came from the efficacy of anorectic drugs. For example, sibutramine (Reductil) targeting the SERT as well as the norepinephrine transporter (NET) has an appetite-suppressing, anorexogenic effect (3). Hence, both monoaminergic systems, and in particular the presynaptically located transporters, are likely to represent key biochemical substrates in the intrinsic control of eating, and their failure in function, or compensatory change in expression, are thought to underlie overeating.

Only few studies have been performed that applied single-photon emission computed tomography (SPECT) or positron emission tomography (PET) with radiotracers for the SERT to unravel altered SERT availability in vivo in obesity or that looked into the association between BMI and SERT. Talbot et al. (11) reported on a PET study with the highly SERT-selective radiotracer [11C]DASB, which was initiated to investigate mechanisms underlying the clinical efficacy of sibutramine. They found SERT occupancy, by clinical doses of sibutramine, of modest magnitude supporting the assumption that SERT inhibition may be necessary for sibutramine’s anti-obesity effect in humans, and suggested that the hypophagic effect requires instead the co-inhibition of both SERT and NET. Given that the serotonergic system is a tonic, modulatory network of fibres stemming from the midbrain raphe nuclei, one might speculate that changes in either the brainstem SERT or the SERT at nerve terminals (e.g., in the diencephalon) are altered when external stimuli disturb the homeostasis to maintain the serotonergic tone. Obesity and the body mass index (BMI) as a marker for overweight might therefore be associated with a change in regional SERT availability in human. First in vivo [11C]DASB PET studies on SERT revealed
contradictory findings, either a positive or an inverse correlation between SERT availability and BMI (12,13), respectively. These studies in healthy volunteers were hampered by a lack of a larger sample of subjects with higher BMI (> 35 kg/m²) so that the curves may have been driven by some outliers in the upper range. A role for the SERT in obesity and BMI is thus plausible, but not yet conclusively demonstrated.

The objective of this study is to analyse extrastriatal SERT binding in an unique European database of [123I]FP-CIT SPECT scans of healthy volunteers to test for an association between SERT availability and BMI with a large BMI range. Recent studies showed that [123I]FP-CIT does not only bind to the dopamine transporter (DAT) in vivo, but also to extrastriatal SERTs (14). Based on our own preliminary [11C]DASB PET data (Hesse et al., 2009), we hypothesize that BMI and SERT binding ratios are positively correlated.

METHODS
This project was part of the collaborative European Association of Nuclear Medicine (EANM) Research Ltd. (EARL) initiative “European Database of [123I]FP-CIT SPECT scans of healthy controls (ENC-DAT)”, which started in 2007 and was successfully completed in 2010 (13 European institutions in 10 European countries recruiting 151 subjects) (15).

Subjects
The subjects were healthy volunteers fulfilling the inclusion and exclusion criteria as previously published (16,17). In brief, participants were between 20 and 90 years, had no evidence or history of neurological and psychiatric disorders as assessed by a neurologist, motor complaints as assessed with the Unified Parkinson’s Disease Rating Scale (UPDRS), a Symptom Checklist-90-R (SCL-90-R) score < 63 to ensure at most minimal psychological problems, a Beck Depression Inventory (BDI) score under 9.5 points, and no evidence for cognitive impairment, as assessed with the Mini-mental state examination (MMSE). They were off any psychotropic medication. Urine based screening tests for drug abuse was obtained in all subjects to exclude the use of illegal substances. They all had T1-MRI and T2-weighted MRI scans for exclusion of significant structural pathologies and for anatomical co-registration with SPECT data (see below).

All subjects underwent a general physical examination, including weight and height measurement for BMI calculation. The protocol was in accordance with the declaration of Helsinki and approved by the Medical Ethical Committees of all participating centres and all subjects provided written informed consent.

SPECT acquisition and data processing
Each subject underwent one SPECT scan 3-4 h after intravenous application of ~185 MBq [123I]FP-CIT (DaTSCAN; GE Healthcare), prior to which they received thyroid blockade. SERT specificity of diencephalon-midbrain [123I]FP-CIT binding was shown in vivo in human by displacement studies using the selective serotonin reuptake inhibitor paroxetine (14,18). Technical data and standardized data acquisition for the SPECT cameras that were used in the trial were described elsewhere (15,19). All scans were reconstructed by the core lab on HERMES (HERMES Medical Solution, Stockholm, Sweden) and corrected for attenuation and scatter.
Region-of-interest (ROI) analysis

For ROI analysis, the SPECT data were transferred to a HERMES workstation and manually co-registered with the MRI scans by using the HERMES MultiModality software following a previously described procedure (20,21). Briefly, the individual MRI scan was reoriented towards the anterior – posterior commissure lines based on a normal standardized MRI. Then, the individual SPECT data were co-registered onto the realigned individual MRI in all three (x, y, z) planes and, the ROIs, which are atlas-based predefined in the normal standardized MRI, were adjusted to the individual anatomy. The uptake in each ROI with the highest mean count density in adjacent slices comprising the entire brain structure, i.e. the SERT-rich regions thalamus/hypothalamus and midbrain/brainstem was determined (one continuous ROI from the thalamus level to the upper brainstem providing two peaks of mean count density, one at the thalamus/hypothalamus and one at the upper brainstem level, Fig. 1). The occipital cortex was used as the reference region representing non-specific binding to calculate the non-displaceable binding ratio (SBR), which is the activity in target-ROI divided by the activity in occipital cortex, minus 1.

Statistical analysis on the interaction between ROI (SBR) and BMI data considering age, gender, and scan time was performed using either PASW/SPSS 20 (IBM, Armonck, NY) or the

Figure 1. Co-registration of the SPECT to the individual MRI data for region-of-interest (ROI) analysis with HERMES MultiModality using mutual information algorithm and manually adopted realignment in three-dimensions (A). The dotted lines indicate the levels of axial slices. Bottom row illustrates co-registered data side-by-side with the target ROI (1) at the level of the hypothalamus (B) and at the midbrain level (C).
software R (http://www.r-project.org/, 2011, 23), version 2.14.0, and is based on a linear model including camera-type (as a factor), gender (22), age (21), BMI (as continuous variables) and the higher order terms gender × age and gender × BMI,. Terms are then dropped in a stepwise fashion based on Akaike’s information criterion and the final model is assessed using a marginal t-statistic. P-values of < 0.05 were considered to be statistically significant.

Voxel-based analysis

Voxel-based analysis was additionally applied using SPM8 running on MATLAB 7.5 for Windows (MathWorks, Natick, MA) (Fig. 2). The procedure has been described in detail elsewhere (17). To obtain individual parametric maps of SERT SBR, the uptake in the occipital cortex was extracted for each scan and SBR calculated for the full brain by (activity per voxel divided by the activity in occipital cortex) minus 1. The ROI for the occipital cortex was drawn manually on the mean scan in ITK-SNAP (version 2.1, PICSL, University of Pennsylvania). A regression analysis was performed in SPM8 with the SBR maps as dependent variable, BMI as independent variable and age, gender, scan time (3 or 4 h post-injection), and camera-type as covariates. Accordingly, comparison of images between groups (BMI ≤ 25 kg/m² versus BMI > 25 kg/m² and BMI ≤ 25 kg/m² versus BMI ≥ 30 kg/m²) was done with ANCOVA with SBR maps as dependent variable, BMI group as independent variable and age, gender, scan time, and camera-type as covariates. All analyses were confined to either diencephalon or brainstem using explicit masking with the target ROI drawn on the mean scan using intensity thresholding in ITK-SNAP (Fig. 2). P-values < 0.05, FWE corrected, were considered significant.

Figure 2. Mean scan with thalamus mask (A) and the midbrain mask (B) for voxel-based SPM analysis.
RESULTS

The final sample eligible for this ENC DAT sub-study (good image quality, full scatter windows, no truncation of data, i.e. scanning of the entire midbrain or occipital cortex) consisted of 127 subjects (58 female) with a mean age of 52.3 ± 18.3 (range 20 - 83) and mean BMI of 25.2 ± 3.8 kg/m² (range 18.2 - 41.1). We did not include one male subject for the entire analysis, because midbrain ROI partly fell outside the scanned part of the brain (as it was registered on the mean brain), which would have affected the voxel-based analysis.

Specific-to-nonspecific [123I]FP-CIT binding ratios in the diencephalon and midbrain did not differ between the groups that were assessed 3 versus 4 h post-injection (p = 0.63 for the thalamus, and p = 0.98 for the midbrain, data not shown in detail)

ROI analysis

The analysis of the diencephalon SBR data shows that the effect of different cameras is large, which resulted in a correction for Infinia camera (GE Healthcare, Fairfield, CT) of 0.5 (p = 0.0006, 95% CI = [0.2, 0.8]) and for the Varicam camera (GE Healthcare, Fairfield, CT) of -0.4 (p = 0.009, 95% CI = [-0.8, -0.1]) as part of the linear model (Fig. 3).

Age was found to be negatively associated with a coefficient of -0.006 per year (p = 0.02, 95% CI = [-0.010, -0.001]). The age dependence was almost entirely driven by two data points,
Figure 4. Negative correlation between SERT availability and age in the thalamus/hypothalamus. The line represents the fit for all values in female and the dotted line the fit for all male values. Note the spread of values which lead to a low $R^2$ (0.06 in female, 0.02 in male subjects) and the two outliers as mentioned in the text.

Figure 5. Positive relationship between SERT availability and BMI in the midbrain. The line represents the fit for all values in female and the dotted line the fit for all male values. Note the spread of values reducing $R^2$ (0.02 in female, 0.08 in male subjects).
however, and the p-value changed to 0.08 and 0.1 after removing these two points (see Fig. 4). BMI and SBR of the diencephalon showed no significant association (p = 0.1 for the full model), especially after removing the two aforementioned outliers (p = 0.8).

The SBR for the midbrain also showed strong effects for the same two cameras with the correction of 0.37 for Infinia (p = 0.009, 95% CI = [0.1, 0.6]) and -0.33 for Varicam (p = 0.03, 95% CI = [-0.6, -0.03]). There was a weak and non-significant association with age with a coefficient of -0.004 SBR/year (p = 0.1), whereas BMI showed little evidence for an association (p = 0.6) although the interaction in the full model between gender and BMI showed a slight trend, whereby men’s SBR values increase more quickly with BMI for men than for women (coefficient for men is: 0.033 m²/kg, p = 0.1 and is zero for women by construction), see Fig. 5 for an illustration using linear regression.

Voxel-based analysis
There was no significant correlation with BMI and midbrain SERT, nor with age and gender. But in the thalamus, a positive correlation between BMI and thalamus SERT in a cluster of 6 voxels, p = 0.020 (FWE corrected p-value < 0.05) (Fig. 6), and a negative correlation between age and thalamus SERT in a cluster of 42 voxels, p = 0.002 (FWE corr, p < 0.05) were found. Comparing subjects based on BMI (BMI ≤ 25 kg/m² vs. BMI > 25 kg/m², and BMI ≤ 25 kg/m² vs. BMI ≥ 30 kg/m², respectively), there was no group difference in midbrain binding. In the thalamus, binding is higher in the BMI > 25 kg/m² group than in BMI ≤ 25 kg/m² group (cluster size: 11 voxels, FWE-corr, p = 0.009), and age and thalamus binding did show a negative correlation (38 voxels, FWE-corr, p = 0.001). For BMI > 30 kg/m² vs BMI < 25 kg/m², the SERT binding is higher in the > 30 kg/m² group in the thalamus (3 voxels, FWE-corr, p = 0.025) and again a negative correlation between age and binding (29 voxels, FWE-corr, p = 0.002) was revealed.

DISCUSSION
In vivo human data of SERT availability in obesity or its correlation with BMI are still sparse and rather contradictory. Since SERT represent a major target of anorectic pharmacotherapy as mentioned above, studies were encouraged to clarify whether there is an association between SERT availability and BMI as a marker of obesity. The main finding of this study was that a firm

![Figure 6](image_url). SPM analysis demonstrating the correlation between thalamic uptake indicating SERT availability and body-mass-index (BMI).
result regarding the association between SERT availability and BMI was not obtained by the study data. However, the results of both voxel-based and ROI-analysis gave some clues for future research on the relationship between BMI and brain serotonin system. So, voxel-based analysis indicates a positive correlation between SERT and BMI in the thalamus. Also, in the ROI analysis the interaction between gender and BMI showed a trend with a higher coefficient for men.

These results are in agreement with previous findings of a positive association between BMI and SERT availability in a [$^{123}$I]nor-β-CIT SPECT study in monozygotic twin pairs with acquired obesity (24). It is interesting that this study also reported a significant effect in the thalamus but not in the midbrain. Like [$^{123}$I]FP-CIT, [$^{123}$I]nor-β-CIT binds in the striatum predominantly to the DATs, but in extrastriatal areas to the SERT, so, these radiotracers have comparable qualities. Our present observations with this non-selective SPECT radiotracer are also in line with our own preliminary data of a positive correlation between SERT availability and BMI, measured with the SERT-specific [$^{11}$C]DASB (12). Contrarily, the study by Erritzoe and co-workers (13) indicated a negative association between BMI, also in subcortical brain areas. The main reason for this discrepancy may be the low numbers of obese subjects in all studies, which makes single data points highly influential. In obesity, however, alterations of the presynaptic serotonergic function, i.e. the SERT, and changes of serotonergic tone were observed in recent animal studies, not only in the brain (8) but also in the gut (25). For example, the study of Huang et al. (9) showed increased SERT in diet-induced obesity, but the results of small laboratory animal studies are not consistent, even in the direction of the changes in SERT expression (6,7).

From a pathophysiological point of view, higher SERT availability at higher BMI theoretically indicates a higher SERT recruitment in healthy persons, most likely due to relatively lower extracellular serotonin. This serotonin imbalance either due to food overload or overactive reward and homeostatic circuits (stress-induced) may lead to higher serotonin recruitment as well, and high SERT can also be a compensatory upregulation in the case of high serotonin levels (26). Nevertheless, at this moment we do not know whether higher SERT availability can be a compensatory mechanism to chronic lower or higher extracellular serotonin concentrations. Indeed, (sub)acute lowering did not induce changes in SERT binding in humans (27), but this does not exclude that chronic changes in serotonin concentration may influence SERT binding. For studying the role of serotonin in obesity, it may be of interest to develop tools to assess extracellular serotonin concentrations, but this approach has not been successful yet (28). Low serotonin levels are associated with hyperphagia and weight gain (29). So, it can also be hypothesized that high SERT availability is a susceptibility for high BMI, as high SERT concentration leads to lower synaptic serotonin levels and thus to hyperphagia. Also, genetic contribution is of crucial interest since preliminary data on the dopamine transporter (DAT) suggest a correlation between DAT availability and BMI only in distinct allele carriers (30). For instance, the SERT promoter polymorphism might be related to obesity as well (31-33). Further, hypermethylation of the SERT promoter region is associated with obesity (34).

Comparing the linear models with and without age, the changes in the coefficient of determination was rather minor. That is, the percentage of the variance in the total model is statistically significant by age in the thalamus (3.4 % explained), but not in the midbrain (1.7 %).

For the interpretation of the present results, however, some drawbacks and limitations have to be mentioned. To overcome the fact that the delineation of the SERT target areas in the diencephalon and midbrain (raphe) is difficult because they are small; also the specific-to-
nonspecific binding ratios in these areas are not that high, we used different (independent) approaches to analyse the data which are either voxel-based (without MRI co-registration) or based on anatomically re-aligned SPECT scans. With both methods the tendency to higher values was shown in SERT-rich brain areas although the regions differed depending on the method used. One reason for the discrepancy might be that the ROIs were including the whole target structure, while the voxel-based analysis provided significant clusters only in a small volume of the thalamus. So, FWE correction allows considering significant clusters of 3-6 voxels, which is at the border of the spatial resolution of gamma cameras. Such explanation is more likely than that different equilibrium conditions in the thalamus and midbrain might have influenced the study results.

In a recent study in healthy controls, we showed that specific-to-nonspecific \(^{[\text{123}}\)FP-CIT binding ratios in the midbrain and diencephalon were significantly higher 2 h compared to 1 h after injection and remained stable between 2 and 3 h after injection (35). Consequently, 3 h after injection is a reasonable time-point to assess extrastriatal SERT binding with \(^{[\text{123}}\)FP-CIT SPECT in vivo, although it has not been formally tested if this ratio is also stable up to 4 h p.i.. As a fact, however, the binding behaviour of FP-CIT in the diencephalon-brainstem is different from that in the striatum (see 17) representing mainly DAT.

It is also speculative to which part of the thalamus the presently observed significant small area in the thalamus belongs. This particular area, however, seems to involve the more midline part of the thalamus (the paraventricular thalamus, the pulvinar) rather than the hypothalamus. Interestingly, this part of the thalamus is responsible for the control response to chronic stress mediated by the serotonergic system and consequently the expression of SERT may play a role (reviewed in 37). As the study by Koskela et al. (24) did apply ROI analysis and not voxel-based analysis, it is unknown whether the reported effect in the thalamus was located in the same thalamic region in that study. Because we find this effect only in the voxel-based analysis in clusters that are not very large, future studies are necessary to replicate this finding. Last but not least as is the case in all the in vivo SERT PET and \(^{[\text{123}}\)FP-CIT imaging studies, the ENCDAT study was also hampered by the limited number of heavily obese subjects, which as mentioned in every case seem to be the crucial data in determining the fitting curve.

CONCLUSIONS

Altogether, the analysis of this unique large European database of \(^{[\text{123}}\)FP-CIT SPECT data suggests that the SERT availability tends to be higher at higher BMI. This shall further be proven in a dedicated cohort with highly obese individuals in order to further shed light on the potential role of the serotonergic system in overweight and consequently to stimulate more studies on SERT as a pathophysiological key substrate in obesity.

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