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High long-term test–retest reliability for extrastriatal ^{11}C-raclopride binding in healthy older adults

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Abstract

In vivo dopamine D2-receptor availability is frequently assessed with ^{11}C-raclopride and positron emission tomography. Due to low signal-to-noise ratios for ^{11}C-raclopride in areas with low D2 receptor densities, the ligand has been considered unreliable for measurements outside the dopamine-dense striatum. Intriguingly, recent studies show that extrastriatal ^{11}C-raclopride binding potential (BP_{ND}) values are (i) reliably higher than in the cerebellum (where D2-receptor levels are negligible), (ii) correlate with behavior in the expected direction, and (iii) showed good test–retest reliability in a sample of younger adults. The present work demonstrates high seven-month test–retest reliability of striatal and extrastriatal ^{11}C-raclopride BP_{ND} values in healthy, older adults ($n = 27$, age: 64–78 years). Mean ^{11}C-raclopride BP_{ND} values were stable between test sessions in subcortical nuclei, and in frontal and temporal cortices ($p > 0.05$). Across all structures analyzed, intraclass correlation coefficients were high (0.85–0.96), absolute variability was low (mean: 4–8%), and coefficients of variance ranged between 9 and 25%. Furthermore, regional ^{11}C-raclopride BP_{ND} values correlated with previously determined ^{18}F-fallypride BP_{ND} values ($\rho = 0.97$ and 0.92 in correlations with and without striatal values, respectively, $p < 0.01$) and postmortem determined D2-receptor densities (including striatum: $\rho = 0.92$; $p < 0.001$; excluding striatum: $\rho = 0.75$; $p = 0.067$). These observations suggest that extrastriatal ^{11}C-raclopride measurements represent a true D2 signal.

Keywords

^{11}C-raclopride, binding potential, positron emission tomography, extrastriatal, reliability

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Introduction

The dopamine (DA) system is implicated in a broad range of functions, including motor control, cognition, and reward.[1–3] It is a target of investigation in several disciplines, such as neuroscience, neurology, and psychiatry. Ever since its discovery in the 1980s,[4–6] the DA D2 receptor (D2DR) antagonist ^{11}C-raclopride has been a widely used radioligand in positron emission tomography (PET) studies of the DA system, e.g.[7-17]. Its wide use is due to its pharmacological profile and high specificity for D2DRs,[18–21] its high reproducibility in measurements of D2DR availability at rest,[22] and its high reliability in measurement of changes in synaptic DA levels via ligand displacement upon tasks and interventions.[23–27]

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^{11}C-raclopride is a moderate affinity ligand with low signal-to-noise ratios in areas with low D2DR levels. Seminal work demonstrated that raclopride binding is several-folds higher in the striatum compared to cortical regions[28] and only a few percent higher in frontal and temporal cortices compared to its inactive enantiomer, FLB472.[29] Therefore, it has not been considered reliable for D2DR assessments outside the DA-dense striatal compartment.[5,29,30] However, cortical raclopride binding depicted a rostral-caudal gradient, which is consistent with DA levels in these brain areas and was displaceable by D2-antagonists.[31] More recent studies show that extrastriatal ^{11}C-raclopride binding potential (BP_{ND}), even though being low, is reliably above zero and within expected ranges[32,33] when comparing with D2 receptor levels quantified postmortem.[28,34] Additional support for the reliability of extrastriatal ^{11}C-raclopride BP_{ND} comes from recent findings showing that striatal, hippocampal, and cortical ^{11}C-raclopride BP_{ND} were interrelated, similarly linked to brain activation during a memory task, and predictive of episodic memory performance.[32,33,35] Furthermore, individuals with lower working memory performance had lower frontal cortical ^{11}C-raclopride BP_{ND} along with less beneficial brain activation patterns at rest and task.[36] In patient groups with DA disorders characterized by frontostriatal dysfunction (Parkinson's and Huntington's diseases), parallel reductions of striatal and cortical ^{11}C-raclopride binding have been reported.[37,38] Moreover, striatal as well as extrastriatal ^{11}C-raclopride displacement was observed in DA release paradigms in healthy individuals.[10,39–43] Taken together, these observations suggest that extrastriatal ^{11}C-raclopride may represent a meaningful D2DR signal, rather than noise.

Measurement reliability can be assessed by consecutive testing within a group of individuals. High test–retest reliability has been shown for striatal ^{11}C-raclopride measurements,[44–46] but also for extrastriatal ^{11}C-raclopride binding in cortical regions and thalamus.[47,48] Alakurtti and colleagues performed their investigations with a high-resolution brain-designated PET scanner in a small sample of younger adults. Consequently, it remains unclear whether their findings can be generalized to other settings, such as when using a scanner with lower resolution or when examining samples of older ages with varying degrees of age-induced DA decline.[49] For this reason, the present work examined ≥6-month test–retest reliability of extrastriatal ^{11}C-raclopride BP_{ND} in a sample of healthy, older adults (n = 27, ages: 64–78 years, 10 men) belonging to the Physical Influences on Brain in Aging (PHIBRA) study.[50] To elucidate the validity of our extrastriatal ^{11}C-raclopride signal, we compared regional ^{11}C-raclopride BP_{ND} values with values determined with the high-affinity ligand ^{18}F-fallypride,[51] and also, with D2DR density values (B_{max}) determined with ^{3}H-raclopride in postmortem autoradiography assessments.[28]

Material and methods

The study was approved by the Swedish Ethical Review Authority (Umeå, Sweden; registration number: 2013-238-31M) and was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from all participants prior to any testing was done.

Sample

The analyses were performed using the participants randomized to the control group of the PHIBRA study, a six-month aerobic exercise intervention (see Jonasson et al. (2017) for details).[50] Between test sessions, individuals included in the present work engaged in exercises of stretching and toning to improve muscle strength, flexibility, and balance. Participants underwent brain assessments, including magnetic resonance imaging (MRI) and PET with ^{11}C-raclopride, at two occasions separated by approximately seven months (mean: 7.3 months; min: 6 months; max: 8 months). Exclusion criteria included factors that affect brain and cognition, such as diabetes; medication known to influence the DA system; neurological, psychiatric, and motor diseases; head trauma; and Mini Mental State Examination scores below 27. Further exclusion criteria consisted of MR-incompatible factors, e.g. claustrophobia and metal implants. All structural MRI images were inspected by a radiologist to screen for structural abnormalities.

BP_{ND} values were excluded for two individuals (from of the original 29), as the percent change between test session was found extreme according to the outlier labeling rule with 2.2 interquartile ranges.[52] Thus, the effective sample consisted of 27 healthy older adults (age: 64–78 years, 10 men; Table 1).

Brain imaging

MRI. MRI was performed with a 3T Discovery MR 750 scanner (General Electric, WI, US), equipped with a 32-channel phased-array head coil. A 3D fast spoiled gradient-echo sequence was used to obtain high-resolution anatomical T1-weighted images. Imaging parameters were 176 slices with 1 mm

thickness, TR = 8.2 ms, TE = 3.2 ms, flip angle = 12°, and field of view = 25 × 25 cm.

The longitudinal image processing pipeline in Freesurfer, version 6[53] was used to process T1 images and achieve subcortical[54] and cortical gray-matter[55] segmentations of regions-of-interest (ROIs) for the two time points. Following cortical parcellation, subregions were merged to represent the following ROIs: orbitofrontal (lateral and medial orbitofrontal), anterior cingulate cortex (ACC; rostral and caudal anterior cingulate), superior frontal, middle frontal (rostral and caudal middle frontal), inferior frontal (pars opercularis, pars triangularis, and pars orbitalis), and temporal (superior, middle, and inferior temporal) cortices (Table 2 and Supplementary Table 1).

Table 1. Sample descriptives.

	Frequency or mean (SD)	min, max
Men	10	64, 78
Women	17	7, 25
Age	69.0 (2.9)	19.4, 37.3
Years of education	13.7 (4.7)	28, 30
Years of education	26.7 (3.5)	
MMSE	29.5 (0.6)	

BMI: body mass index; max: maximum; min: minimum; MMSE: Mini Mental State Examination; SD: standard deviation.

PET. PET was performed with a Discovery PET/CT 690 (General Electric, WI, US) and ^{11}C-raclopride. Head movements were minimized with individually fitted thermoplastic masks that were attached to the bed surface. A 55 min, 18-frame dynamic PET scan was acquired during resting-state conditions following intravenous bolus injection of approximately 250 MBq ^{11}C-raclopride (baseline: 276.00 ± 12.22 MBq; follow-up: 269.10 ± 10.91 MBq; $p > 0.05$). Mass of injected raclopride was lower at baseline (0.13 ± 0.06 μg) than at follow-up (0.48 ± 0.15 μg; $t(19) = -12.28$, $p < 0.001$), due to differences in specific activity of batches (baseline: 771.78 ± 341.70; follow-up: 190.96 ± 70.64 GBq/μmol; $t(19)=7.70$, $p < 0.001$). A CT scan (20 mA, 120 kV, 0.8 s/revolution) preceded ligand injection for attenuation-correction purposes.

Attenuation- and decay-corrected images (47 slices, field of view = 25 cm, 256 × 256-pixel transaxial images, voxel size = 0.977 × 0.977 × 3.27 mm^3) were reconstructed with the iterative point-spread function ordered subset maximization (PSF-OSEM) algorithm VUE Point HD-SharpIR (GE[56]; six iterations, 24 subsets, 3.0 mm post filtering), yielding full width at half maximum (FWHM) of 3.2 mm.[57]

The following procedures were performed to determine ^{11}C-raclopride BP_{ND} in striatal and extrastriatal regions. PET images were motion corrected and co-registered to the structural T1-weighted images from the corresponding session (baseline and follow-up) using the Statistical Parametric Mapping software (SPM8). Within T1-segmented subcortical and cortical structures BP_{ND} was calculated with orthogonal regression reference Logan analysis[58] and time–activity

Table 2. Test–retest statistics for ^{11}C-raclopride BP_{ND} measurements performed approximately seven months apart.

	Baseline Mean (SD)	CV (%)	Follow-up Mean (SD)	CV (%)	VAR (%) mean (SD)	min; max	ICC	*r*
Caudate	2.276 (0.306)	13.4	2.215 (0.283)	12.8	6.4 (4.6)	0.4; 16.7	0.91	0.82
Putamen	3.384 (0.336)	9.9	3.326 (0.290)	8.7	4.1 (3.4)	0.3; 12.9	0.91	0.82
Hippocampus	0.267 (0.052)	19.5	0.264 (0.048)	18.2	7.7 (5.3)	0.0; 27.4	0.95	0.91
Amygdala	0.393 (0.049)	12.5	0.393 (0.051)	13.0	6.7 (6.1)	0.0; 21.7	0.86	0.74
Pallidus	1.384 (0.149)	10.8	1.352 (0.142)	10.5	6.0 (4.7)	0.7; 18.4	0.85	0.75
Thalamus	0.486 (0.059)	12.1	0.487 (0.054)	11.1	4.7 (3.4)	0.0; 11.5	0.94	0.90
Orbitofrontal	0.249 (0.046)	18.5	0.255 (0.044)	17.3	7.0 (6.0)	0.0; 20.5	0.93	0.88
ACC	0.256 (0.047)	18.4	0.261 (0.038)	14.6	7.0 (4.7)	0.0; 16.0	0.93	0.83
Sup. frontal	0.193 (0.045)	23.3	0.192 (0.036)	18.8	8.2 (6.5)	0.0; 24.5	0.93	0.88
Mid. frontal	0.227 (0.042)	18.5	0.226 (0.037)	16.4	5.3 (3.9)	0.0; 13.4	0.96	0.93
Inf. frontal	0.208 (0.051)	24.5	0.207 (0.050)	24.2	7.9 (8.4)	0.0; 29.7	0.95	0.92
Temporal	0.277 (0.038)	13.7	0.283 (0.038)	13.4	4.9 (3.8)	0.0; 16.3	0.95	0.91

CV: coefficient of variance; ICC: intraclass correlation coefficient; max: maximum; min: minimum; *r*: Pearson's correlations coefficient (adjusted for gray-matter volume); SD: standard deviation; VAR: absolute variability. Note: $p > 0.05$ for comparisons of mean values between sessions.

curves with the median of ROI voxel values from each time frame. Logan regression was based on frame 10–18 (18–55 min). Cerebellar gray matter served as the reference area, due to its negligible D2DR expression.[5,34]

Statistical analyses

Median ^{11}C-raclopride BP_{ND} was extracted for each ROI and subject, averaged over hemispheres, and entered into analyses. Results for the sample are presented with frequencies, means, standard deviations (SDs), and minimum and maximum values. Differences in mean values for ^{11}C-raclopride BP_{ND}, gray-matter volumes, injected radioactivity dose (MBq), and specific radioactivity (GBq/μmol) were compared between sessions with paired sample-tests.

Reliability was assessed with the intraclass correlation coefficient (ICC), and also, partial correlations (covariate: regional gray-matter volumes) with the Pearson's correlation coefficient (r). Test–retest absolute variability (VAR, %) between sessions was calculated by entering BP_{ND} values from the two sessions in the equation below

$$VAR = \frac{2|test - retest|}{test + retest} \cdot 100$$

Furthermore, the coefficient of variance (CV), defined as the ratio of SD to the mean for the sample (in %), was calculated to compare dispersion of values around the mean for the regions analyzed.

For indications of validity, our regional ^{11}C-raclopride BP_{ND} values were compared with previously determined regional values of ^{3}H-raclopride B_{max}[28] and ^{18}F-fallypride BP_{ND} (available at https://osf.io/h67k4/). Whereas ^{18}F-fallypride values were found for all 12 brain regions reported in Table 2 of the present work, ^{3}H-raclopride B_{max} for the superior frontal cortex was an outlier (>3 SD from the other cortical values) and the inferior frontal cortex was not reported by Hall et al.,[28] and thus, these data points are missing (Figure 1(a) and (b)). Associations among these values are described with the Spearman's rank correlation coefficient (ρ). As DA constituents decline in aging,[49] ^{18}F-fallypride data were attained for a subsample aged >60 years (mean: 69.4, SD: 6.8, $n = 29$) for approximate age-matching with the PHIBRA data. Furthermore, ^{18}F-fallypride BP_{ND} values were averaged over subregions and hemispheres to represent the ROIs. Values for B_{max} based on ^{3}H-raclopride from Hall et al.[28] (Figure 1(b) in the original publication) were digitized with the PlotDigitizer software (http://plotdigitizer.sourceforge.net).

Results

High seven-month test–retest reliability for striatal and extrastriatal ^{11}C-raclopride binding

Test–retest statistics demonstrated high reliability for ^{11}C-raclopride BP_{ND} measurements in both striatal and extrastriatal regions (Table 2), with minor differences between hemispheres (Supplementary Table 1). No between-scan differences were observed for mean values in any of the regions ($p > 0.05$). CV indicated that the extent of variability in relation to the means was similar for cortical and subcortical regions, albeit lowest for the putamen (~9%) and slightly elevated for frontal cortical regions (~20%). Mean VAR was similar among regions, ranging between approximately 4 and 8%, and with comparable minimum and maximum values. ICCs were all >0.85, and thus in the good-to-excellent range.[59,60] Adjusting for gray-matter volumes, first-order correlations indicated high coherence for within-subject BP_{ND} values from the two sessions (rs: 0.74–0.93). Notably, similar values were achieved in zero-order correlations, i.e. without adjustment for volumes (rs for putamen: 0.85, caudate: 0.85, hippocampus: 0.91, amygdala: 0.74, globus pallidus: 0.75, thalamus: 0.89, orbitofrontal: 0.87, ACC: 0.89, superior: 0.89, middle: 0.93, inferior frontal: 0.91, and temporal: 0.91 cortices). Aside from the hippocampus,[50] volumes for all other structures remained similar between test sessions ($p > 0.10$).

Due to the significant differences between mass of injected raclopride at the baseline and follow-up sessions, we performed zero-order correlations among the percent change in injected raclopride and (1) percent change in ^{11}C-raclopride BP_{ND} values and (2) VAR in ^{11}C-raclopride BP_{ND} values. No significant associations were observed for any of the ROIs in Table 2.

Relation between ^{11}C-raclopride BP_{ND} and previously determined D2-receptor levels

^{11}C-raclopride BP_{ND} values from our study were compared with previously determined ^{3}H-raclopride B_{max}[28] and ^{18}F-fallypride BP_{ND} (https://osf.io/h67k4/) in the corresponding regions. High correlations were found between our ^{11}C-raclopride BP_{ND} measures and ^{3}H-raclopride B_{max} over regions when including striatal and pallidal ROIs ($\rho = 0.92$, $p < 0.001$; Figure 1(a)), but also, among low D2DR density-regions ($\rho = 0.75$, $p = 0.067$; Figure 1(b)). In addition, high correlations were found between our ^{11}C-raclopride BP_{ND} measures and ^{18}F-fallypride BP_{ND} values over regions, with small differences in the strength of the correlation when excluding high-density regions ($\rho = 0.97$; $p < 0.001$ and $\rho = 0.92$; $p < 0.01$ with and without striatal and pallidal ROIs, respectively; Figure 1(c) and (d)).

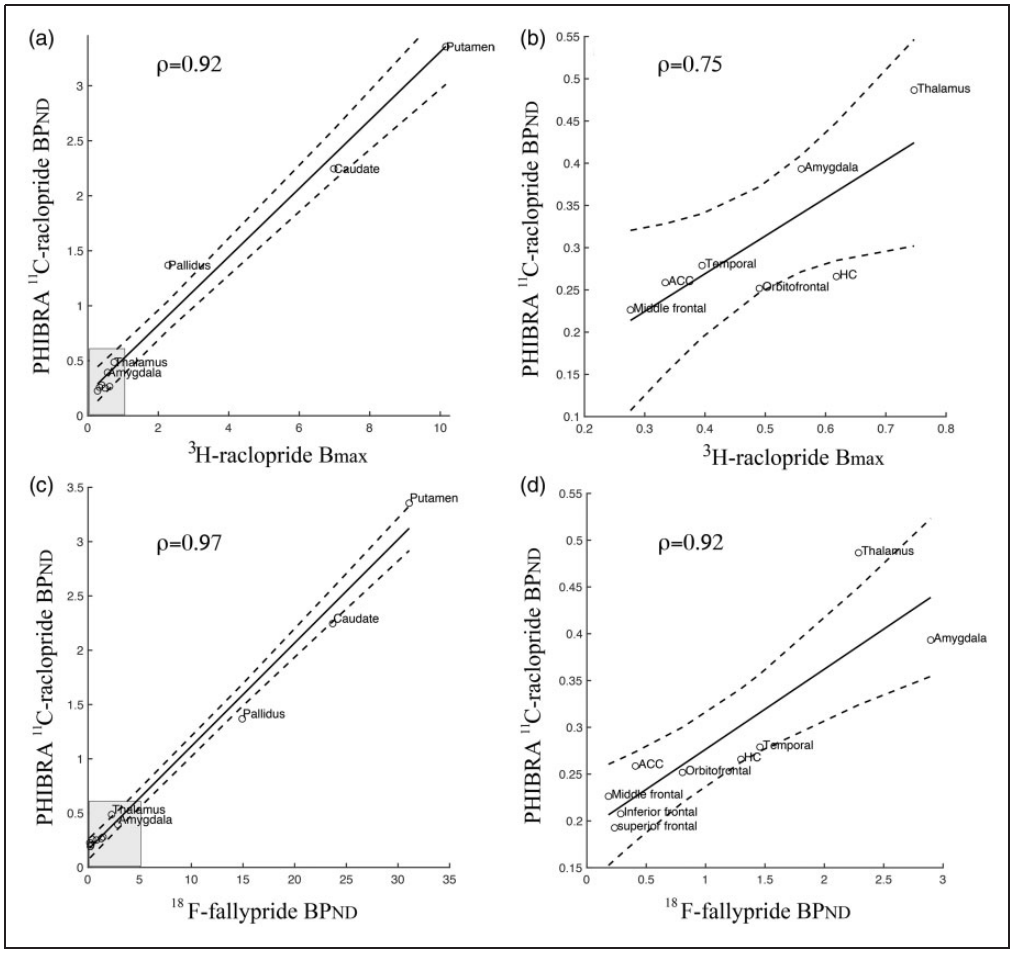

Figure 1. Regional coherence between our ^{11}C-raclopride BP_{ND} measures and previously determined ^{3}H-raclopride B_{max}[28] and ^{18}F-fallypride BP_{ND}[51]. Scatter plots illustrate associations when including striatum and globus pallidus (a), and low uptake-regions (b), which are visualized in the gray box in (a). Similarly, high correlations were found between our ^{11}C-raclopride BP_{ND} measures and ^{18}F-fallypride BP_{ND} over regions when including striatum and globus pallidus (d), and only the low uptake-regions (d).

Discussion

The use of high-affinity ligands such as ^{11}C-FLB457[61] and fallypride[62] has been recommended for extrastriatal D2DR assessments. Notably, high-affinity ligands come with their own limitations, including unreliable striatal BP_{ND} estimations with ^{11}C-FLB457[63] and unreliable measurements of DA release with ^{18}F-fallypride displacement.[64] Hence, it is well-motivated to further investigate the feasibility of extrastriatal ^{11}C-raclopride assessments. Here, we demonstrate high test–retest reliability for striatal as well as extrastriatal measurements performed seven months apart in older adults. Striatal and extrastriatal values were comparable in terms of degree of reliability, absolute variance, and dispersion of BP_{ND} values around the mean, with small differences between hemispheres. Importantly, ^{11}C-raclopride BP_{ND} values throughout frontal, temporal, and subcortical regions were ranked according to previously determined ^{18}F-fallypride BP_{ND},[51] as well as ^{3}H-raclopride B_{max} from postmortem autoradiography.[28] As such, these data add evidence for feasibility and validity of extrastriatal ^{11}C-raclopride assessment.

Test–retest reliability measures the consistency over measurement occasions. Test scores can vary due to variability in true scores and measurement errors. With the estimated striatal D2DR decline being around 5–10% per decade,[3,49] D2DR levels were not expected to change considerably over seven months in this group of healthy, older adults. Any observation of variability would then likely arise from measurement error. In line with previous reports, we noted high test–retest reliability for striatum,[44–46,65] but also for extrastriatal regions.[47,48,66] This stability may relate to factors such as high camera resolution and sensitivity, minimal movement artifacts (achieved via masks and movement correction of images), and small differences in blood flow between sessions.[67] A recent finding

shows, however, that effects from changes in blood flow are negligible.[68] When it comes to data modeling, BP_{ND} values estimated with Logan analysis have been shown to be reliable and comparable to those estimated with simplified reference tissue modeling.[69,70] The underlying reasons for the large variability in specific activity between batches at the baseline and follow-up sessions remain unknown but could be a consequence of a service of the system that took place between the two test sessions. At other PET sites, fluctuations in specific activity over time have been related to, e.g. changes in the flow of the carrier gas from the target.[71] That said, the levels of injected mass of raclopride were at tracer doses, not exceeding those of previous reports, e.g. [5,48,72-73], and thus, the number of available receptors should have remained largely unaffected throughout the experiments. This is supported by the stable mean values for ^{11}C-raclopride BP_{ND} values between test sessions.

To ensure minimal variability of volumes between sessions, the longitudinal pipeline of the Freesurfer software was used. It is well known that the volume of ROIs may cause under- and overestimations of BP values.[74] Partial volume effects, caused by limited spatial resolution of the scanner and reconstruction algorithms, give rise to varying recovery of the signal in different regions, where structures having high surface-to-volume ratios are most vulnerable.[75,76] FWHM of the PSF-OSEM algorithm has been estimated to be 3.2 mm on the scanner used in the present study.[57] Furthermore, higher signal recovery was found for the reconstruction algorithm used here, PSF-OSEM, when compared to filtered back-projection, and did not induce a bias in the estimation of BP depending on the level of radioactivity.[76] Despite the varying morphology of the structures analyzed, reliability was constant over regions and correlations between ^{11}C-raclopride BP_{ND} values from the consecutive trials did not change when controlling for gray-matter volume.

When scrutinizing the statistics presented here, ^{11}C-raclopride BP_{ND} values for subcortical, temporal, and frontal cortical regions were coherent between test sessions at the group level. ICC values ranged from 0.85 to 0.96 and were thus in the range of excellent[59,60] and even higher than in the reports by Alakurtti et al.[47,48] The high ICC values indicate that variance arise from inter- rather than intra-individual variation. Furthermore, the VAR of BP_{ND} values as well as the dispersion of values around the mean was similar among regions with high and low D2DR expression. The coefficient of variation was, however, slightly elevated for frontal cortical ROIs (~20%) when comparing with striatal, thalamic, and temporal regions (~10%), which is on a par with previous reports.[47] In further comparisons among studies, the values for VAR for subcortical and cortical regions were similar to previous reports[47,65,66] and comparable to test–retest experiments with high-affinity D2DR ligands.[77,78] Consequently, it seems that ^{11}C-raclopride measurements are robust and consistent in regions with varying numbers of D2DRs. Since the present work replicates previous findings in young adults[47,48,66] reliability is not compromised by the well-known age-related DA decline.[49]

It is critical to point out that high reliability does not imply validity of measurements, which in this context would translate to D2DR levels. With its moderate affinity to D2DRs, relatively low signal-to-noise ratio, and only slightly elevated binding when compared with its inactive enantiomer in areas with low D2DR levels,[29] ^{11}C-raclopride has been considered excellent for striatal and unfit for extrastriatal D2DR measurements. Early work showed minor accumulation of ^{11}C-raclopride in cortical areas[30,79] that was only a few percent higher than binding of its inactive enantiomer.[29] Low binding would, then again, be reasonable given that cortical D2DR levels constitute only a few percent of the striatal levels.[28,34,80] Our ^{11}C-raclopride BP_{ND} measures in striatal as well as extrastriatal regions were ranked according to previously determined ^{18}F-fallypride BP_{ND} and ^{3}H-raclopride B_{max} measures, thereby indicating that extrastriatal ^{11}C-raclopride BP_{ND} reflects the presence of D2DRs. Noteworthy, the BP_{ND} values were higher in several subcortical nuclei, but not cortical regions, when determined with fallypride as compared to raclopride. Consequently, the striatal-to-extrastriatal BP_{ND} ratios differed several-folds between the two D2DR ligands. This may relate to ligand characteristics; however, it remains for future research to understand the source of such observations. Using data from a multiligand study,[81] Egerton et al.[67] did not observe similar coherence between extrastriatal ^{11}C-raclopride and ^{11}C-FLB457 binding. As pointed out by the authors, the lack of association may relate to the small sample size ($n = 10$). Small sample sizes are a limitation of most PET studies, and possibly, even more troublesome for the use of ^{11}C-raclopride in extrastriatal regions with low D2DR levels.

Other indications of validity come from studies showing ^{11}C-raclopride displacement during tasks in theoretically expected regions, including in prefrontal regions during working memory,[10] in ACC and substantia nigra during a planning task,[67] and in motor regions upon motor actions.[42] Furthermore, *in-scanner* interventions have resulted in extrastriatal ^{11}C-raclopride displacement during reward-settings,[39,82,83] but also upon pharmacological DA interventions.[40,41,43,66,84] Findings from a well-powered ^{11}C-raclopride study have shown that both striatal and

extrastriatal D2DR correlate and predict cognitive performance,[32,33,36] with observed allelic group differences when stratifying the sample according to a D2DR polymorphism.[85] Finally, 11C-raclopride binding was found to be organized according to anatomical and functional DA pathways, which further supports the validity of extrastriatal ^{11}C-raclopride measurements.[86]

Concluding remarks

The findings presented here and elsewhere open up the possibility of a reliable extrastriatal ^{11}C-raclopride signal that is displaceable by interventions.[67] For definite conclusions of the nature of the extrastriatal signal, further observations of extrastriatal ^{11}C-raclopride displacement upon D2DR manipulation are needed in well-powered settings.

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Authors' contribution

NK and LJ performed research. C-JB, KR, LJ, and LN designed and funded the research; NK, LJ, JJ, GP, AS, and MA analyzed data. NK wrote the manuscript, which was edited by all authors.

Supplemental material

Supplemental material for this paper can be found at the journal website: http://journals.sagepub.com/home/jcb

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References

1. Salamone JD. Complex motor and sensorimotor functions of striatal and accumbens dopamine: involvement in instrumental behavior processes. *Psychopharmacology* 1992; 107: 160–174.
2. Arias-Carrión O, Stamelou M, Murillo-Rodríguez E, et al. Dopaminergic reward system: a short integrative review. *Int Arch Med* 2010; 3: 24–24.
3. Bäckman L, Nyberg L, Lindenberger U, et al. The correlative triad among aging, dopamine, and cognition: current status and future prospects. *Neurosci Biobehav Rev* 2006; 30: 791–807.
4. Farde L, Ehrin E, Eriksson L, et al. Substituted benzamides as ligands for visualization of dopamine receptor binding in the human brain by positron emission tomography. *Proc Natl Acad Sci USA* 1985; 82: 3863–3867.
5. Farde L, Hall H, Ehrin E, et al. Quantitative analysis of D2 dopamine receptor binding in the living human brain by PET. *Science* 1986; 231: 258–261.
6. Ehrin E, Farde L, de Paulis T, et al. Preparation of 11C-labelled raclopride, a new potent dopamine receptor antagonist: preliminary PET studies of cerebral dopamine receptors in the monkey. *Int J Appl Radiat Isot* 1985; 36: 269–273.
7. Volkow ND, Gur RC, Wang GJ, et al. Association between decline in brain dopamine activity with age and cognitive and motor impairment in healthy individuals. *Am J Psychiatry* 1998; 155: 344–349.
8. Lou HC, Rosa P, Pryds O, et al. ADHD: increased dopamine receptor availability linked to attention deficit and low neonatal cerebral blood flow. *Dev Med Child Neurol* 2004; 46: 179–183.
9. Rajji TK, Mulsant BH, Nakajima S, et al. Cognition and dopamine D2 receptor availability in the striatum in older patients with schizophrenia. *Am J Geriatr Psychiatry* 2017; 25: 1–10.
10. Sawamoto N, Piccini P, Hotton G, et al. Cognitive deficits and striato-frontal dopamine release in Parkinson's disease. *Brain* 2008; 131: 1294–1302.
11. Cools R. Dopaminergic modulation of cognitive function-implications for L-DOPA treatment in Parkinson's disease. *Neurosci Biobehav Rev* 2006; 30: 1–23.
12. Howes OD and Kapur S. The dopamine hypothesis of schizophrenia: version III – the final common pathway. *Schizophr Bull* 2009; 35: 549–562.
13. Solanto MV. Dopamine dysfunction in AD/HD: integrating clinical and basic neuroscience research. *Behav Brain Res* 2002; 130: 65–71.
14. Dunlop BW and Nemeroff CB. The role of dopamine in the pathophysiology of depression. *Arch Gen Psychiatry* 2007; 64: 327–337.
15. Cools R and D'Esposito M. Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biol Psychiatry* 2011; 69: e113–e125.
16. Goldman-Rakic PS. The cortical dopamine system: role in memory and cognition. *Adv Pharmacol* 1998; 42: 707–711.
17. Arnsten AF. Catecholamine regulation of the prefrontal cortex. *J Psychopharmacol* 1997; 11: 151–162.
18. Hall H, Kohler C, Gawell L, et al. Raclopride, a new selective ligand for the dopamine-D2 receptors. *Prog Neuropsychopharmacol Biol Psychiatry* 1988; 12: 559–568.

19. Seeman P and Ulpian C. Dopamine D1 and D2 receptor selectivities of agonists and antagonists. *Adv Exp Med Biol* 1988; 235: 55–63.
20. Ögren SO, Hall H, Köhler C, et al. The selective dopamine D2 receptor antagonist raclopride discriminates between dopamine-mediated motor functions. *Psychopharmacology* 1986; 90: 287–294.
21. Farde L, Wiesel FA, Jansson P, et al. An open label trial of raclopride in acute schizophrenia. Confirmation of D2-dopamine receptor occupancy by PET. *Psychopharmacology* 1988; 94: 1–7.
22. Laruelle M. Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. *J Cereb Blood Flow Metab* 2000; 20: 423–451.
23. Koepp MJ, Gunn RN, Lawrence AD, et al. Evidence for striatal dopamine release during a video game. *Nature* 1998; 393: 266–268.
24. Wang GJ, Volkow ND, Fowler JS, et al. Reproducibility of repeated measures of endogenous dopamine competition with [11C]raclopride in the human brain in response to methylphenidate. *J Nucl Med* 1999; 40: 1285–1291.
25. Bäckman L, Nyberg L, Soveri A, et al. Effects of working-memory training on striatal dopamine release. *Science* 2011; 333: 718.
26. Jonasson LS, Axelsson J, Riklund K, et al. Dopamine release in nucleus accumbens during rewarded task switching measured by [(1)(1)C]raclopride. *Neuroimage* 2014; 99: 357–364.
27. Martinez D, Kim JH, Krystal J, et al. Imaging the neurochemistry of alcohol and substance abuse. *Neuroimaging Clin N Am* 2007; 17: 539–555.
28. Hall H, Sedvall G, Magnusson O, et al. Distribution of D1- and D2-dopamine receptors, and dopamine and its metabolites in the human brain. *Neuropsychopharmacology* 1994; 11: 245–256.
29. Farde L, Pauli S, Hall H, et al. Stereoselective binding of 11C-raclopride in living human brain – a search for extrastriatal central D2-dopamine receptors by PET. *Psychopharmacology* 1988; 94: 471–478.
30. Farde L, Halldin C, Stone-Elander S, et al. PET analysis of human dopamine receptor subtypes using 11C-SCH 23390 and 11C-raclopride. *Psychopharmacology* 1987; 92: 278–284.
31. Lidow MS, Goldman-Rakic PS, Rakic P, et al. Dopamine D2 receptors in the cerebral cortex: distribution and pharmacological characterization with [3H]raclopride. *Proc Natl Acad Sci USA* 1989; 86: 6412–6416.
32. Nyberg L, Karalija N, Salami A, et al. Dopamine D2 receptor availability is linked to hippocampal-caudate functional connectivity and episodic memory. *Proc Natl Acad Sci USA* 2016; 113: 7918–7923.
33. Lövden M, Karalija N, Andersson M, et al. Latent-profile analysis reveals behavioral and brain correlates of dopamine-cognition associations. *Cereb Cortex* 2018; 28: 3894–3907.
34. Camps M, Cortés R, Gueye B, et al. Dopamine receptors in human brain: autoradiographic distribution of D2 sites. *Neuroscience* 1989; 28: 275–290.
35. Salami A, Garrett DD, Wahlin A, et al. Dopamine D2/3 binding potential modulates neural signatures of working memory in a load-dependent fashion. *J Neurosci* 2019; 39: 537–547.
36. Salami A, Rieckmann A, Karalija N, et al. Neurocognitive profiles of older adults with working-memory dysfunction. *Cereb Cortex* 2018; 28: 2525–2539.
37. Ribeiro MJ, Thobois S, Lohmann E, et al. A multitracer dopaminergic PET study of young-onset parkinsonian patients with and without parkin gene mutations. *J Nucl Med* 2009; 50: 1244–1250.
38. Politis M, Pavese N, Tai YF, et al. Microglial activation in regions related to cognitive function predicts disease onset in Huntington's disease: a multimodal imaging study. *Hum Brain Mapp* 2011; 32: 258–270.
39. Kaasinen V, Aalto S, Nagren K, et al. Expectation of caffeine induces dopaminergic responses in humans. *Eur J Neurosci* 2004; 19: 2352–2356.
40. Piccini P, Pavese N and Brooks DJ. Endogenous dopamine release after pharmacological challenges in Parkinson's disease. *Ann Neurol* 2003; 53: 647–653.
41. Stokes PR, Egerton A, Watson B, et al. Significant decreases in frontal and temporal [11C]-raclopride binding after THC challenge. *Neuroimage* 2010; 52: 1521–1527.
42. Garraux G, Peigneux P, Carson RE, et al. Task-related interaction between basal ganglia and cortical dopamine release. *J Neurosci* 2007; 27: 14434.
43. Berry AS, White RL 3rd, Furman DJ, et al. Dopaminergic mechanisms underlying normal variation in trait anxiety. *J Neurosci* 2019; 39: 2735–2744.
44. Nordström A-L, Farde L, Pauli S, et al. PET analysis of central [11 C]raclopride binding in healthy young adults and schizophrenic patients – reliability and age effects. *Hum Psychopharm Clin Exp* 1992; 7: 157–165.
45. Nyberg S, Farde L and Halldin C. Test-retest reliability of central [11C]raclopride binding at high D2 receptor occupancy. A PET study in haloperidol-treated patients. *Psychiatry Res* 1996; 67: 163–171.
46. Yoder KK, Albrecht DS, Kareken DA, et al. Test-retest variability of [(1)(1)C]raclopride-binding potential in nontreatment-seeking alcoholics. *Synapse* 2011; 65: 553–561.
47. Alakurtti K, Johansson JJ, Joutsa J, et al. Long-term test-retest reliability of striatal and extrastriatal dopamine D2/3 receptor binding: study with [(11)C]raclopride and high-resolution PET. *J Cereb Blood Flow Metab* 2015; 35: 1199–1205.
48. Alakurtti K, Aalto S, Johansson JJ, et al. Reproducibility of striatal and thalamic dopamine D2 receptor binding using [11C]raclopride with high-resolution positron emission tomography. *J Cereb Blood Flow Metab* 2011; 31: 155–165.
49. Karrer TM, Josef AK, Mata R, et al. Reduced dopamine receptors and transporters but not synthesis capacity in normal aging adults: a meta-analysis. *Neurobiol Aging* 2017; 57: 36–46.
50. Jonasson LS, Nyberg L, Kramer AF, et al. Aerobic exercise intervention, cognitive performance, and brain structure: results from the Physical Influences on

Brain in Aging (PHIBRA) Study. *Front Aging Neurosci* 2017; 8: 336.
51. Seaman KL, Smith CT, Juarez EJ, et al. Differential regional decline in dopamine receptor availability across adulthood: linear and nonlinear effects of age. *Hum Brain Mapp* 2019; 40: 3125–3138.
52. Hoaglin DC and Iglewicz B. Fine-tuning some resistant rules for outlier labeling. *J Am Stat Assoc* 1987; 82: 1147–1149.
53. Reuter M, Schmansky NJ, Rosas HD, et al. Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage* 2012; 61: 1402–1418.
54. Fischl B, Salat DH, Busa E, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002; 33: 341–355.
55. Desikan RS, Segonne F, Fischl B, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 2006; 31: 968–980.
56. Bettinardi V, Presotto L, Rapisarda E, et al. Physical performance of the new hybrid PETCT Discovery-690. *Med Phys* 2011; 38: 5394–5411.
57. Wallsten E, Axelsson J, Sundstrom T, et al. Subcentimeter tumor lesion delineation for high-resolution 18F-FDG PET images: optimizing correction for partial-volume effects. *J Nucl Med Technol* 2013; 41: 85–91.
58. Logan J, Fowler JS, Volkow ND, et al. Distribution volume ratios without blood sampling from graphical analysis of PET data. *J Cereb Blood Flow Metab* 1996; 16: 834–840.
59. Cicchetti DV. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. *Psychol Assessment* 1994; 6: 284–290.
60. Koo TK and Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med* 2016; 15: 155–163.
61. Halldin C, Farde L, Hogberg T, et al. Carbon-11-FLB 457: a radioligand for extrastriatal D2 dopamine receptors. *J Nucl Med* 1995; 36: 1275–1281.
62. Mukherjee J, Shi B, Christian BT, et al. 11C-Fallypride: radiosynthesis and preliminary evaluation of a novel dopamine D2/D3 receptor PET radiotracer in non-human primate brain. *Bioorg Med Chem* 2004; 12: 95–102.
63. Olsson H, Halldin C, Swahn C-G, et al. Quantification of [11C]FLB 457 binding to extrastriatal dopamine receptors in the human brain. *J Cereb Blood Flow Metab* 1999; 19: 1164–1173.
64. Slifstein M, Kegeles LS, Xu X, et al. Striatal and extrastriatal dopamine release measured with PET and [(18)F] fallypride. *Synapse* 2010; 64: 350–362.
65. Hietala J, Nagren K, Lehikoinen P, et al. Measurement of striatal D2 dopamine receptor density and affinity with [11C]-raclopride in vivo: a test-retest analysis. *J Cereb Blood Flow Metab* 1999; 19: 210–217.
66. Hirvonen J, Aalto S, Lumme V, et al. Measurement of striatal and thalamic dopamine D2 receptor binding with 11C-raclopride. *Nucl Med Commun* 2003; 24: 1207–1214.
67. Egerton A, Mehta MA, Montgomery AJ, et al. The dopaminergic basis of human behaviors: a review of molecular imaging studies. *Neurosci Biobehav Rev* 2009; 33: 1109–1132.
68. Sander CY, Mandeville JB, Wey HY, et al. Effects of flow changes on radiotracer binding: simultaneous measurement of neuroreceptor binding and cerebral blood flow modulation. *J Cereb Blood Flow Metab* 2019; 39: 131–146.
69. Fischer K, Sossi V, Schmid A, et al. Noninvasive nuclear imaging enables the in vivo quantification of striatal dopamine receptor expression and raclopride affinity in mice. *J Nucl Med* 2011; 52: 1133–1141.
70. Logan J, Fowler JS, Volkow ND, et al. Graphical analysis of reversible radioligand binding from time-activity measurements applied to [N-11C-methyl]-(-)-cocaine PET studies in human subjects. *J Cereb Blood Flow Metab* 1990; 10: 740–747.
71. Andersson J, Truong P and Halldin C. In-target produced [11C]methane: increased specific radioactivity. *Appl Radiat Isot* 2009; 67: 106–110.
72. Kegeles LS, Abi-Dargham A, Frankle WG, et al. Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. *Arch Gen Psychiatry* 2010; 67: 231–239.
73. Lammertsma AA, Bench CJ, Hume SP, et al. Comparison of methods for analysis of clinical [11C]raclopride studies. *J Cereb Blood Flow Metab* 1996; 16: 42–52.
74. Smith CT, Crawford JL, Dang LC, et al. Partial-volume correction increases estimated dopamine D2-like receptor binding potential and reduces adult age differences. *J Cereb Blood Flow Metab* 2019; 39: 822–833.
75. Dewaraja YK, Ljungberg M and Koral KF. Monte Carlo evaluation of object shape effects in iodine-131 SPET tumor activity quantification. *Eur J Nucl Med* 2001; 28: 900–906.
76. Jonasson LS, Axelsson J, Riklund K, et al. Simulating effects of brain atrophy in longitudinal PET imaging with an anthropomorphic brain phantom. *Phys Med Biol* 2017; 62: 5213–5227.
77. Mukherjee J, Christian BT, Dunigan KA, et al. Brain imaging of 18F-fallypride in normal volunteers: blood analysis, distribution, test-retest studies, and preliminary assessment of sensitivity to aging effects on dopamine D-2/D-3 receptors. *Synapse* 2002; 46: 170–188.
78. Vilkman H, Kajander J, Nagren K, et al. Measurement of extrastriatal D2-like receptor binding with [11C]FLB 457 – a test-retest analysis. *Eur J Nucl Med* 2000; 27: 1666–1673.
79. Hall H, Farde L and Sedvall G. Human dopamine receptor subtypes – in vitro binding analysis using 3H-SCH 23390 and 3H-raclopride. *J Neural Transm* 1988; 73: 7–21.
80. Suhara T, Sudo Y, Okauchi T, et al. Extrastriatal dopamine D2 receptor density and affinity in the human brain measured by 3D PET. *Int J Neuropsychopharmacol* 1999; 2: 73–82.

81. Ito H, Takahashi H, Arakawa R, et al. Normal database of dopaminergic neurotransmission system in human brain measured by positron emission tomography. *Neuroimage* 2008; 39: 555–565.
82. Takahashi K, Mizuno K, Sasaki AT, et al. Imaging the passionate stage of romantic love by dopamine dynamics. *Front Hum Neurosci* 2015; 9: 191.
83. Lippert RN, Cremer AL, Edwin Thanarajah S, et al. Time-dependent assessment of stimulus-evoked regional dopamine release. *Nat Commun* 2019; 10: 336.
84. Mehta MA, Montgomery AJ, Kitamura Y, et al. Dopamine D2 receptor occupancy levels of acute sulpiride challenges that produce working memory and learning impairments in healthy volunteers. *Psychopharmacology* 2008; 196: 157–165.
85. Karalija N, Papenberg G, Wahlin A, et al. C957T-mediated variation in ligand affinity affects the association between (11)C-raclopride binding potential and cognition. *J Cogn Neurosci* 2019; 31: 314–325.
86. Papenberg G, Jonasson L, Karalija N, et al. Mapping the landscape of human dopamine D2/3 receptors with [(11)C]raclopride. *Brain structure & function*. Epub ahead of print 23 August 2019. DOI: 10.1007/s00429-019-01938-1.