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ORIGINAL ARTICLE

Establishing test–retest reliability of an adapted [18F]fallypride imaging protocol in older people

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[18F]fallypride is a high-affinity dopamine D2/3 receptor tracer with the ability to reliably quantify D2/3 receptor sites in both striatal and corticolimbic regions. The translational potential of [18F]fallypride imaging is, however, limited by the lengthy scanning sessions (60–80 minutes duration over a total of 3–4 hours) required by current protocols. The aims of our study were to adapt [18F]fallypride imaging for use in clinical populations with neurological and neuropsychiatric disorders, by reducing the duration of individual scanning sessions; and to establish the reproducibility and reliability of our adapted protocol in healthy older people. Eight participants (five male and three female; mean age = 75.87 ± 4.39 years) were scanned twice, 4–6 weeks apart. [18F]fallypride binding potential was determined from image data collected during three sampling times: 0–30; 60–90; and 210–240 minutes post injection. High reproducibility and reliability (test–retest variability < 8%; intraclass correlation coefficient > 0.8) were observed in all but the prefrontal regions, and remained so when sampling times were reduced to 20 minutes (0–20; 70–90; 220–240 minutes). The adapted protocol is feasible for use across neuropsychiatric disorders in which dopamine has been implicated and is sufficiently sensitive to detect within-subject changes between 2.7% and 5.5% in striatal and limbic regions.

INTRODUCTION

Since dopamine D2/3 receptors were first visualized in vivo in humans,[1] positron emission tomography (PET) tracers that target dopamine D2/3 receptors have provided crucial insights into the pathophysiology and treatment of psychiatric and neurological disorders.[2,3] Such tracers have been instrumental in guiding treatment strategies in schizophrenia, by establishing a 'therapeutic window' of striatal D2/3 receptor occupancy by antipsychotic drugs.[4,5] More recently, the development of high-affinity D2/3 receptor tracers such as [18F]fallypride[6] and [11C]FLB457[7] has shifted the focus of D2/3 receptor imaging toward extrastriatal regions where receptor density is 10–100 times lower than the striatum.[8] This has allowed the clinical relevance of corticolimbic D2/3 receptor occupancy to be explored, has raised important questions regarding the contribution of temporal cortical occupancy to therapeutic response,[9,10] and remains a focus of investigation across a range of atypical antipsychotic drugs.

Another major development in imaging technology has been the use D2/3 PET tracers to image endogenous neurotransmitter release. The observation that D2/3 tracers compete with endogenous dopamine for receptor sites has been used in imaging paradigms to investigate the sensitivity of the dynamic system after pharmacological or behavioral challenge.[11] High-affinity D2/3 receptor tracers are increasingly used in this respect as they offer the opportunity to explore the role of corticolimbic dopamine release in human behavior[12,13] and in a range of neurological and psychiatric illnesses including schizophrenia-spectrum disorders[14] and Parkinson's Disease.[15]

[18F]fallypride is unique among D2/3 receptor tracers, as it can provide stable estimates of both striatal and extrastriatal receptor availability within the same scanning session.[6] However, techniques currently used to quantify [18F]fallypride binding involve multiple sampling periods (each lasting 60–80 minutes) over a total scan duration of 3-4 hours, to allow tracer uptake to achieve a plateau within the striatum where receptor sites are more densely concentrated.[6,16] These imaging protocols are not feasible for use in many clinical populations, particularly older, cognitively impaired individuals or those with movement disorders. Adapting [18F]fallypride imaging for use in clinical populations who are unable to tolerate lengthy scanning sessions would widen its potential for use in understanding disease mechanisms, drug occupancy, and dopamine release in response to pharmacological and behavioral challenge.

The aims of the study were as follows:

(i) To adapt [18F]fallypride imaging by reducing the length of individual scanning sessions to 30 minutes.
(ii) To establish the test–retest reliability of the adapted protocol in healthy older people.
(iii) To investigate whether sampling times could be further reduced to 20-minute sessions without reducing reliability.

