Spatial Covariance of Cholinergic Muscarinic M1/M4 Receptors in Parkinson’s Disease

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ABSTRACT: Background: Parkinson’s disease (PD) is associated with cholinergic dysfunction, although the role of M1 and M4 receptors remains unclear.
Objective: To investigate spatial covariance patterns of cholinergic muscarinic M1/M4 receptors in PD and their relationship with cognition and motor symptoms.
Methods: Some 19 PD and 24 older adult controls underwent 123I-iodo-quinuclidinyl-benzilate (QNB) (M1/M4 receptor) and 99mTc-exametazime (perfusion) single-photon emission computed tomography (SPECT) scanning. We implemented voxel principal components analysis, producing a series of images representing patterns of intercorrelated voxels across individuals. Linear regression analyses derived specific M1/M4 spatial covariance patterns associated with PD.
Results: A cholinergic M1/M4 pattern that converged onto key hubs of the default, auditory–visual, salience, and sensorimotor networks fully discriminated PD patients from controls ($F_{1,41} = 135.4$, $P < 0.001$). In PD, we derived M1/M4 patterns that correlated with global cognition ($r = −0.62$, $P = 0.008$) and motor severity ($r = 0.53$, $P = 0.02$). Both patterns emerged with a shared topography implicating the basal forebrain as well as visual, frontal executive, and salience circuits. Further, we found a M1/M4 pattern that predicted global cognitive decline ($r = 0.46$, $P = 0.04$) comprising relative decreased binding within default and frontal executive networks.
Conclusions: Cholinergic muscarinic M1/M4 modulation within key brain networks were apparent in PD. Cognition and motor severity were associated with a similar topography, inferring both phenotypes possibly rely on related cholinergic mechanisms. Relative decreased M1/M4 binding within default and frontal executive networks could be an indicator of future cognitive decline. © 2021 The Authors. Movement Disorders published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

Key Words: Parkinson’s disease; muscarinic receptors; SPECT; spatial covariance; cholinergic M1/M4 receptors; networks

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Parkinson’s disease (PD) is a neurodegenerative disorder affecting 1 person of every 1000 in their fifth decade and rising to 19 out of every 1000 in their eighth decade or older. The main clinical symptoms are abnormal involuntary movements, bradykinesia, rigidity, and tremor. Patients also display non-motor symptoms, with high levels of mild cognitive impairment (MCI) even at incident diagnosis and increased likelihood of progression to dementia over time. The cholinergic system is severely affected in PD, even at its early stages, with widespread denervation. These cholinergic abnormalities are associated with a wide array of clinical features including motor symptoms, levodopa-induced dyskinesias, cognitive deterioration, sleep abnormalities, autonomic dysfunction, and altered olfaction.

Molecular imaging of the cholinergic system in PD has focused on the cognitive changes, motivated mainly by the cholinergic hypothesis of dementia. PET studies assessing acetylcholinesterase activity have consistently shown cortical reductions of between 10% and 23% in PD relative to controls. Using a nonselective muscarinic receptor tracer, [11C-N-methyl-4-piperidyl benzilate, increased frontal binding has also been reported. However, there remains a paucity of in vivo studies of selective muscarinic ligands examining the relationship between receptor expression and cognition. At postmortem, similar levels of M1, M2, and M4 receptor expressions in striatum, globus pallidus, and substantia innominata were found between PD and controls. In contrast, elevated M2-M3-M4 expressions in prefrontal cortex, with higher M3 but similar M1 levels in striatum, were shown in PD relative to controls.

Brain connectivity plays an important role in the symptomatology of neurodegenerative disorders. In PD, several cholinergic networks have been proposed and thought to affect attention, visuoperceptual, and memory domains. In terms of muscarinic M1/M4 cholinergic receptors, alterations in limbic–paralimbic and salience networks have been described in PD dementia (PDD). One approach of examining brain connectivity is by spatial covariance, where such procedures have studied the effects of progression, treatment, and cognition in PD.

We applied spatial covariance analysis to (R, R) [123I-iodo-quinuclidinyl-benzilate (QNB) single-photon emission computed tomography (SPECT) scans, a ligand with high binding affinity for M1 and M4 receptors, to derive discrete patterns that distinguish PD from healthy individuals and in PD that correlate with global cognition, motor severity, and cognitive decline.

