Cholinergic and perfusion brain networks in Parkinson disease dementia

ABSTRACT

Objective: To investigate muscarinic M1/M4 cholinergic networks in Parkinson disease dementia (PDD) and their association with changes in Mini-Mental State Examination (MMSE) after 12 weeks of treatment with donepezil.

Methods: Forty-nine participants (25 PDD and 24 elderly controls) underwent $^{123}$I-QNB and $^{99m}$Tc-exametazime SPECT scanning. We implemented voxel principal components (PC) analysis, producing a series of PC images of patterns of interrelated voxels across individuals. Linear regression analyses derived specific M1/M4 and perfusion spatial covariance patterns (SCPs).

Results: We found an M1/M4 SCP of relative decreased binding in basal forebrain, temporal, striatum, insula, and anterior cingulate ($F_{1,47} = 31.9, p < 0.001$) in cholinesterase inhibitor-naïve patients with PDD, implicating limbic-paralimbic and salience cholinergic networks. The corresponding regional cerebral blood flow SCP showed relative decreased uptake in temporoparietal and prefrontal areas ($F_{1,47} = 177.5, p < 0.001$) and nodes of the frontoparietal and default mode networks (DMN). The M1/M4 pattern that correlated with an improvement in MMSE ($r = 0.58, p = 0.005$) revealed relatively preserved/increased pre/medial/orbitofrontal, parietal, and posterior cingulate areas coinciding with the DMN and frontoparietal networks.

Conclusion: Dysfunctional limbic-paralimbic and salience cholinergic networks were associated with PDD. Established cholinergic maintenance of the DMN and frontoparietal networks may be prerequisite for cognitive remediation following cholinergic treatment in this condition. Neurology® 2016;87:1-8

GLOSSARY

AIC = Akaike information criterion; CAMCOG = Cambridge Cognitive Examination; CAMCOGexec = Cambridge Cognitive Examination executive function subscale; CAMCOGmemory = Cambridge Cognitive Examination memory subscale; ChEI = cholinesterase inhibitor; DLB = dementia with Lewy bodies; DMN = default mode network; MMSE = Mini-Mental State Examination; NBM = nuclear basalis of Meynert; NPI = Neuropsychiatric Inventory; PC = principal component; PD = Parkinson disease; PDD = Parkinson disease dementia; rCBF = regional cerebral blood flow; SCP = spatial covariance pattern; SN = salience network; SSF = subject scaling factor.

In Parkinson disease (PD), development of dementia (PDD) occurs in up to 80% of people 15–20 years after PD diagnosis, with 50% developing cognitive impairment within 6 years. In PDD, cholinergic dysfunction is strongly implicated in cognitive deficits, fluctuating cognition, and visual hallucinations. Reductions in choline acetyltransferase are marked in PDD compared to Alzheimer disease and PD, while clinically, cholinesterase inhibitors (ChEIs) can ameliorate cognition and visual hallucinations. However, response is variable with some efficacy.

Since the brain is a networked entity, pathologic change in one area may influence other topographically distant regions. Indeed, distributed network dysfunction is now considered a key contributor to symptoms that manifest in neurodegenerative dementias. In PDD, theoretical models of dysfunctional neural networks have been proposed. In particular, several cholinergic networks arising from the nuclear basalis of Meynert (NBM) projecting to...
specific brain regions are thought to affect major
cognitive domains, e.g., attention (NBM →
neocortex), visuoperceptual (NBM → parieto-
ocipital, parahippocampal-fusiform), and
memory (NBM → medial temporal).7 One
way to examine functional brain connectivity
is by spatial covariance analysis.

We applied spatial covariance to (R, R) 123I-
QNB SPECT data,8 acquired in ChEI-naive
patients with PDD, to investigate disease-
associated M1/M4 cholinergic networks. Also,
as cholinergic network dysfunction is implicated
in cognitive impairment and amelioration of
cholinergic function is an important aspect of
treatment, we derived an M1/M4 covariance
pattern that correlated with a change in Mini-
Mental State Examination (MMSE) score, after
12 weeks of treatment with the ChEI (donepez-
ril), to probe the clinical significance of these
networks.

**METHODS**

**Standard protocol approvals, registrations,
and patient consents.** Study approval was from the UK
Department of Health’s Administration of Radioactive Substances
Advisory Committee and Newcastle, North Tyneside, and
Northumberland research ethics committees. All participants or
nearest relatives gave written informed consent for the study
including treatment.

**Participants.** The study comprised 49 individuals (25 PDD and
24 similarly aged controls). Patients were recruited from outpa-
tient movement disorder clinics in Newcastle-upon-Tyne and
Gateshead, while healthy controls were from patient spouses and
friends in this and other studies. Participants had physical,
neurologic, and neuropsychiatric assessments, including mental
state, history, physical examination, and, for patients, blood screen
with B12 and folate levels. The study battery administered included
the MMSE,9 Neuropsychiatric Inventory (NPI),10 and Cambridge
Cognitive Examination (CAMCOG)11 with memory and executive
function subscales (CAMCOGmemory, CAMCOGexec).

