No link between striatal dopaminergic axons and dopamine transporter imaging in Parkinson's disease

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Abstract

Background: Brain dopamine transporter (DAT) binding has been considered a possible biomarker for nigrostriatal degeneration in Parkinson's disease (PD).

Objective: To investigate if DAT binding is associated with the number of dopaminergic neurites in the putamen.

Methods: Tyrosine hydroxylase positive (TH+) nerve fibers were counted from *postmortem* putamen sections taken from 14 parkinsonism patients who had been scanned with DAT SPECT *antemortem*. The fiber counts were correlated with the putamen DAT binding and substantia nigra neuron counts.

Results: The putamen DAT specific binding ratio (SBR) did not correlate with the putamen TH+ axon counts (r=0.00, p=1.0; PD patients: r=0.07, p=0.86). The nigra neuron counts had a positive correlation with the putamen TH+ axon counts.

Conclusions: Striatal DAT imaging does not associate with axonal nor somal loss of the nigrostriatal neurons in PD. It may reflect dopaminergic activity rather than number of surviving neurons or their striatal projection axons.

Introduction

The nigrostriatal dopaminergic tract degenerates in Parkinson's disease (PD), but the level of damage along the pathway may not be the same at each point in time. There is a large body of evidence from imaging, genetic and *postmortem* studies that suggests the axons, not the somas, of the dopaminergic neurons are first damaged in PD.[1,2] This retrograde ('dying back') degeneration process starts at the striatal end of the nigrostriatal tract, which could also impact correlations between striatal and nigral imaging markers. Indeed, in patients with early PD, there appears to be a marked loss of dopamine transporter (DAT) in the striatum with a concurrent preservation of nigral DAT levels.[3] In line with this observation, our previous results suggested that *postmortem* nigral neuron numbers and *antemortem* striatal DAT binding do not correlate in PD[4]; this finding could be associated with the dying back phenomenon.

Given the apparent retrograde degeneration process of the nigrostriatal pathway in PD, we considered it possible that, although striatal and nigral markers do not correlate, there could be a positive relationship between striatal DAT binding and the number of striatal nerve fibers (axons). Decreased DAT binding could represent more damage to the striatal terminals rather than the loss of nigral nerve cell bodies.[5] This correlation would agree with the dying back phenomenon and would validate striatal DAT imaging as a marker of striatal dopaminergic axon density rather than a marker of nigral neuron numbers. Conversely, if striatal DAT binding does not correlate with striatal axon numbers, then the result would point to a role for DAT imaging as a functional measurement of striatal dopaminergic activity.

Methods

Neuropathology

Fourteen patients with neuropathologically confirmed PD (n=10) or atypical parkinsonism (n=4) were included in the study. The present sample is a partial subsample from a previously published study (n=18).[4] Putamen samples were not available for six patients who were included in the previous study. Two patients were excluded from the earlier study due to the lack of substantia nigra pars compacta (SNc) samples, but these patients were included in the present study. Initial neuropathological examinations were performed in the Department of Pathology at Turku University Hospital, Finland, in 2004-2015. For this study, brain samples from all included patients were re-examined by neuropathologists, who used previously described neuropathological diagnostic criteria.[6,7] Patients were scanned with DAT SPECT *antemortem* for diagnostic purposes, as previously described[4]. The mean interval was 5.2 (3.4) years between scanning and death. The results were separately calculated for patients with a shorter interval of 3.2 (1.6) years (n=9). The mean duration of the disease at the time of death was 6.7 (3.2) years from diagnosis. Two patients were receiving antiparkinsonian medications at the time scanning (missing data for 4 patients). All but one patient was treated with levodopa at the time of death (missing data for one patient). Clinical and demographic details are presented in Table 1.

Formalin-fixed, paraffin-embedded tissue from the putamen samples was taken from either the right or left hemisphere (7 patients each). The hemisphere and location of the samples were selected by the neuropathologist during the routine examination on the basis of representativeness. Data were obtained from the coronal slides of postcommissural striatum in which the globus pallidus interna and externa were present (Fig 1A). To identify

dopaminergic axons, putamen samples were cut at 8 μm sections. The staining for tyrosine hydroxylase (TH) was done as previously described.[8] The slides were re-evaluated prior to immunohistochemistry to confirm that the tissue was representative and that it was of sufficient quality. The outlines were drawn on putamen and this area was sectioned into 9 subregions to aid in the counting (Fig 1B). In each putaminal subregion, two independent examiners annotated three standardized areas of the visual-field (20x magnification) to obtain a total area of approximately 1.0 mm^2 for each region. In these magnification windows, TH-positive nerve fibers or cross-sections of fibers were quantified in 27 annotated areas in each putamen (Fig 1C and 1D). The interrater agreement of fiber counts between two independent examiners was good (ICC=0.739, absolute agreement) or excellent (ICC=0.815, consistency). Fiber counts were reported for six putaminal regions based on different regional neuronal/axonal densities: three in medial-lateral and three in rostrocaudal direction.

SPECT imaging and image analyses have been described previously.[4] Specific binding ratio (SBR) calculations were done on the DAT SPECT data. Spatial resolutions of the scanners ranged between 8.0 and 10.5 mm.

Statistical Analyses

Mean putamen SBRs were correlated with the corresponding total putamen fiber counts. The analyses were performed separately for anterior and posterior putamen SBRs. The correlations were analyzed with Spearman's rank correlation coefficients or Pearson's partial correlation coefficients as appropriate. The level of statistical significance was set at two-tailed $p<0.05$.

The study was approved by the Ethics Committee of our institution. Our institution does not require written patient consent for retrospective studies.

Results

The putamen SBR did not correlate with the total putamen TH+ fiber counts in all patients (r=0.00, p=1.0, n=14) or in PD patients (r=0.07, p=0.86, n=10). Correlations remained nonsignificant when the time interval between SPECT and death was used as covariate in the analysis (r=0.27, p=0.37), when the symptom duration at the time of the scan was used as a covariate (r=0.07, p=0.82), when only the FP-CIT SBRs were used (r=0.00, p=1.0, n=12) or when the scanner was used as a covariate (r=0.34, p=0.31). Correlations between total putamen TH+ fiber counts and mean putamen SBR remained nonsignificant when intervals death to neuropathology (r=-0.04, p=0.90) or death to autopsy (r=-0.02, p=0.94) were used as covariates. In addition, in patients with shorter interval between SPECT and death, correlations were nonsignificant (r=0.21, p=0.62). The putamen SBR (mean, anterior or posterior) did not correlate with fiber counts in any of the six putaminal subregions in all patients (r=-0.24 to 0.11, p>0.42) or in PD patients (r=-0.29 to 0.23, p>0.43) (Fig 1E).

The SNc TH+ neuron count correlated with the medial and central putamen fiber count in all patients (central in rostrocaudal direction r=0.65, p=0.02) and in PD patients (medial r=0.68, p=0.04; central in rostrocaudal direction r=0.80, p=0.01) (Fig 1F). There were no significant correlations between the SNc neuromelanin containing neuron counts and fiber counts (r=0.25, p=0.44).

Discussion

These results provide no evidence for the hypothesis that putamen DAT binding is associated with the number of putamen dopaminergic axons. Our earlier study, which included some of the same patients, showed that striatal DAT binding did not correlate with the number of substantia nigra neurons.[4] The lack of correlation could have been due to differences in the temporal development of striatal and nigral neuropathology in relation to retrograde axonal degeneration. If the striatum is the initial site of nigrostriatal damage in PD, and the loss of dopaminergic markers in the striatum is more severe than in the substantia nigra in early PD, DAT binding deficits in the striatum would correlate with the number of striatal axons but would not correlate with the number of nigral cell bodies. Nevertheless, our present results clearly show no correlation between striatal DAT binding and the striatal dopaminergic neuron axon counts. This finding suggests that, with our patients having a mean duration of motor symptoms of 1.5 years at the time of imaging, who generally have approximately 50% loss in striatal DAT binding as compared with age-matched healthy individuals[9], there is no relationship between tracer binding and the amount of dopaminergic striatal axons.