**Patients and Methods**

**Standard Protocol Approvals, Registrations, and Patient Consents**

Approval was from the UK Department of Health’s Administration of Radioactive Substances Advisory Committee (ARSAC) and Newcastle, North Tyneside, and Northumberland Research Ethics Group. Participants and/or nearest relative gave written informed consent.

**Participants**

The investigation consisted of 43 individuals (19 PD, 24 older healthy controls). Patients were recruited from outpatient movement disorder clinics in Newcastle-upon-Tyne and Gateshead, UK. Older cognitively intact controls were enrolled from patient spouses, friends, and volunteers. Participants had physical, neurological, and neuropsychiatric assessments, including mental state, history, physical examination and, for patients, blood screen with B12 and folate levels. PD diagnoses were carried out by a movement disorder specialist (D.B.) using the UK Parkinson’s Disease Society Brain Bank criteria. All patients were diagnosed with probable PD, each with a positive DaTSCAN. The only permissible PD medications were levodopa and carbidopa/benserazide. Participants on any of the following medications were excluded from the study: antipsychotics, cholinesterase inhibitors, anti-cholinergics, and antidepressants.

Motor severity was assessed using Part III (motor examination) of the Unified Parkinson’s Disease Rating Scale (UPDRS). Cognitive function was evaluated with the Mini-Mental State Examination (MMSE) and Cambridge Cognitive Examination (CAMCOG) tests. At the time of QNB imaging, the PD group had a range of cognitive dysfunction, which is typical for incident PD cohorts. Patients were classified as cognitively intact or cognitively impaired with the latter defined as mild cognitive impairment (MCI) or PD dementia (PDD). Level I PD-MCI criteria were used to retrospectively classify the former and PDD consensus criteria for the latter, with the following cutoffs chosen from CAMCOG scores: 86 ≤ MCI < 95 and ≤ 85 for dementia. Patients consisted of 6 PD-intact, 6 PD-MCI, and 7 PD-dementia.

The patient cohort was also part of a larger longitudinal PD and dementia study, where a series of baseline and annual repeat assessments were conducted. Serial cognitive data for the current PD sample was available (n = 18), where the CAMCOG/MMSE assessment nearest to the [123I-QNB scan (mean ± SD, 39.8 ± 29.6 days) was selected as baseline and the next annual follow-up assessment as repeat (interval 1.0 ± 0.03 years).

**Radiochemistry**

Employing the technique of Lee et al, (R, R) [123I-123I-QNB radiosynthesis was performed, the details of which have been previously described.
Acquisition

Individuals were scanned with a triple-head gamma camera (Picker 3000XP), 5 hours post-injection of (R, R) $^{123}$I-QNB using a previously reported imaging protocol.27 Within 4 weeks of the (R, R) $^{123}$I-QNB scan, participants undertook 99mTc-exametazime regional cerebral blood flow (rCBF) SPECT scanning.27

Spatial Preprocessing

Scans were registered to match specific $^{123}$I-QNB and 99mTc-exametazime SPECT templates in standard MNI space using the image registration tool FLIRT (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT). Information regarding the template images has been reported.26,28

The registered images were then smoothed with a 10 mm FWHM 3D Gaussian filter.

Spatial Covariance

Spatial covariance was simultaneously applied to ‘n’ preprocessed (registered and smoothed) $^{123}$I-QNB SPECT scans using covariance software (http://www.nitrc.org/projects/gcva_pca/),29 capturing the major sources of variation, producing (n−1) principal component (PC) images organized in a descending order of decreasing variance. A mask image defined the brain volume subspace for voxel analyses. Global means (within brain mask) for each subject were computed resulting linear combinations with lowest AIC determined how many SSFs (PCs), should be expressed by the SCPQNB image by the corresponding voxel value in the subject’s QNB scan followed by summation of these products to produce a score.

Spatial covariance was then applied to the perfusion scans, primarily to assess whether the $M_1/M_4$ disease pattern (SCPQNB) differed from perfusion. Therefore, positive and negative weights of these images were interpreted as concomitant relative/normalized increased and decreased rCBF/perfusion, respectively. The analysis generated a SCPRCBF that separated PD from controls, with subject expression scores (SSFRCBF).

We then produced in PD, $M_1/M_4$ SCPs that correlated with baseline global cognition (CAMCOG), motor (UPDRS III), and global cognitive progression (ΔCAMCOGbaseline – repeat) scores. This involved conducting separate covariance analyses of just the PD patients, generating a set of PD-specific PCs, expressed by the SSFs, which were entered into a regression model as predictors, with either CAMCOG, UPDRS III, or cognitive progression scores as the dependent. The resulting linear combinations with lowest AIC defined the $M_1/M_4$ ‘cognitive’, ‘motor’, and ‘progression’ SCPs, with individual pattern expressions scores of SSFCOGNITIVES, SSFMOTOR, and SSFPROGRESSION, respectively.

Stability and reliability of the SCPs were assessed by bootstrap resampling (1000 iterations) to identify significant areas with high confidence. This transforms the voxel weights of each SCP into Z maps, computed as the ratio of voxel weight and bootstrap incurred standard deviation. The Z-statistic follows roughly a standard normal distribution where a one-tailed $P < 0.05$ infer a threshold of $|Z| \geq 1.64$.31 Labelling of maps used the MNI brain atlas integrated within the medical image viewer ‘Mango’ (http://ric.uthscsa.edu/mango/).

Univariate Analysis

QNB and perfusion scans were investigated using statistical parametric mapping (SPM12, http://www.fil.ion.ucl.ac.uk/spm). Respective brain masks from the covariance method defined voxels for univariate analysis and calculation of mean global uptake (used for intensity normalization). Two-sample $t$ test assessed differences in scaled QNB and rCBF between PD and controls. Results were thresholded using the family-wise error correction ($P_{FWE} \leq 0.05$).

Statistical Analyses

Analysis used IBM SPSS v. 25.0.0.1 and R (v. 4.0.3, https://www.R-project.org/). Baseline variables were tested for normality and variance homogeneity using Shapiro–Wilk and Levene’s tests, respectively. The data
were examined using parametric (ANOVA F, Welch’s ANOVA W) and non-parametric (Mann–Whitney U, \( \chi^2 \)) tests. For related measures, differences were assessed with the Wilcoxon signed rank test. Correlations were assessed with Pearson coefficients. Where appropriate, Benjamini–Hochberg multiple comparisons correction (\( P_{BH} \)) with a 5% false discovery rate was applied.

## Results

### Demographics and Clinical Characteristics

Table 1 shows the demographic and clinical characteristics. Groups were matched for gender and age, while for global cognition PD was slightly worse than controls (\( P \leq 0.003 \)). For cognitive progression, CAMCOGtotal scores were significantly lower at repeat compared to baseline (\( \Delta = -4.5, P = 0.008 \)), likewise for MMSE (\( \Delta = -1.4 \)), though not significant (\( P = 0.2 \)).

### Spatial Covariance

\( PC_{1,2,3,6,11} \) formed the SCP\(_{QNB} \) that distinguished PD from controls (Fig. 1a,b), accounting for 26.1% of the total image variance. SSF\(_{QNB} \) scores were standardized and offset such that the control mean/SD were 0/1 respectively. Scores significantly differed between groups (mean ± SD; controls = 0 ± 1.0, PD = 3.7 ± 1.1, \( F_{1,41} = 135.4, P < 0.001 \), Fig. 1c). The disease pattern was characterized by relative preserved/increased M1/M4 binding (warm colors) in inferior/middle/superior temporal, parahippocampus, fusiform, striatum, and anterior cingulate. Table S1 details regions contributing to the M1/M4 disease pattern.

\( PC_{1,2,3,4} \) derived the associated SCP\(_{rCBF} \) (Fig. 1d), accounting for 19.2% of the total image variance. SSF\(_{rCBF} \) scores were similarly standardized and differed between groups (controls = 0 ± 1.0, PD = 1.9 ± 1.7, \( W_{1,28} = 19.8, P < 0.001 \), Fig. 1e). The rCBF disease pattern involved relative increased rCBF (warm colors) in cerebellum, midbrain, pallidum, parahippocampus, precenral gyrus, and medial orbitofrontal cortex with relative decreased rCBF (cold colors) in anterior/posterior cingulate, caudate, inferior parietal, precuneus, and medial/lateral prefrontal areas. Table S2 details regions of the rCBF disease pattern.

We generated M1/M4 patterns in PD that individually correlated with baseline CAMCOG, UPDRS III, and \( \Delta \)CAMCOGbaseline-repeat scores and termed them ‘cognitive’, ‘motor’, and ‘progression’ patterns, respectively. The cognitive pattern converged onto the composite image PC\(_{2,3} \) and accounted for 13.9% of the total PD image variance. The pattern (Fig. 2a) consisted of relative preserved/increased M1/M4 binding (warm colors) in fusiform, insula, occipital lobe, inferior parietal, and precuneus with relative decreased binding (cold colors) in superior/middle frontal gyri, dorsomedial prefrontal cortex, medial frontal gyrus, superior parietal, superior temporal gyrus, anterior cingulate, parahippocampus, caudate, and basal forebrain. Figure 2b depicts the pattern expression scores (SSF\(_{PC23} \)) plotted as a function of CAMCOG (\( r = -0.62, P_{BH} = 0.008 \)). Table S3 details regions contributing to the cognitive pattern. PC\(_2 \) (Fig. 2c) formed the motor pattern, accounting for 11.2% of the total PD image variance. This pattern comprised of relative preserved/increased M1/M4 binding (warm colors) in inferior/middle/superior temporal gyri, insula, parahippocampus, lingual gyrus, striatum, fusiform, and precuneus with relative decreased binding (cold colors) in inferior/middle/
superior frontal gyri, dorsomedial prefrontal cortex, medial orbitofrontal cortex, pre/post central gyri, inferior/superior parietal, cuneus, anterior cingulate, and basal forebrain. Figure 2d depicts the pattern expression scores (SSFPC2/motor) plotted as a function of UPDRS III ($r = 0.53$, $P_{BH} = 0.02$). Table S4 highlights regions contributing to the motor pattern. The baseline pattern associated with global cognitive decline in PD, that is, the progression pattern converged onto PC9, and accounted for 5.0% of the total PD image variance ($n = 18$, Fig. 2e). The pattern included relative preserved/increased $M_1/M_4$ binding (warm colors) in anterior cingulate, inferior temporal gyrus, insula, pre/post central gyri, superior parietal, superior frontal
gyrus, and left lingual gyrus with relative decreased binding (cold colors) in basal forebrain, dorsomedial prefrontal cortex, parahippocampus, posterior cingulate, ventral precuneus, fusiform, inferior parietal, ventral orbitofrontal cortex, middle frontal gyrus, right middle occipital, and left superior/middle temporal gyri. Figure 2f illustrates the pattern expression scores ($SSF_{PC_{9}/\text{progression}}$) plotted as a function of $\Delta$CAMCOG$_{\text{baseline-repeat}}$ ($r = 0.46$, $P_{\text{BH}} = 0.04$). Table S5 details regions contributing to the progression pattern.
Univariate Analysis

Supporting information (Fig. S1a,b) depicts the QNB and rCBF univariate results. Significant reduction in M1/M4 binding in PD compared to controls was observed in fusiform, middle temporal, and parahippocampus (Table S6). Significant hypoperfusion in PD relative to controls was found in precuneus and middle frontal (Table S7). No differences were observed in QNB or rCBF where PD > controls.

Discussion

Our aim was to undertake a spatial covariance perspective of (R, R) 123I-QNB SPECT scans, a component of the ascending cholinergic system, in PD and healthy older individuals. Typically, univariate methods provide information about the regional changes in uptake, while often ignoring the network aspects of the brain. Since we undertook the spatial covariance approach, it seemed intuitive to interpret the results in terms of functional networks, and no attempt was made to offer a regional quantitative evaluation of M1/M4 receptors. We therefore derived a M1/M4 disease-related pattern that largely differed from rCBF. We also identified baseline M1/M4 patterns that separately correlated with global cognition, motor severity, and cognitive progression, which could represent signatures of M1/M4 expressions for cognitive and motor dysfunction as well as cognitive decline in PD.

A pattern emerged from 123I-QNB images that fully discriminated PD from controls. The M1/M4 pattern consisted of concomitant relative decreased and preserved/increased binding in several brain regions. The decreased pattern converged on lingual, fusiform, and lateral temporal cortex (visuospatial and auditory); striatum (motor); parahippocampus (memory); and anterior cingulate (salience). The preserved/increased pattern converged on lateral/medial orbitofrontal, posterior cingulate, precuneus, parietal, occipital, and pre/post central regions. These involved a constellation of brain regions within key hubs of the default mode (DMN) and sensorimotor networks, suggesting these M1/M4 circuits are intact at this stage of the disease or upregulated in response to the cholinergic deficits. Overall the PD disease pattern infers a possible modulation of muscarinic receptors within DMN, salience, auditory-visuospatial, and sensorimotor networks. The frontal pattern aligns with previous reports of increased frontal muscarinic receptor expression in PD,6,7 therefore a frontal positive covariance signal may indicate compensation. Expectedly, although in independent cohorts, there was shared topography between the current M1/M4 pattern and our previously reported M1/M4 pattern in PDD,12 that is relative decreased binding in fusiform, striatal, and lateral temporal with lateral preserved/increased binding in lateral orbitofrontal and occipitoparietal regions. However, there were variations that were likely attributed to differences in disease/cognitive severity, namely a more extensive decreased M1/M4 pattern within anterior cingulate, insula, basal forebrain, and medial temporal areas, inferring, as dementia develops, that deficits in relative M1/M4 expression extend into basal forebrain, salience, and memory circuits. The univariate findings showed reduced M1/M4 binding between PD and controls in middle temporal, fusiform, and parahippocampus, regions also captured within the covariance topography.

The corresponding rCBF covariance pattern involved relative increased activity in cerebellum, pons, globus pallidus, precentral, parahippocampal, and medial orbitofrontal regions with relative decreased activity in inferior parietal, precuneus, posterior cingulate, caudate, and medial/lateral prefrontal cortices. Regions of relative reduction appear to include DMN hubs (precuneus, posterior cingulate, inferior parietal, medial prefrontal), where theories infer its contribution to cognition,32 while regions of relative increase seem to implicate mainly motor circuits. Earlier PD covariance studies with SPECT (99mTc-ECD) and PET (18F-FDG) showed broadly similar topographies to the present results. For SPECT, increased relative perfusion in cerebellum and lentiform nucleus with relative decreased perfusion in prefrontal areas,33 while for PET, increased relative uptake in cerebellum, pons, and pallidum along with decreased relative uptake in inferior parietal, precuneus, and medial prefrontal.13 Variation between these and our results most likely stem from differences in target tracer, partial volume effects, and imaging/analysis methods. Univariate-wise, differences were observed in the bilateral precuneus and left middle frontal, where others have similarly revealed perfusion deficits within these regions in PD.54-56

We found a M1/M4 pattern that correlated with baseline global cognition (cognitive pattern) and consisted of relative preserved/increased binding in fusiform, insula, occipital, precuneus, and inferior parietal with relatively decreased binding in lateral/medial prefrontal, superior/medial temporal, anterior cingulate, caudate, and basal forebrain regions. Relative preserved/increased M1/M4 receptor expression broadly centred on components of the posterior DMN and visual networks, while decreased receptor expression focused on elements of auditory/speech, memory, executive, and salience circuits. Salience networks are important for initializing cognitive control and providing a switch function for recruitment of other relevant functional networks such as DMN and executive/attention.37,38 Specifically, dopamine-mediated salience dysfunction along with the parahippocampus has been shown to contribute to the memory impairment in PD,39 while salience dysfunction is increasingly being implicated as a driver for somatic symptoms disorders in this disease.40 Loss of functional connectivity without
structural damage in salience network regions have also been observed in PD-MCI. This is of potential interest, with the current findings appearing to suggest that changes in cholinergic-mediated signalling may be involved in misattribution of salience in PD deprioritizing the central executive networks. Our finding of muscarinic receptor expression alteration in limbic and neocortical regions correlates with Lewy body pathology that develops in these areas with disease progression. Reduced M1/M4 receptor expression within the dorsomedial prefrontal cortex (dmPFC), an executive hub, is also of relevance since in the earliest stages of cognitive decline in PD, reduced functional connectivity has been shown between posterior DMN sites and this region. In addition, this structure is part of the so-called ‘metabolic cognitive pattern’ of PD. Thus, the clear functional deficit within the dmPFC may further support the view of the neurotrophic effects of acetylcholine mediated through muscarinic receptors and its link with cognition. The preserved/upregulated M1/M4 pattern within posterior DMN and visual networks could be compensatory responses to the now apparent and early deterioration that occurs in the basal forebrain in PD.

The M1/M4 motor pattern shared some of the topographical features of the M1/M4 cognitive pattern, with additional regions also making a significant contribution. The motor pattern similarly characterized relative preserved/increased M1/M4 binding within insula, precuneus, and ventral visual regions as well as relative decreased binding in basal forebrain, anterior cingulate, and frontal executive hubs. Other sites contributing to the pattern included preserved/increased binding in striatal, auditory/speech, and parahippocampal regions with decreased relative binding within sensorimotor and lateral/medial orbitofrontal areas. The spatial commonality between the cognitive and motor M1/M4 patterns provides credence to the developing view of co-dependence between cognitive and motor processes in Lewy body disease. For example, there is increasing evidence that cholinergic dysfunction contributes to motor/gait disturbances in PD, with one study showing that reduced short-latency afferent inhibition (a surrogate measure of cholinergic activity) predicted slower gait speed in early PD, thus pointing toward the involvement of non-motor mechanisms in gait.

In terms of M1/M4 receptor expression, a visual-executive-salience topography emerged as being associated with severity of cognitive and motor symptoms in PD, and perhaps indicates, from a cholinergic perspective, that motor and cognitive symptoms are both influenced by these cholinergic pathomechanisms.

The M1/M4 progression pattern predicted future global cognitive decline, measured by ΔCAMCOGbaseline-repeat. The pattern comprised of relative preserved/increased binding within salience, visual, and sensorimotor regions and relative decreased binding in fusiform, dorsolateral/dorsomedial prefrontal, lateral/medial orbitofrontal, ventral precuneus, posterior cingulate, inferior parietal, parahippocampus, and basal forebrain. The relative decreased binding pattern fell mainly within regions of the DMN and executive networks, inferring that a cholinergic-mediated reduction of M1/M4 receptors within these sites is a marker of future cognitive decline. Others have also demonstrated specific imaging biomarkers as predictors of cognitive dysfunction in de novo PD. In a large magnetic resonance imaging study, basal forebrain atrophy was shown to predict cognitive decline, while another reported prefrontal atrophy correlated with attentional impairment. These studies reinforce the notion that early basal forebrain neurodegeneration and its cholinergic projections to specific brain regions are important contributors to the emergence of cognitive dysfunction in PD. This supports our findings where we have direct evidence that reduced M1/M4 receptor expression at baseline within DMN and frontoparietal networks were prerequisite for cognitive remediation following cholinergic treatment in PDD. Therefore it could be argued that baseline M1/M4 expression integrity within default and frontoparietal hubs are possible key determinants of cognitive decline and treatment outcomes in PD.

There were study limitations that cannot be overlooked: the patient sample was relatively small, results were not validated in a second independent patient cohort, and global cognitive scores only 1 year apart were used to assess progression. As such, the findings need to be interpreted as tentative. We did not correct for partial volume effects and so regional specificity of the results could be affected. Uncertainty regarding which receptor subtype is affected (M1 vs. M4) is another limitation. There was a minority of participants who improved cognitively; however, this is not unexpected since in incident PD cohorts cognition may improve or fluctuate. Strengths were: scanning (muscarinic, perfusion) and clinically assessing PD patients free from cholinergic medications.

In summary, we identified a number of cholinergic muscarinic receptor networks in PD. Cognition and motor severity were associated with a similar topography, inferring both phenotypes possibly rely on related cholinergic mechanisms. Relative decreased M1/M4 receptor expression within DMN and frontal executive hubs could be an indicator of future cognitive decline.
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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.