Diagnosis was carried out consensually between 2 clinicians
using the diagnostic criteria for PDD.12 Patients with PDD were
on levodopa and carbidopa or benserazide combination therapy
and were naive to ChEI treatment at the time of QNB imaging.
Participants on any of the following medications were excluded
and were naive to ChEI treatment at the time of QNB imaging.
Participants on levodopa and carbidopa or benserazide combination therapy
were reintroduced into a regression model as predictor variables
with group as the dependent parameter. Akaike information criteria (AICs)
determined how many PCs should be included to reach optimal
bias-variance tradeoff.17 The set of PCs yielding the lowest AIC
value was used to derive the SCPQNB. The degree to which each
participant expressed the SCPQNB was by the SSFQNB.

The same approach was applied to the 99mTc-exametazime
SPECT images. Therefore, positive and negative weights were
interpreted as concurrent increased and decreased rCBF,
respectively. The analysis produced the SCPrCBF that best
separated PDD from controls, while each participant expressed the
SCP rCBF by his or her SSFrCBF.

Following their 123I-QNB scan, the majority of patients (n = 18)
were then treated with the ChEI donepezil titrated up to the
standard daily clinical dose of 10 mg. After a period of 12 weeks,
patients underwent repeated MMSE assessments. We derived
a ChEI-naive M1/M4 SCP that correlated with ΔMMSEref,b which described the percentage change in MMSE relative to base-
line. This involved conducting a separate analysis, generating a series
of PCs expressed by each participant by the SSFs, which in turn
were introduced into a regression model as predictor variables
with ΔMMSEref,b as the response parameter. The resulting linear
combination with the smallest AIC value generated the SCP ΔMMSE
(R2 = 0.34, p = 0.005), where each individual expressed the
pattern by the SSF ΔMMSE.

Stability and reliability of the SCPs were assessed by bootstrap
resampling (1,000 iterations) to identify areas that contributed to
the patterns with high confidence. This transforms the voxel
weights of each SCP into Z maps, computed as the ratio of voxel
weight and bootstrap SD. The Z-statistic follows roughly a stan-
dard normal distribution where a one-tailed p ≤ 0.05 iners
a threshold of |Z| ≥ 1.64.18

**Statistical analyses.** Continuous variables were tested for normality
using visual inspection of histograms and Shapiro-Wilk test.
Demographic, clinical, and imaging measures were assessed, where
applicable, using parametric (analysis of variance) and nonparametric
χ2 tests. Correlations were performed using Pearson r coefficients.
Statistical tests were interpreted as significant if $p < 0.05$. Data analysis used the Statistical Package for Social Sciences (Chicago, IL) software (SPSS version 22.0; http://www-01.ibm.com/software/analytics/spss/products/statistics/).

### RESULTS

Demographics and clinical characteristics. Table 1 shows demographic and clinical characteristics of the study sample. Groups were similar in age and sex, while as expected, all other variables differed ($p < 0.001$).

**Spatial covariance analysis.** The SCP$_{QNB}$ that distinguished PDD from controls is shown in figure 1, A and B. SSF$_{QNB}$ scores were higher in PDD than controls (mean ± SD; controls = 1.5 ± 2.5, PDD = 6.2 ± 3.3, $F_{1,47} = 31.9, p < 0.001$; figure 1C). The pattern was mainly characterized by concomitant decreases in M1/M4 binding (blue regions) in basal forebrain, temporal, striatal, insula, and anterior cingulate together with concomitant preserved or increases (red regions) in frontal and parieto-occipital areas. Table e-1 on the Neurology® Web site at Neurology.org presents detailed description of specific regions contributing to the M1/M4 disease-related pattern.

The associated SCP$_{CBF}$ that differentiated PDD from controls is illustrated in figure 2, A and B, where SSF$_{CBF}$ scores differed between groups (controls = 0.4 ± 0.9, PDD = 6.2 ± 1.9, $F_{1,47} = 177.5, p < 0.001$; figure 2C). The pattern mainly comprised relative decreased rCBF (blue) in temporoparietal and

<table>
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<tr>
<th>Table 1 Participant characteristics</th>
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<tr>
<td><strong>Controls</strong></td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>M:F</td>
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<td>Age, y</td>
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<tr>
<td>MMSE</td>
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<tr>
<td>CAMCOG</td>
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<tr>
<td>CAMCOG$_{memory}$</td>
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<td>CAMCOG$_{exec}$</td>
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<tr>
<td>NPI</td>
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<tr>
<td>NPI$_{hall}$</td>
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<tr>
<td>UPDRS III</td>
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</tbody>
</table>

Abbreviations: CAMCOG = Cambridge Cognitive Examination; CAMCOG$_{exec}$ = Cambridge Cognitive Examination executive function subscale; CAMCOG$_{memory}$ = Cambridge Cognitive Examination memory subscale; MMSE = Mini-Mental State Examination; NA = not applicable; NPI$_{hall}$ = