Eur J Nucl Med Mol Imaging (2014) 41:1938–1946
DOI 10.1007/s00259-014-2785-8

ORIGINAL ARTICLE

Extrastriatal binding of [^{123}I]FP-CIT in the thalamus and pons: gender and age dependencies assessed in a European multicentre database of healthy controls

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Received: 15 December 2013 / Accepted: 15 April 2014 / Published online: 8 May 2014

Abstract
Purpose Apart from binding to the dopamine transporter (DAT), [^{123}I]FP-CIT shows moderate affinity for the serotonin transporter (SERT), allowing imaging of both monoamine transporters in a single imaging session in different brain areas. The aim of this study was to systematically evaluate extrastriatal binding (predominantly due to SERT) and its age and gender dependencies in a large cohort of healthy controls.
Methods SPECT data from 103 healthy controls with well-defined criteria of normality acquired at 13 different imaging centres were analysed for extrastriatal binding using volumes of interest analysis for the thalamus and the pons. Data were

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examined for gender and age effects as well as for potential influence of striatal DAT radiotracer binding.
Results Thalamic binding was significantly higher than pons binding. Partial correlations showed an influence of putaminal DAT binding on measured binding in the thalamus but not on the pons. Data showed high interindividual variation in extrastriatal binding. Significant gender effects with 31 % higher binding in women than in men were observed in the thalamus, but not in the pons. An age dependency with a decline per decade (±standard error) of 8.2±1.3 % for the thalamus and 6.8±2.9 % for the pons was shown.
Conclusion The potential to evaluate extrastriatal predominant SERT binding in addition to the striatal DAT in a single imaging session was shown using a large database of [^{123}I]FP-CIT scans in healthy controls. For both the thalamus and the pons, an age-related decline in radiotracer binding was observed. Gender effects were demonstrated for binding in the thalamus only. As a potential clinical application, the data could be used as a reference to estimate SERT occupancy in addition to nigrostriatal integrity when using [^{123}I]FP-CIT for DAT imaging in patients treated with selective serotonin reuptake inhibitors.

Keywords Dopamine transporter · Serotonin transporter · [^{123}I]FP-CIT · Extrastriatal binding · SPECT · Gender difference · Age effects

Introduction

Imaging of the dopaminergic system provides a valuable tool for discriminating neurodegenerative Parkinsonian syndromes with an associated presynaptic dopaminergic deficit from diseases without presynaptic neurodegeneration (e.g. essential tremor) [1–3]. The use of dopamine transporter (DAT) SPECT has recently been extended to the discrimination between patients with suspected dementia with Lewy bodies (DLB) and those with Alzheimer's disease [4].

[^{123}I]N-ω-Fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane ([^{123}I]FP-CIT) is the most widely used radiotracer for DAT imaging. It is commercially available and approved for use in patients with Parkinsonian syndromes (Europe and the US) and suspected DLB (Europe). [^{123}I]FP-CIT shows high affinity for DAT but also moderate affinity for the serotonin transporter (SERT) [5]. Binding of radiolabelled tropane derivatives in most extrastriatal regions (ESTR) has been primarily attributed to SERT binding. The thalamus has about 20 times more SERT than DAT binding sites [6–8]. In an autoradiography study of the human brain [9], the selective serotonin reuptake inhibitor (SSRI) citalopram almost entirely displaced [^{125}I]RTI-55 ([^{125}I]2β-carbomethoxy-3β-(4-iodophenyl)tropane, [^{125}I]β-CIT) in the midbrain, thalamus and hypothalamus, indicating that radiotracer uptake in these regions is almost exclusively associated with SERT and not with DAT. With a preference for DAT sites (3.5 nM) over SERT sites (10 nM) [5], approximately 70 % of [^{123}I]FP-CIT uptake in the thalamus should result from SERT binding. Consistently, applied to SPECT imaging, Ziebell et al. [10] observed a loss of thalamic binding potential of the nondisplaceable radioligand by 63 % when blocking SERT with citalopram. For semiquantitative evaluation using a ratio method in transient equilibrium (as generally applied in clinical routine), even 86 – 90 % of thalamus signal could be attributed to SERT binding [10, 11]. The first successful attempts to establish [^{123}I]FP-CIT for SERT imaging studied the central serotonergic system in 6 [12] and 19 [13] healthy volunteers. Initial applications in patients indicated potential clinical usefulness showing reduced extrastriatal SERT availability in patients with Parkinson's disease and particularly in those with DLB [14, 15].

In comparison to other cocaine analogues suitable for SPECT imaging, [^{123}I]FP-CIT allows the combined evaluation of both DAT and SERT in a single scan. The ideal time window for imaging the extrastriatal SERT is between 2 and 3 h after injection [13]. For DAT imaging with [^{123}I]FP-CIT, the pseudoequilibrium is reached 3 h after injection [16], resulting in ideal imaging conditions for both monoamine transporters 3 h after injection. This, in addition to its widespread availability, makes [^{123}I]FP-CIT attractive for imaging not only the striatal DAT but also the extrastriatal SERT in the diagnostic work-up of patients. As a potential clinical application, SERT occupancy could be estimated in patients treated with SSRIs undergoing DAT SPECT to measure nigrostriatal integrity. Depression is a common nonmotor symptom in Parkinson's disease and its treatment is challenging [17]. Higher pretreatment SERT availability, higher SSRI-induced occupancy of SERT [18] and SERT interplay between the median raphe nucleus and projection areas [19] have been

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associated with treatment response. Knowledge of SERT availability could allow individualization of treatment strategies (for example by optimized selection of the most suitable antidepressant with a serotonergic, noradrenergic, dopaminergic or monoamine oxidase inhibiting mechanism).

For accurate interpretation of [^{123}I]FP-CIT binding to SERT, knowledge of potential influencing factors such as age or gender dependencies is essential. An age-related decline in SERT binding has been described using other radioligands [20–27]. However, post-mortem studies have not confirmed effects of aging on SERT mRNA availability [28–32].

The aim of this study was to explore age and gender influences on extrastriatal binding of [^{123}I]FP-CIT to SERT in healthy volunteers using the data from a well-defined cohort of a large multicentre trial.

Methods

Subjects

Included in this prospective study were 103 healthy individuals free of neurological and psychiatric diseases (46 women, 57 men; age range 21 – 83 years, mean 51.8±17.8 years). The study population is part of the ENC-DAT (European Normal Control Database of DaTSCAN) database established as an initiative of the Neuroimaging Committee of the European Association of Nuclear Medicine and includes data from 13 different imaging centres. All subjects had to fulfil predefined criteria of normality without any significant clinical abnormalities in medical history, blood chemistry, neurological examination (including the Unified Parkinson's Disease Rating Scale score) and psychiatric evaluation including the Symptom Checklist-90 revised (score <63) [33] and the Beck Depression Inventory (score <9) self-assessment scales [34]. Cognitive deficits as assessed by the Mini-Mental State Examination (score ≥28) were ruled out. Furthermore, a history of Parkinsonian syndromes in first-degree relatives was an exclusion criterion. The study population had a balanced gender and age distribution. The average ages were comparable between the women (51.3±17.7 years) and the men (52.1±18.1 years).

Each subject underwent MRI examination according to the protocols of the respective imaging centre to detect potential structural pathologies. Pregnancy was ruled out in the women by urinary pregnancy testing. In addition, all subjects underwent urine drug testing. Approval of the ethics committee and the local authorities was obtained and all subjects signed written informed consent. Further details have been reported previously [35].

SPECT imaging

Stringent quality control protocols were applied at each of the 13 imaging sites [36]. Uniformity and centre of rotation were continuously maintained. In addition, ^{123}I SPECT images of an anthropomorphic striatal phantom (Radiology Support Devices Inc., Long Beach, CA) were acquired in each of the participating centres according to a protocol published elsewhere [37] to calibrate the gamma camera systems on 17 imaging systems: four Siemens ECAM, three GE Infinia, three Philips IRIX, two Siemens SYMBIA, two Trionix Triad XLT 20, one GE Millennium VG, one Siemens MultiSPECT, and one Mediso x-Ring/4HR.

[^{123}I]FP-CIT (180±16 MBq) was injected intravenously as a bolus. SPECT images were acquired 3.0±0.3 h after tracer injection. Acquisition parameters were: fixed rotational radius between 13 and 15 cm, matrix 128 × 128, angular sampling ≤3° (360° rotation), and hardware zoom of 1.23 to 2.00 to achieve a pixel size of 2 – 3 mm. The photopeak was set to 159 keV ±10 %. The acquisition time was chosen to obtain at least 2 million counts per study. Further information on SPECT system configuration and the scanning procedure has been reported previously [35].

In the subset of the ENC-DAT database reported here, scans were reconstructed using HERMES hybrid reconstruction (Hermes Medical Solutions, Stockholm, Sweden) using ordered subsets expectation maximization (OSEM) with 16 iterations and four or five subsets depending on the number of projections. To obtain a high level of standardization of scans with different camera systems and to reduce partial volume effects when evaluating small structures such as the pons, all scans were corrected for

1. Attenuation: Chang method [38] with automatically generated ellipses, and an attenuation coefficient μ of 0.146 has been used, the high value for μ corresponding to the "narrow beam attenuation coefficient" that is recommended when scatter correction is applied [39].
2. Scatter: A Monte-Carlo based scatter correction was applied using the HERMES Monte Carlo simulator [40]. Basically this method uses reconstruction-based scatter compensation with Monte Carlo modelling of scatter. It includes the simulation of the pathways of a large number of photons accelerated by a coarse grid and intermittent scatter modelling without simulation of septal penetration.
3. Resolution recovery: Correction for depth-dependent detector response was done separately for each camera model using calculated point-spread functions based on the exact geometry of the respective collimator as well as the scanning geometry of the detector orbit [41].

Three-dimensional gaussian postfiltering was applied using a kernel with full-width at half-maximum (FWHM) of 7 mm, resulting in an estimated image FWHM of approximately 8 – 10 mm (more details are provided by Dickson et al. [36]).

Automated semiquantitative evaluation method

Semiquantitative evaluation of the SPECT data was performed using the brain analysis software (BRASS, version 3.6; Hermes Medical Solutions, Stockholm, Sweden). The software automatically performs spatial coregistration of individual patient images to a [^{123}I]FP-CIT healthy control template using a registration algorithm based on mutual information and optimized for [^{123}I]FP-CIT images. The accuracy of the coregistration of scans to the template was confirmed in all subjects. The template itself was derived using the 103 normal control scans, which were spatially normalized to the Montreal Neurological Institute standard MRI template (MNI; McGill University, Montreal QC, Canada) [42]. Next, a standard set of 3-D VOIs defining the thalamus and the putamen based on the Automated Anatomical Labelling (AAL) atlas [43] as well as a spherical VOI for the pons was created. An occipital cortex (OC) VOI served as the reference region. The respective VOIs are shown in Fig. 1. All VOIs were applied to the SPECT data, and manually adjusted (drag and drop) when necessary to ensure positional accuracy. The pons VOI was positioned to cover the uptake in the area of the raphe nuclei. Mean counts per voxel were calculated for each VOI. Specific binding (SB) ratios in the predefined ESTR in relation to the OC were calculated according to the formula:

$$SB = \frac{ESTR - OC}{OC}$$

Statistics

Descriptive statistics are given as means and standard deviation. Comparisons between and within groups were tested with independent group or paired Student's *t* tests, respectively. The relationship between age and SERT binding was analysed using linear regression and described by Pearson correlation coefficients. To verify differences in the slopes of the linear regression curves between male and female subjects and therefore different gender profiles of age effects, the effect of gender on the slopes of the regression lines was tested by analysis of covariance investigating the significance of the interaction between the classification effect (gender) and the covariate (measured SB). Analyses of covariance was used to determine the influence of gender, age

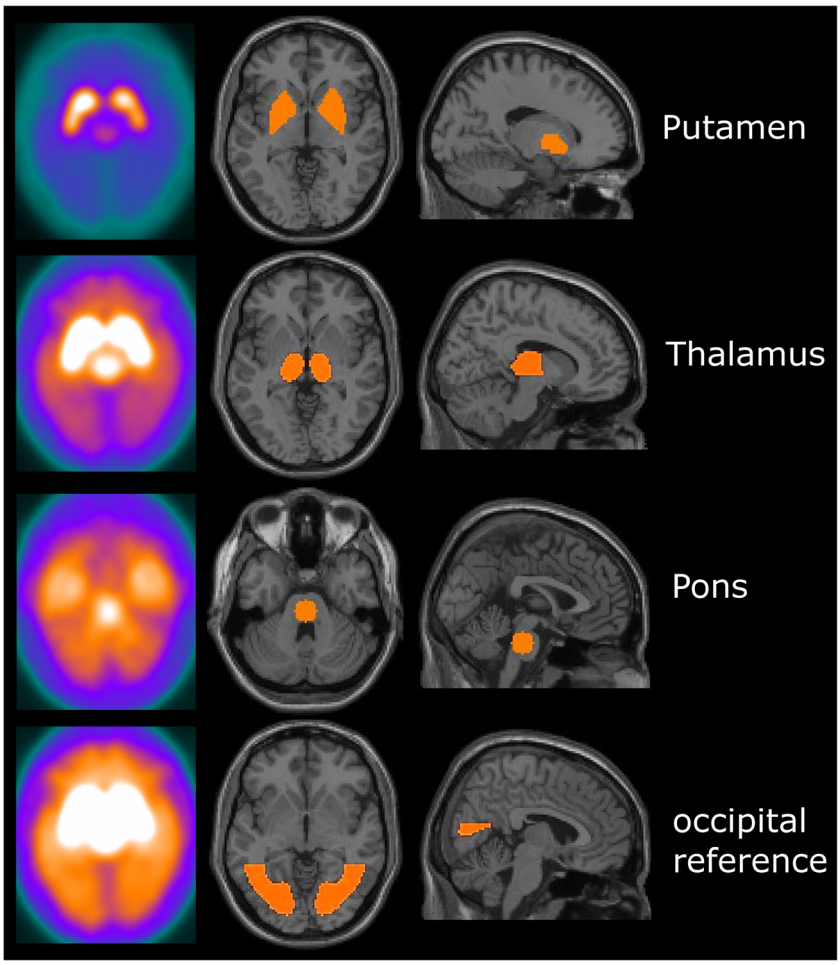

Fig. 1 VOIs used for semiquantitative analysis and corresponding SPECT template (*left column* colour scale thresholds adjusted to depict the respective structures)

and striatal binding on extrastriatal binding. To control for confounding factors, partial correlations were calculated. All statistical analyses were performed using SPSS software version 13.0 (SPSS Inc, Chicago, IL).

Results

The age distributions of the men and women were balanced (p=0.814). Measured specific SERT binding ratios in both the thalamus and pons showed considerable variability across the full age range (Fig. 2) with thalamic binding ratios in general being significantly higher than pons binding ratios (0.35±0.17 vs. 0.26±0.21, p=0.001).

Mean SB ratios in the thalamus were significantly higher in women than in men (0.40±0.15 vs. 0.31±0.17, p=0.003). Mean SB ratios in the pons were slightly higher in men than in women (0.29±0.19 vs. 0.22±0.23), but this difference did not reach the level of significance (p=0.077). A linear fit showed an age-related decline in [^{123}I]FP-CIT binding ratios in both the thalamus and the pons (Fig. 2). The corresponding regression parameters, Pearson correlation coefficients and p values are given in Table 1 (for comparison these values are also given for the putamen). Correlation coefficients were stronger for the thalamus than for the pons. Correlations were significant for the entire cohort as well as for men and women, with only one exception: the correlation between age and SB ratios in the pons in women failed to reach the level of significance. Extrastriatal binding ratios showed a reduction with normal aging with average (±standard error) declines per decade of 8.2±1.3 % in the thalamus and 6.8±2.9 % in the pons.

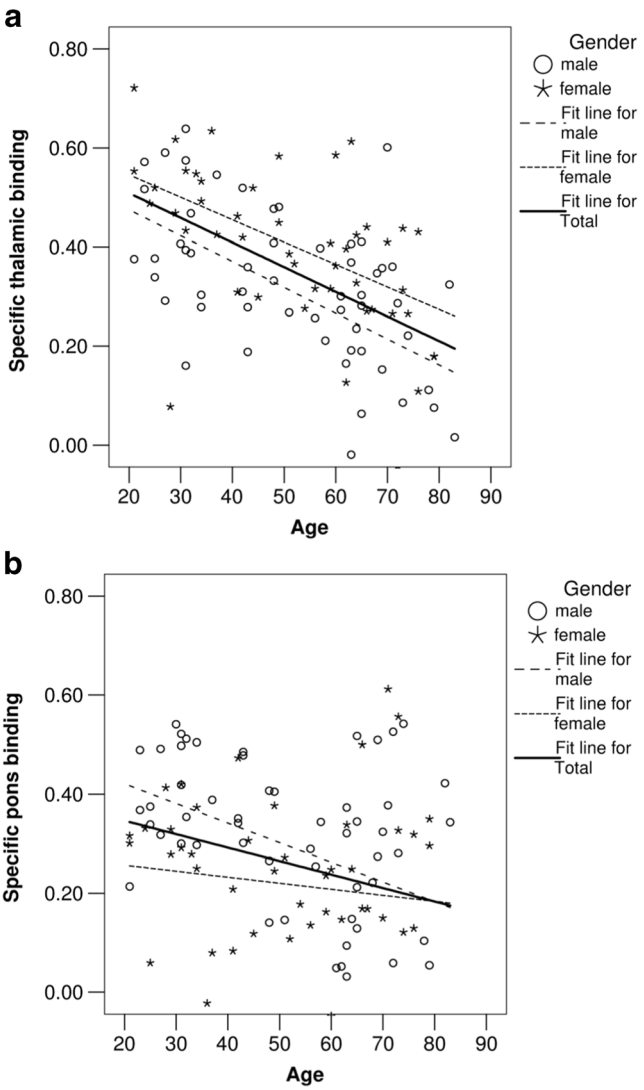

Fig. 2 Correlations between age and [^{123}I]FP-CIT binding ratios in (**a**) the thalamus and (**b**) the pons

The declines in binding ratios per decade in the thalamus and in the pons were not different between men (thalamus 9.0 ±1.9 %, pons 7.9±2.7 %) and women (thalamus 7.1±1.6 %, analysis of covariance p=0.61; pons 4.3±7.0 %, p=0.10). Specific thalamic binding ratios were also correlated with putaminal binding ratios (Pearson's correlation coefficient 0.35, p<0.001), whereas no correlation was found between pons and putaminal binding ratios (correlation coefficient 0.11, p=0.29; Fig. 3). Controlling for putaminal binding ratios with partial correlations, age effects remained stable in both the thalamus (p<0.001) and pons (p=0.043).

Discussion

To our knowledge this is the largest study to systematically evaluate extrastriatal binding of the commercially available DAT tracer [^{123}I]FP-CIT in a cohort of healthy controls with a wide age range and with well-defined criteria of normality. The study population had balanced age distribution across the whole age range as well as an even gender distribution. The measured specific thalamic [^{123}I]FP-CIT binding ratios were similar to those recently reported by Borgers et al. [12] and Koopman et al. [13]. As expected, SB ratios in SERT-rich ESTR were considerably lower than in the DAT-rich striatum, owing to the lower affinity of [^{123}I]FP-CIT for SERT than for DAT [5, 44] and the overall lower expression of SERT in the brainstem and thalamus in comparison to the striatal DAT expression [45, 46].

There was high variability (high standard deviation compared to the average) of extrastriatal radiotracer binding across the entire age range. A drug-induced reduction due to potential blocking effects of SSRIs [10, 11] or other psychotropic drugs were excluded as all subjects were drug-naive and urine drug screening revealed no abnormalities. Also psychiatric disorders as potential confounding factors were ruled out. In line with our results, similar high interindividual variations in extrastriatal binding have also been found using other SPECT and PET tracers [10, 13, 47–50]. Ziebell et al. [10] discussed potential reasons for this variability. It could be attributed to

Table 1 Parameters of linear regression analysis of age with thalamic, pons and putaminal (for comparison) radiotracer binding ratios for the entire cohort as well as for the subgroups men and women

Region	Gender	Slope ± standard error	Constant	Pearson coefficient	*p* value
Thalamus	Both	−0.0050±0.0008	0.61±0.04	−0.526	<0.001
	Female	−0.0045±0.0010	0.64±0.06	−0.547	<0.001
	Male	−0.0052±0.0011	0.58±0.06	−0.542	<0.001
Pons	Both	−0.0027±0.0012	0.40±0.06	−0.226	0.022
	Female	−0.0012±0.0020	0.28±0.11	−0.092	0.542
	Male	−0.0040±0.0013	0.50±0.07	−0.369	0.005
Putamen	Both	−0.0110±0.0022	2.70±0.12	−0.439	<0.001
	Female	−0.0131±0.0034	2.85±0.18	−0.386	0.003
	Male	−0.0093±0.0030	2.58±0.17	−0.503	<0.001

variation in SERT density, the relatively low [^{123}I]FP-CIT binding affinity to SERT with consequently relatively low binding ratios, or relative DAT versus SERT density ratios in the analysed regions.

We observed a significant correlation between thalamic (but not pons) radiotracer binding and putaminal binding. A similar finding was reported by Ryding et al. [26] for the thalamus using ([^{123}I]β-CIT). Visual image analysis revealed potential spill-in of striatal counts into the thalamic VOI. Despite applying resolution modelling and scatter correction, spill-in of counts cannot be entirely eliminated [51] and might have led to a stronger observed correlation than could be attributed to direct thalamic DAT radiotracer binding, since DAT density in the thalamus is known to be relatively low [52]. Potential spill-in might limit the specific analysis of SERT in the thalamus and (apart from potential biological binding differences, partial volume effects and VOI sizes) could also contribute to the higher radiotracer uptake observed in the thalamus than in the pons. Eventually this might also account for the significantly higher thalamic uptake in women than in men, due to the well-documented gender dependency of DAT binding [53, 54]. Borgers et al. [12] analysed binding of [^{123}I]FP-CIT in the hypothalamus in healthy controls, patients with pituitary insufficiency and patients with hypothalamic impairment, but found no differences in binding ratios in the hypothalamus among these groups, although SERT appears to play a key role in the hypothalamus [55]. The assumed spill-in of counts from striatal DAT binding in combination with the overall low specific [^{123}I]FP-CIT binding ratios in the thalamus may have eventually masked the expected group differences. In our subjects, the pons region did not appear to be influenced by striatal binding. Although higher radiotracer binding in women than in men has been reported in this region for other radiotracers [53, 56], and despite preclinical evidence of protective effects of oestrogens on SERT [57], we were not able to show significant gender effects on [^{123}I]FP-CIT binding. We found a significant age dependency of [^{123}I]FP-CIT binding in both the thalamus and the pons. These age effects remained significant after controlling for potential spill-in from the putamen using partial correlation analyses.

Post-mortem studies with [^{3}H]imipramine [29–31] and [^{3}H]paroxetine [28, 32] did not reveal any age-dependent

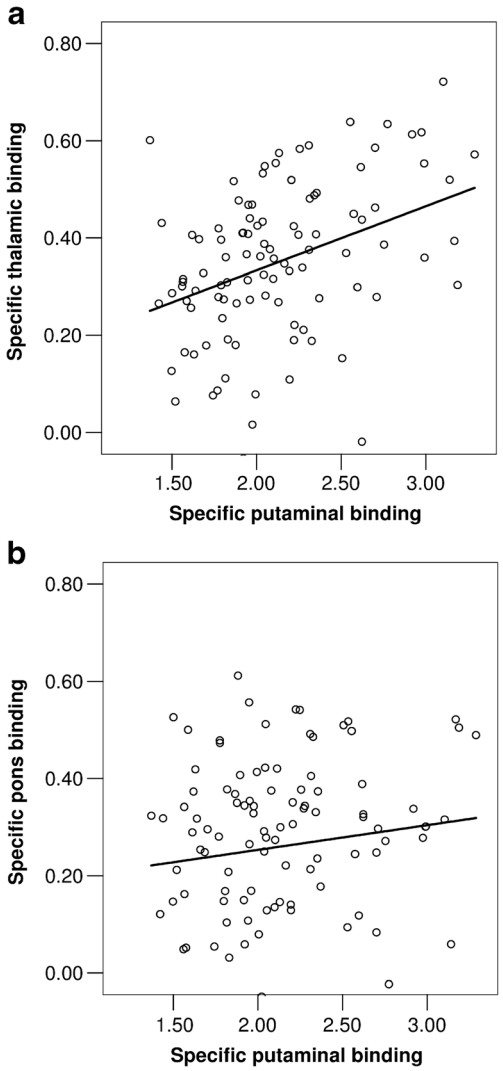

Fig. 3 Correlations between putaminal [^{123}I]FP-CIT binding ratios and (**a**) thalamic binding ratios and (**b**) pons binding ratios

decline in SERT expression in the thalamus or brainstem. The inability of these studies to demonstrate age-dependency could potentially be explained by their limited sample size, low age range of the subjects and limited selectivity of the ligands used. Most neuroimaging studies have confirmed effects of healthy aging on SERT. Using [^{123}I]β-CIT, several studies have shown a decline in thalamus binding per decade in the range 3.2 % to 7.2 % and in midbrain binding in the range 4.2 % to 8.3 % [22, 25, 27]. We obtained higher values (8.6 % in the thalamus and 7.1 % in the pons) which lie in a similar range to those reported for DAT [58–61]. Binding loss with healthy aging seems to vary depending on which radiotracer is used (possibly attributable to different levels of specific to nonspecific binding as well as the contribution of SERT to the reference region used). Using the more SERT-selective tracer 2-((2-((dimethylamino)methyl)phenyl)thio)-5-[^{123}I]iodophenylamine ([^{123}I]ADAM), a low decline of only 3.0 % per decade in the midbrain was reported by Newberg et al. [24] and using [^{123}I]2β-carbomethoxy-3β-(4-iodophenyl)nortropane ([^{123}I]Nor-β-CIT) a decline of 2.0 % per decade was reported by Kuikka et al. [23]. In young subjects up to 35 years of age, Buchert et al. found no relevant influence of aging on SERT [56] using [^{11}C]McN5652 and PET. Aging effects in young subjects appear not to be relevant. On the other hand, the finding could also indicate a nonlinear relationship between age and SERT binding that cannot be entirely ruled out based on our data. Nonlinear fits did not provide usable results in our study population due to the observed high variation of extrastriatal binding.

There are some limitations to consider when interpreting our results. First, [^{123}I]FP-CIT has higher affinity to DAT than to SERT [5, 44]. There is evidence of the presence of some DAT expression in the midbrain [62], potentially also resulting in a small influence on the pons binding observed. We controlled for this statistically using partial correlations. Age effects remained stable when controlling for DAT binding ratios measured in the putamen. For [^{123}I]β-CIT, attempts have been made to separate DAT and SERT binding based on the different uptake kinetics and on SERT blocking with selective SSRI [26]. Using this method, Ryding et al. observed no age dependency of SERT binding, but the sample size (23 healthy controls) is considerably lower than in our study. More accurate separation could be achieved using more selective SERT ligands such as [^{123}I]ADAM [63]. Second, our results were based on a variety of different imaging equipment, potentially explaining some of the interindividual variation observed. Phantom calibration [37] was validated for striatal DAT binding only, and not for extrastriatal SERT-rich regions. However, extensive efforts for standardization of acquisition protocols and camera-specific resolution modelling in the reconstruction process were made, and all scans were processed and evaluated in a single centre. Third, apart from resolution modelling in the reconstruction process, no additional MRI-based recovery correction was performed to reduce potential pons atrophy effects with aging. Previous studies examining age-related volumetric decline in the brainstem, however, have shown no overall volume loss in this area [64, 65].

Fourth, we did not correct for other potential confounding factors such as seasonal variability in SERT availability [56]. Also polymorphism in the SERT promoter gene has been reported, that can potentially regulate SERT expression [66] possibly modulated by a family history of axis-I disorders. A family history of axis-I disorders was an exclusion criterion. Fifth, in line with most other groups, we used the OC as the reference region for both SERT and DAT analysis which contains a low (but presumably not relevant) concentration of SERT [45, 67]. Age effects could be underestimated if age-related decline in the occipital reference region occurs in a similar proportion as in the thalamus or pons. We did not use the cerebellum as the reference region, since attenuation correction in this area is problematic and would have introduced additional variability. In addition, in clinical routine imaging, the cerebellum is often not covered entirely, limiting the use of a cerebellar reference region.

Conclusion

Imaging with the radioligand [^{123}I]FP-CIT, that is commercially widely available and approved for specific applications, may allow the evaluation of DAT and SERT in a single imaging session. [^{123}I]FP-CIT binding in the thalamus as well as in the pons showed high interindividual variation most likely hampering accurate interpretation of SERT expression on an individual basis. The additional information on SERT binding, however, may provide new insights into neurological and psychiatric diseases such as shown for Parkinson's disease and DLB [14, 15] in research trials in which [^{123}I]FP-CIT and SPECT are used. The data could also be used as a reference to estimate SERT occupancy in addition to nigrostriatal integrity when using [^{123}I]FP-CIT for DAT imaging in patients treated with selective SSRIs to individualize treatment of depression, a common nonmotor symptom in Parkinson's disease.

Acknowledgments The participating centres thank GE Healthcare and the German Parkinson Association for their financial contribution to this study, ABX-CRO for managing the network activities and the Executive Committee of the EANM for establishing EANM Research Ltd. (EARL) as an administrative framework for this project.

The authors also thank the personnel of each Nuclear Medicine Centre responsible for the quality controls and acquisition of the SPECT data.

Disclosure Prof. Jan Booij and Koen Van Laere have acted as neuroimaging consultants for GE Healthcare in the context of [^{123}I]FP-CIT scans.

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