Previously, similar studies have been conducted with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys. In line with our results, stereologically measured striatal fiber lengths in MPTP-monkeys have not correlated with striatal terminal PET measures, but an association has been observed with nigral cell counts.[10] In addition, although diffusion MRI measures have correlated with nigral neurons and striatal fiber density in the MPTP model, PET measures of striatal tracer uptake have not.[11] Currently, neuropathological data from human subjects who have undergone functional dopamine imaging are extremely rare, and the main limitation in our study is the small number of

patients. Therefore, caution must be applied to our findings, although our sample was comparable with previous clinicopathological imaging studies (reviewed in[4]). It should also be noted that we did not count fibers with an unbiased stereological method but by manually from a section, which could have induced some bias together with the possibility of *postmortem* variation in tissues. However, two independent investigators counted the fibers and the interrater reliability of the measurements was good to excellent. Although the fiber counts did not correlate with striatal DAT binding, they did correlate with the number of nigral neurons, which suggests that the method, the sample size and the quality of the samples were sufficient to detect associations between different nigrostriatal measures. A limitation of any clinicopathological *postmortem* study is the time interval between the measurements. Also in our study, the delays limit the interpretation, although they were used as covariates in the analyses also separately for patients with shorter and longer intervals. Finally, the results might be different in early PD when the level of damage is less severe.[12,13] Studies with rare *postmortem* data of patients dying at early stages of PD are therefore greatly needed. Given the significant degree of uncertainty due to our sample size and disease progression, we cannot exclude a detectable relationship between DAT and nerve terminals in larger samples of patients, especially at earlier stages of PD.

If DAT imaging does not reflect the counts of cell bodies, dendrites or axons, one has to consider the possibility that the imaging marker reflects dopamine or dopaminergic activity as opposed to neuronal structures. DAT appears to have an active role in the termination of synaptic dopamine signals, and also in the release of dopamine.[14] Thus, DAT imaging could reflect synaptic dopamine levels or DAT expression, a hypothesis which should be investigated in detail in future imaging studies.

To conclude, there appears to be no correlation between striatal DAT binding and the number of *postmortem* striatal axons or nigral neurons. The precise mechanism of changes in DAT binding signal in PD remains to be elucidated, but the amount of synaptic dopamine may have a key role. These results do not support the use of DAT imaging as a surrogate marker for striatal axonal loss.

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Author roles

1. Research project: A. Conception, B. Organization, C. Execution;
2. Statistical analysis: A. Design, B. Execution, C. Review and critique;
3. Manuscript preparation: A. Writing the first draft, B. Review and critique

E.H: 1B, 1C, 2B and 3A

L.S: 1B and 3B

K.O: 1B and 3B,

M.G: 1B and 3B

T.N: 1B and 3B

J.J: 1C and 3B

V.K: 1A, 1B, 1C, 2A, 2C and 3B

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Dr. Gardberg serves as an advisory board member of Abbvie, has received research funding from the Finnish Medical Society and the Perklén foundation, travel expenses from Abbvie and is a shareholder in companies Orion and Oriola.

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References

1. Tagliaferro P, Burke RE. Retrograde Axonal Degeneration in Parkinson Disease. *J Parkinsons Dis*. 2016;6(1):1-15.
2. Kordower JH, Olanow CW, Dodiya HB, et al. Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. *Brain*. 2013;136(Pt 8):2419-2431.
3. Fazio P, Svenningsson P, Cselényi Z, Halldin C, Farde L, Varrone A. Nigrostriatal dopamine transporter availability in early Parkinson's disease. *Mov Disord*. 2018;33(4):592-599.
4. Saari L, Kivinen K, Gardberg M, Joutsa J, Noponen T, Kaasinen V. Dopamine transporter imaging does not predict the number of nigral neurons in Parkinson disease. *Neurology*. 2017;88(15):1461-1467.
5. Jankovic J. Parkinson's disease tremors and serotonin. *Brain*. 2018;141(3):624-626.
6. Joutsa J, Gardberg M, Röyttä M, Kaasinen V. Diagnostic accuracy of parkinsonism syndromes by general neurologists. *Park Relat Disord*. 2014;20(8):840-844.
7. Dickson DW, Braak H, Duda JE, et al. Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. *Lancet Neurol*. 2009;8(12):1150-1157.
8. Kraemmer J, Kovacs GG, Perju-Dumbrava L, Pirker S, Traub-Weidinger T, Pirker W. Correlation of striatal dopamine transporter imaging with post mortem substantia nigra cell counts. *Mov Disord*. 2014;29(14):1767-1773.
9. Kaasinen V, Vahlberg T. Striatal dopamine in Parkinson disease: A meta-analysis of

imaging studies. *Ann Neurol*. 2017;82(6):873-882.

10. Tian L, Karimi M, Brown CA, Loftin SK, Perlmutter JS. In vivo measures of nigrostriatal neuronal response to unilateral MPTP treatment. *Brain Res*. 2014;1571:49-60.
11. Shimony JS, Rutlin J, Karimi M, et al. Validation of diffusion tensor imaging measures of nigrostriatal neurons in macaques. *PLoS One*. 2018;13(9):e0202201.
12. Blesa J, Pifl C, Sánchez-González MA, et al. The nigrostriatal system in the presymptomatic and symptomatic stages in the MPTP monkey model: A PET, histological and biochemical study. *Neurobiol Dis*. 2012;48(1):79-91.
13. Karimi M, Tian L, Brown CA, et al. Validation of nigrostriatal positron emission tomography measures: Critical limits. *Ann Neurol*. 2013;73(3):390-396.
14. Leviel V. Dopamine release mediated by the dopamine transporter, facts and consequences. *J Neurochem*. 2011;118(4):475-489.

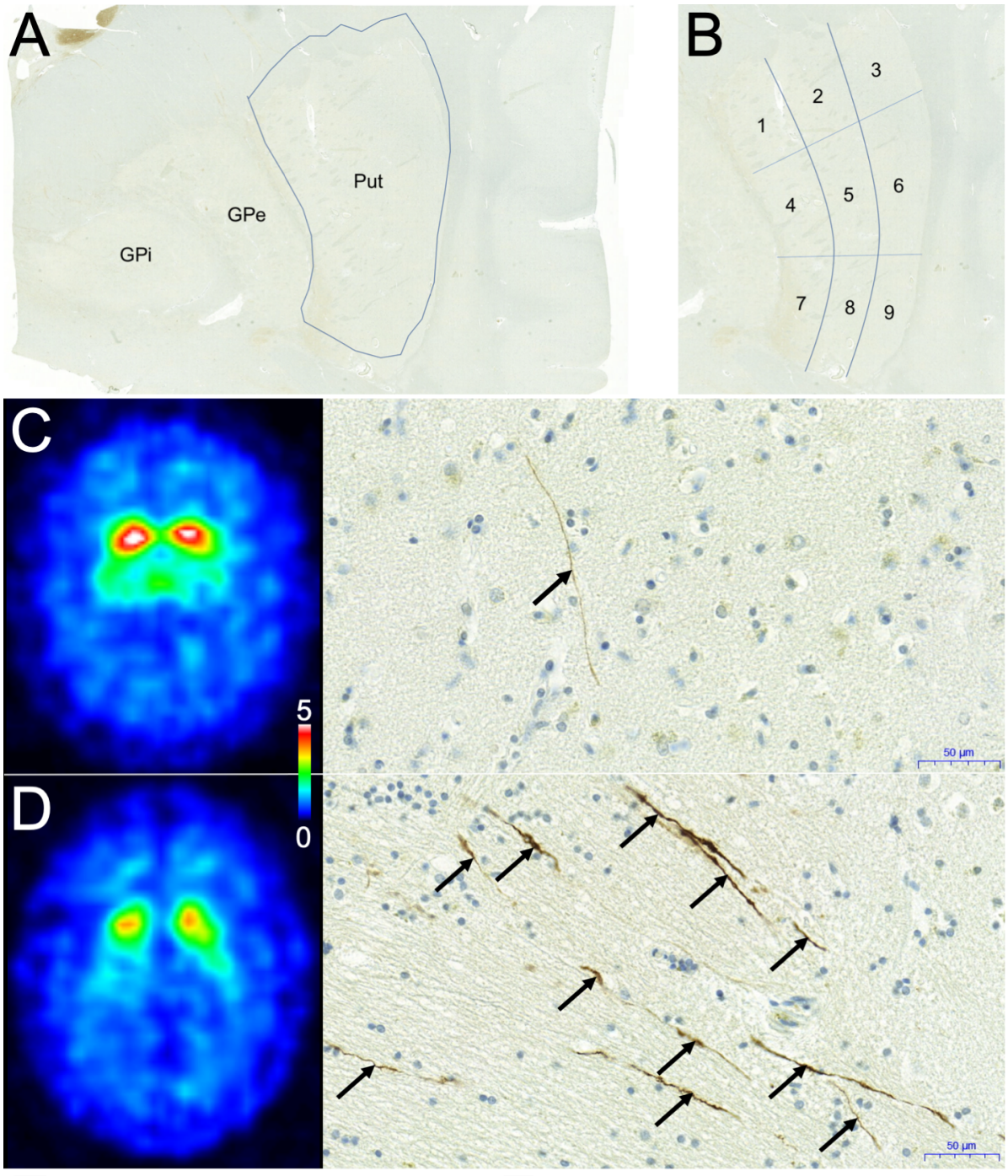
Figure legends

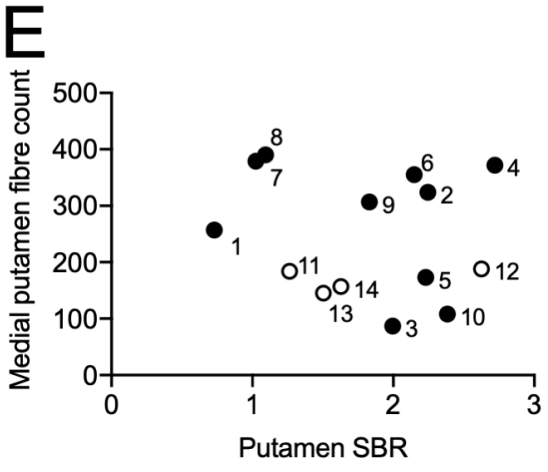
Figure 1.

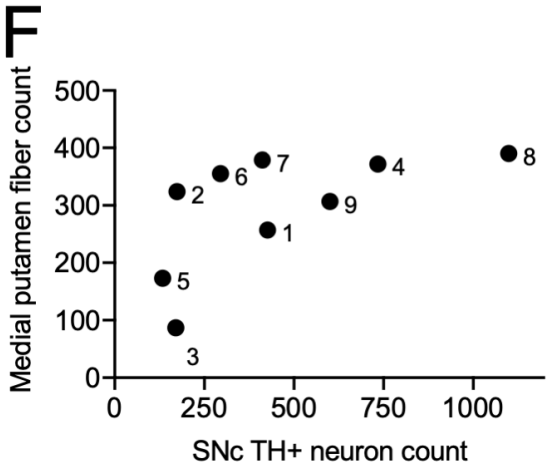
A. A representative coronal section of the postcommissural striatum immunohistochemically stained for TH and used for fiber counting. The outer margin of the putamen (Put) is delineated. Putamen counting was performed from coronal sections that included the globus pallidus interna (GPi) and externa (GPe). **B.** The putamen was divided into nine sections for fiber counting: 1. medial-cranial 2. central-cranial 3. lateral-cranial 4. medial-central 5. central-central 6. lateral-central 7. medial-caudal 8. central-caudal 9. lateral-caudal. **C.** Representative SPECT image of PD patient (case 3) with higher DAT binding and low fiber putamen sample (total TH+ fibre count 133). The color scale represents the striatal-to-occipital cortex binding ratio. Arrow denote the fiber that was counted. Note the higher DAT binding with lower fiber counts. For clinical details, see Table 1. **D.** Representative SPECT image of PD patient (case 8) with lower DAT binding and high fiber putamen sample (total TH+ fibre count 543). Note the lower DAT binding with higher fiber counts. **E.** Scatter plot demonstrating the nonsignificant correlation between the putamen SBR and the medial putamen fiber counts (r=-0.05, p=0.88). Corresponding case numbers to individualize the symbols are available in Table 1. Solid circles = PD, open circles = atypical parkinsonism. **F.** Correlation between the substantia nigra TH+ neuron counts and the medial putamen fiber counts in PD patients (r=0.68, p=0.04).

Table 1.

Main demographic and clinical characteristics of the studied sample. SBR = specific binding ratio, PD = Parkinson's disease, MSA = multiple system atrophy, CBD = corticobasal degeneration, PSP = progressive supranuclear palsy.


A
Put
GPe
GPi
B
1
2
3
4
5
6
7
8
9
C
5
0
D
50 µm
50 µm


E
Medial putamen fibre count
500
400
300
200
100
0
0
1
2
3
Putamen SBR
8
7
6
4
2
9
1
11
14
5
12
13
3
10


F
Medial putamen fiber count
500
400
300
200
100
0
0
250
500
750
1000
SNc TH+ neuron count
8
7
6
4
2
9
1
5
3

Case	Code	Sex	Diagnosis	Age at death (yrs)	Motor symptoms duration at SPECT (yrs)	Interval between scan and death (yrs)	Interval between death and autopsy (days)	Interval between death and neuropathological examination (days)	Putamen SBR	No. of putamen TH+ fibers	No. of SNc TH+ neurons
1	N21-15	m	PD	76	3.0	1.2	7	43	0.73	428	426
2	N22-04	m	PD	78	0.5	4.3	4	31	2.25	578	174
3	N49-11	m	PD	74	1.0	9.8	7	50	2.00	133	170
4	N84-15	m	PD	68	0.5	3.8	2	21	2.71	784	735
5	N30-09	f	PD	80	2.8	7.0	8	34	2.23	261	134
6	N41-08	m	PD	58	0.5	7.3	6	29	2.15	604	295
7	N60-11	m	PD	74	5.0	0.8	8	41	1.03	605	412
8	N64-12	m	PD	77	2.0	5.1	1	42	1.10	543	1099
9	N7-13	m	PD	75	0.5	5.2	3	51	1.83	542	601
10	N87-10	m	PD	81	1.0	12.8	6	39	2.39	190	-
11	N103-08	m	MSA	48	0.5	2.8	4	31	1.27	245	285
12	N114-08	f	CBD	72	1.0	2.2	5	27	2.63	331	631
13	N42-12	m	PSP	63	2.4	7.1	7	36	1.51	295	414
14	N53-05	m	PSP	63	1.0	3.2	4	18	1.63	295	-
Mean (SD)	-	-	-	71 (9.4)	1.5 (1.3)	5.2 (3.4)	5.1 (2.2)	35.2 (9.9)	1.82 (0.62)	417 (193)	448 (281)