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MATERIALS AND METHODS

Sample

Eight healthy older adults (five male and three female; mean age $= 75.87 \pm 4.39$ years) were recruited to the study. Participants were 'healthy controls' identified from the Dementia Case Register database within the South London and Maudsley (SLaM) NHS Trust, funded by the National Institute of Health Research (NIHR Biomedical Research Centre). All participants gave written informed consent. The study was approved by the Joint South London and Maudsley and the Institute of Psychiatry NHS Research Ethics Committee. Permission to administer [18F]fallypride was given by the Administration of Radioactive Substances Advisory Committee. Before their involvement, a full medical and psychiatric history was taken from participants, including dementia screening using the Mini-Mental State Examination.[17] Exclusion criteria included (i) current or past neurological or psychiatric illness, including drug and substance abuse; (ii) Mini-Mental State Examination score < 26; (iii) history of any cerebrovascular event; (iv) use of estrogen replacement therapy or other medications, which may have affected brain dopamine activity; (v) needle phobia; and (vi) other medical conditions that might affect the ability to tolerate a brain scan, such as significant cardiorespiratory disease or severe kyphosis. Structural imaging (T1-weighted magnetic resonance imaging (MRI)) was carried out at the Centre for Neuroimaging Sciences to exclude intracranial abnormalities.

PET Imaging Procedure

Participants were scanned twice at rest, 4–6 weeks apart, on a GE (GE Healthcare, Hatfield, UK) VCT Discovery PET-CT camera (FWHM 5 mm), at St Thomas' PET Centre. A molded head rest and straps were used to minimize head movement and an external webcam was used to detect significant head movements that could degrade the quality of image data. [18F]fallypride was administered via a single bolus intravenous injection of 250 MBq. Each scanning session consisted of three dynamic scans in three-dimensional (3D) mode, each lasting 30 minutes, and preceded by a low-dose computed tomography scan for attenuation correction. Image data were collected during three scanning sessions, determined from previous [18F]fallypride studies in young adults;[16,18,19] 0–30 minutes to provide an input function to model a reference region approach; 60–90 minutes to capture peak tracer binding within extrastriatal regions; and 210–240 minutes to ensure that tracer binding had achieved a plateau in the striatum of all participants (in a small proportion of people, the tracer may take up to 210 minutes to achieve equilibrium)[20] and to achieve a good model fit. The initial 3 minutes of the first scanning session acquired frames with short duration (1×10 seconds, 10×5 seconds, 6×10 seconds, 3×20 seconds). The remaining 87 minutes of scanning were acquired using frame lengths of 1 minute.

Image Analysis

Data were analyzed using a simplified reference tissue model[21] and the cerebellum as a reference region. This approach has been previously validated for the quantification of [18F]fallypride BP_{ND}[22] and found to have good reproducibility and reliability.[23] Preprocessing was performed using SPM8 (www.fil.ion.ucl.ac.uk/spm/software/spm8/) and all other analyses using Matlab (www.mathworks.com). [18F]fallypride images were processed using SPM8 in Matlab. Non-attenuation corrected, fully 3D iteratively reconstructed PET scans (GE 'VuePoint' reconstruction algorithm, 4 ierations, 28 subsets, 4.8 mm Hanning 3D-filter) were used for frame-by-frame realignment. These transformations were then applied to attenuation-corrected (AC) filtered back projected (Fourier-rebinned 2D reconstruction, with geometric, deadtime, scatter, and random correction, 4.8 Hanning Transaxial filter, GE Scanner) PET images, which were used for quantification, and AC-VuePoint PET images (4 iterations, 28 subsets, 4.8 mm Hanning 3D-filter) used for warping atlases. All images were reconstructed to $128 \times 128 \times 47$ voxels with dimensions $2 \times 2 \times 3.27 \text{ mm}^3$.

Method 1

Image data collected over the three sampling times (0–30; 60–90; and 210–240 minutes) were used to quantify binding of [18F]fallypride to dopamine D2/3 receptors, expressed as BP_{ND}.[24] The cerebellar reference region was defined using the Automated Anatomical Labeling atlas[25] and included all cerebellar regions apart from the vermis. Regions of interest (ROI) in areas with specific binding were defined using the Tzioritzi atlas[26] and included the caudate, putamen, medial and inferior temporal gyri, thalamus, amygdala, hippocampus, orbitofrontal cortex, and anterior cingulate gyrus.

In addition to the Tzioritzi atlas, we used a pre-existing template that defines the striatum in terms of its functional connections, and which has been previously used to quantify [11C] Raclopride BP_{ND} in people with Alzheimer's disease.[27] The striatal subdivisions were defined in standard space (Montreal Neurological Institute, MNI), using the parameters described by Martinez et al.[28] The boundary between the ventral (limbic) striatum (inferiorly) and dorsal caudate and dorsal putamen (superiorly) was defined using the anterior commissure–posterior commissure transaxial plane. The ventral (limbic) striatum was sampled from the anterior boundary of the striatum to the level of the anterior commissure coronal plane. The transaxial plane was used to subdivide the dorsal caudate and putamen into associative (caudate and putamen rostral to the anterior commissure and the caudate caudal to the anterior commissure) and sensorimotor (putamen caudal to the anterior commissure) striatum.

We chose not to coregister MRI with PET data, as our specific aim was to establish the most widely applicable analysis method that would be suitable for use in older, cognitively impaired individuals, in whom MRI may be contraindicated or difficult to tolerate. As an alternative, atlases (and the striatal functional subdivisions template) were warped to subject space via a PET [18F]fallypride template in standard (MNI) space. The [18F]fallypride template was created from six healthy young subjects,[29] using the following method:

1. Structural (MRI) data were spatially normalized to MNI space using the unified segmentation algorithm in SPM.
2. These transforms were applied to each of the coregistered summed (3–30 min) AC-VuePoint PET images.
3. A mean [18F]fallypride template was calculated by scaling each transformed PET image by the subject global mean, and then taking the mean of the six PET images.

Method 2

Sampling times were reduced from 30 to 20-minute blocks (0–20; 70–90; and 220–240 minutes). All other aspects of the image analysis were identical to Method 1.

Statistical Analysis

All statistical analysis was carried out using SPSS 19 (www.spss.com). A paired t-test was used to compare the administered dose of [18F]fallypride between test (scan 1) and retest (scan 2) scans. The reliability of the test–retest values was determined by calculating an intraclass correlation coefficient (ICC)[30] for each region sampled. The absolute variability of test–retest reproducibility was calculated as follows: $(2|scan1 - scan2|)/(scan 1 + scan 2) \times 100$. Percentage variability in mean BP_{ND} between Methods 1 and 2 was determined by: $(|Method1\_scan1 - Method2\_scan1|/Method1\_Scan1) \times 100$ and expressed as the mean and s.d. across subjects. Power calculations were performed using 'PS' software.[31] The regional percentage change in [18F]fallypride BP_{ND} detectable in a typical within-subjects comparison (sample size $= 15$; paired t-test) was calculated using a probability (power) of 0.8, and an associated type I error probability (α) of 0.05. Percentage variability in mean BP_{ND} was used as an estimate of within-subject s.d.

RESULTS

Administered dose of [18F]fallypride was 244.1 ± 7.3 MBq. There were no significant differences in administered dose between scan 1 and scan 2 (mean difference $= 0.5 \pm 10.8$ MBq, $P = 0.9$).

Reliability Analysis

Method 1. Time–activity curves for Method 1 (30-minute sampling times) are shown in Figure 1 (all ROI defined using the Tziorotzi atlas), Figure 2 (extrastriatal ROI, defined using the Tzioritzi atlas), and Figure 3 (striatal functional subdivisions), and represent the AC filtered back projected data on which the kinetic analysis was carried out. Mean percentage test–retest differences (% variability) and reliability (ICC) of regional [18F]fallypride binding for Method 1 are presented in Table 1. Reproducibility was excellent within the caudate, putamen, amygdala, and inferior temporal gyrus, where $<5\%$ variability was observed.
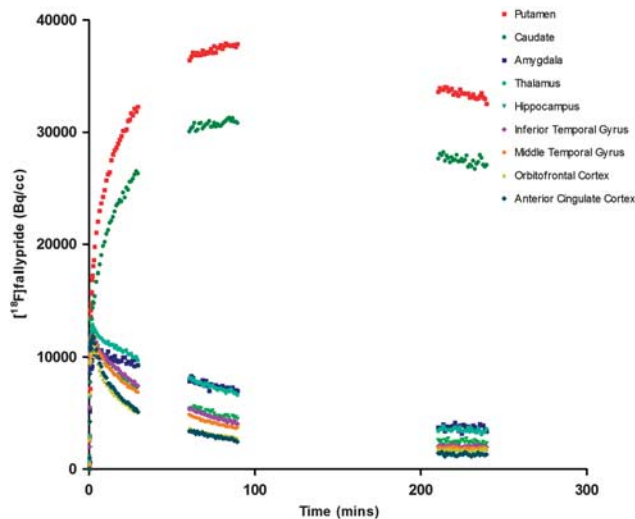
Figure 1. Time–activity curves (Method 1) are shown for regions of interest defined using the Tziortzi atlas and represent [18F]fallypride uptake in a single participant.
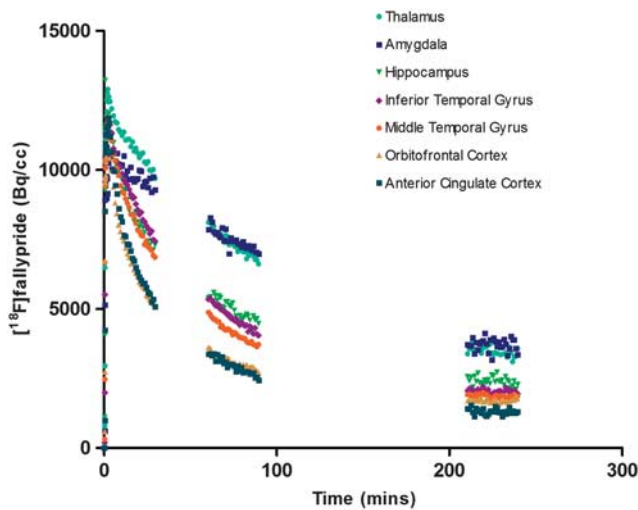

Figure 3. Time–activity curves (Method 1) are shown for the striatal functional subdivisions and represent [18F]fallypride uptake in a single participant.

Figure 2. Time–activity curves (Method 1) are shown for extrastriatal regions of interest and represent [18F]fallypride uptake in a single participant.

All of the other regions examined showed high reproducibility (<8% variability), with the exception of the orbitofrontal cortex (15.04% variability) and anterior cingulate gyrus (28.84% variability). Reliability (ICC) ranged from 0.804 (limbic striatum) to 0.988 (inferior and middle temporal gyri).

Method 2. Mean percentage test–retest differences (% variability) and reliability (ICC) of regional [18F]fallypride binding for Method 2 (20-minute sampling times) are presented in Table 2. Reproducibility remained excellent (<5% variability) in the caudate and amygdala, and high (<8% variability) in all but the limbic striatum (8.25% variability) and prefrontal regions (orbitofrontal cortex = 17.45%; anterior cingulate gyrus = 37.35%). Intraclass correlation coefficient values ranged from 0.794 (anterior cingulate gyrus) to 0.986 (inferior and middle temporal gyri). When regional 'test' BP_{ND} values were compared across the two methods (shown in Table 2) mean variability was 3.19% or less in all regions sampled apart from the anterior cingulate gyrus (14.47%) (shown in Table 2).
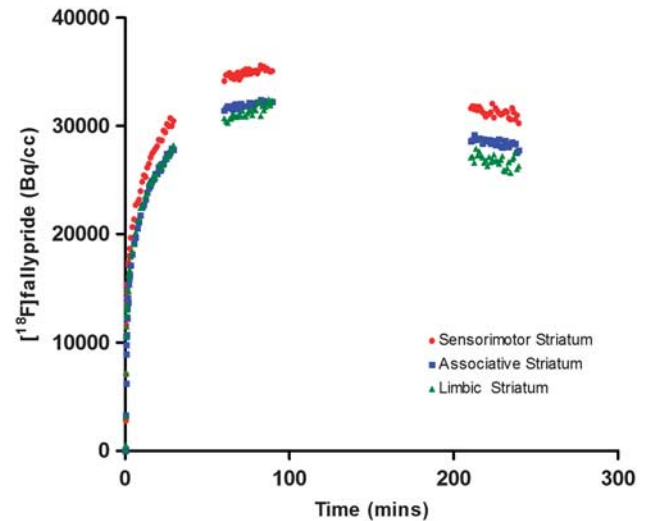
Table 1. Test–retest reproducibility of regional [18F]fallypride binding using method 1 (3 × 30 minutes)

Region	Test[a]	Retest[a]	Variability %[b]	ICC
Sensorimotor striatum	19.73 (2.66)	19.70 (2.06)	5.56 (2.69)	0.864
Associative striatum	17.50 (2.39)	17.58 (1.77)	5.05 (5.33)	0.845
Limbic striatum	16.90 (2.83)	17.04 (2.00)	7.12 (7.37)	0.804
Putamen	21.19 (2.82)	21.50 (2.15)	4.99 (3.76)	0.879
Caudate	16.57 (2.77)	16.71 (2.29)	3.44 (3.87)	0.961
Amygdala	1.77 (0.41)	1.76 (0.38)	4.08 (4.64)	0.976
Thalamus	1.48 (0.28)	1.47 (0.24)	6.10 (6.70)	0.903
Hippocampus	0.82 (0.21)	0.79 (0.19)	7.35 (6.40)	0.950
Inferior temporal gyrus	0.57 (0.21)	0.58 (0.20)	4.83 (7.61)	0.988
Middle temporal gyrus	0.39 (0.17)	0.39 (0.17)	6.40 (9.45)	0.988
Orbitofrontal cortex	0.21 (0.10)	0.23 (0.11)	15.04 (15.68)	0.923
Anterior cingulate gyrus	0.18 (0.10)	0.18 (0.09)	28.84 (39.59)	0.822

Abbreviation: ICC, intraclass correlation coefficient.
[a]BP_{ND} given as mean across subjects (s.d.). [b]Absolute variance given as mean across subjects (s.d.) = (|scan 1 − scan 2|/(scan 1 + scan 2)/2)*100.

Power Analysis

The results of a power analysis for a within-subject study design and a sample size of 15 (paired *t*-test; $\alpha 0.05$, power = 0.8) are shown in Table 3. This indicates that method 1 would be sufficiently sensitive to detect changes of 5% or less in all regions apart from the limbic striatum (5.5%), hippocampus (5.7%), orbitofrontal cortex (11.7%), and anterior cingulate gyrus (22.4%). Method 2 could detect changes of 5% or less across the caudate, putamen, dorsal striatal subdivisions, inferior temporal gyrus, and amygdala; and larger changes in the other regions.

DISCUSSION

This study has shown high reproducibility and reliability of an adapted [18F]fallypride protocol across the striatum, consistent with previous test–retest studies on [18F]fallypride in young adults, which have used full modeling with arterial sampling[6] or a simplified reference tissue approach[23] to image analysis. [18F]fallypride BP_{ND} was also highly reproducible in the amygdala, temporal cortex, thalamus, and hippocampus, comparable with previous data on [18F]fallypride.[6,23] In contrast, reproducibility in prefrontal regions was poor, particularly in the anterior cingulate

Table 2. Test–retest reproducibility of regional [18F]fallypride binding using Method 2 (3 × 20 minutes)

Region	Test[a]	Retest[a]	Variability %[b]	ICC	Method 1 versus method 2[d]
Sensorimotor striatum	19.82 (3.04)	19.70 (2.06)	6.37 (3.70)	0.839	1.77 (1.13)
Associative striatum	17.55 (2.64)	17.56 (1.84)	5.80 (5.91)	0.837	1.66 (0.93)
Limbic striatum	16.94 (3.06)	16.99 (2.06)	8.25 (6.97)	0.802	1.56 (0.89)
Putamen	21.27 (3.19)	21.49 (2.21)	5.68 (4.86)	0.857	1.69 (0.98)
Caudate	16.62 (3.06)	16.68 (2.35)	4.43 (4.43)	0.946	1.72 (0.90)
Amygdala	1.76 (0.42)	1.75 (0.38)	4.70 (5.30)	0.967	1.02 (0.74)
Thalamus	1.47 (0.29)	1.46 (0.23)	6.60 (7.25)	0.890	1.13 (0.74)
Hippocampus	0.81 (0.21)	0.79 (0.18)	7.67 (7.84)	0.935	1.38 (1.11)
Inferior temporal gyrus	0.56 (0.21)	0.57 (0.20)	5.28 (9.96)	0.986	2.89 (2.60)
Middle temporal gyrus	0.38 (0.18)	0.38 (0.17)	7.94 (10.47)	0.986	3.19 (2.76)
Orbitofrontal cortex	0.21 (0.10)	0.22 (0.11)	17.45 (14.23)	0.915	2.70 (2.41)
Anterior cingulate gyrus	0.17 (0.11)	0.17 (0.09)	37.35 (54.16)	0.794	14.47 (20.50)

Abbreviation: ICC, intraclass correlation coefficient.
[a]BP_{ND} given as mean across subjects (s.d.). [b]Absolute variance given as mean across subjects (s.d.) = (|test − retest|/(test + retest)/2)*100. [d]Given as mean percentage difference between Method 1 and Method 2 test BP_{ND} (s.d.) = (|Method1_test − Method2_test|/Method1_test) × *100.

Table 3. Regional detectable within-subject (%) change in [18F]fallypride binding[a]

Region	Method 1	Method 2
Sensorimotor striatum	4.3	5.0
Associative striatum	3.9	4.5
Limbic striatum	5.5	6.4
Putamen	3.9	4.4
Caudate	2.7	3.4
Amygdala	3.2	3.7
Thalamus	4.7	5.1
Hippocampus	5.7	6.0
Inferior temporal gyrus	3.8	4.1
Middle temporal gyrus	5.0	6.2
Orbitofrontal cortex	11.7	13.6
Anterior cingulate gyrus	22.4	29.1

[a]$n = 15$; power = 0.8; $\alpha = 0.05$.

cortex, where a higher mean and wider range of variability was observed compared with the other regions sampled. These findings are consistent but more marked than those of Cropley et al,[23] who similarly reported a high variability in the anterior cingulate (21.8 ± 3.8%) in healthy young adults. This is not unexpected, given the relatively low signal to noise ratio of [18F]fallypride in this region and the additional impact of age upon D2/3 receptor availability in our sample,[6,32] which is more pronounced in the anterior cingulate than other cortical regions.[32] Reducing individual sampling times to 20 minutes produced the same pattern of reproducibility across striatal and extrastriatal regions and increased test–retest variability minimally in all but the prefrontal regions.

A [18F]fallypride imaging technique that requires only brief periods in the scanner could significantly enhance the translational potential of [18F]fallypride imaging across a range of neuropsychiatric disorders in which dopamine has been implicated, including patients with dementia, Parkinson's disease, movement disorders, and disorders of impulsivity. Our primary interest in adapting [18F]fallypride imaging was to develop a technique suitable for use in D2/3 receptor occupancy studies in older and/or cognitively impaired patients. Older people are extremely susceptible to antipsychotic side effects, including extrapyramidal symptoms, falls, sedation, and increased cerebrovascular mortality.[33,34] The potential mechanisms underpinning this heightened sensitivity are poorly understood, although several theories have been proposed, including alterations in

central pharmacokinetics and reduced D2/3 receptor reserve in the ageing brain.[35] It has been suggested that the 'therapeutic window' of striatal D2/3 receptor occupancy may be lower in older people,[36] but this has not been tested robustly using a within-subject approach, nor has it been extended to extrastriatal regions, such as the temporal cortex, that have been implicated in treatment response in young psychotic adults.[9]

Our adapted protocol is sufficiently sensitive to detect small percentage changes in all but the prefrontal regions, and is therefore a suitable approach to establish the relative contribution of limbic versus striatal occupancy in elderly patients during antipsychotic treatment. This includes patients with schizophrenia-like illness, who are prescribed very low doses (1/10th of the dose used in young adults) of antipsychotic medication[33] and in whom a relatively low regional receptor D2/3 receptor occupancy might be anticipated. The fact that the length of individual scanning times can be reduced to 20 minutes without significantly compromising reliability increases its feasibility for use in patients with dementia. It could be argued that patients with cognitive or neurological impairment are likely to have more variable data than healthy older participants and that longer scanning sessions may be necessary to obtain data of sufficient quality. However, we anticipate that shorter sessions will maximize patient cooperation and reduce head movement confounds. The next stage of our research will be to pilot the protocol as a pretreatment (baseline) protocol in people with dementia who are about to begin antipsychotic medication, both to assess tolerability and the quality of the data collected over 30- versus 20-minute sampling times. We also aim to adapt and optimize an interrupted scanning protocol, which will be suitable for use post antipsychotic treatment, as occupancy by antipsychotic medication will reduce the number of available D2/3 receptor sites and shorten the time taken to achieve equilibrium, particularly within striatal regions.[16,18] Similar considerations will need to be taken into account when adapting the protocol for other types of pharmacological interventions, which may alter tracer kinetics and affect times of peak uptake and washout.

The decision not to use MRI data for coregistration with PET images was based on our previous experience of imaging older participants, particularly those with cognitive impairment,[37] in whom a multiple scan approach is more challenging and tolerance for confined spaces is generally poor. As an alternative, regions that were predefined on an atlas were warped to participants' PET images using a [18F]fallypride template and an automated procedure. This method is similar to the approach previously used to quantify [11C] Raclopride BP_{ND} in people with Alzheimer's disease[27] and has the advantage of

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removing any subjectivity in the placing of ROIs. However, the fact that the templates originated from structural and functional imaging data on healthy young adults could have impacted upon the warping process. Although high tracer signal forms the key component of the process, the accuracy of warping, particularly in smaller, noisier regions, may have been reduced. Partial volume effects, particularly relevant in an older sample,[38] are also likely to have affected the accuracy of tracer quantification. These factors are likely to have contributed to the high test–retest variability in the anterior cingulate gyrus. However, the method of image analysis does not appear to have affected the pattern of reproducibility across other brain regions, as our findings are consistent with previously conducted test–retest studies that used the participant's MRI[23] or PET-MR coregistered images[6] to define ROI. A further consideration is the fact that the sampling times used to quantify cortical and striatal BP$_{ND}$ were based upon [^{18}F]fallypride studies carried out in young adults. Given that time to achieve peak tracer binding is dependent upon receptor density, and may be affected by age-related factors including peripheral clearance, regional cerebral blood flow, and blood brain barrier transport, it remains possible that the adapted protocol did not optimally capture the peak in all participants. These methodological limitations are less important for within-subject study designs, but will need to be addressed to maximize the potential of the protocol for use in between-subject comparisons, or to correlate regional BP$_{ND}$ with behavioral measures.

In addition to receptor occupancy, we wanted to establish the sensitivity of our protocol to detect changes in endogenous dopamine after pharmacological or behavioral intervention. Amphetamine displacement studies carried out in young adults have found that [^{18}F]fallypride is less sensitive to changes in cortical dopamine levels than other high-affinity tracers such as [^{11}C]FLB457, because of its relatively low signal to noise ratio in these regions.[39] A sufficiently robust effect has, however, been demonstrated across striatal and limbic (hippocampal and amygdala) regions.[12,19,23] A power calculation, using a standard sample size of 15 (previous studies have included 10–25 subjects),[12,13,15,19,39] indicates that our adapted protocol will be sufficiently sensitive to detect within-subject changes between 2.7% and 5.5% within striatal and limbic regions using method 1, and between 3.4% and 6.4% using method 2. As discussed earlier, we have not yet established whether these findings will generalize to cognitively or neurologically impaired populations, and this issue is particularly relevant for studies that aim to quantify endogenous dopamine release, where small changes in BP$_{ND}$ are anticipated. Given the fact that sensitivity to detect within-subject change is crucial for studies of this type, it may be advisable to collect data over the longer (30 minute) sampling times used in method 1 to image endogenous neurotransmission.

We have successfully adapted [^{18}F]fallypride imaging, using an interrupted scanning protocol that considerably shortens scanning times. The high reproducibility and reliability of this protocol means that it could be applied not only to D2/3 receptor occupancy studies but also to image endogenous neurotransmission in striatal and limbic regions. Future work will aim to pilot the protocol in clinically relevant populations and to refine the image analysis method to maximize its potential for use in between-subject study designs.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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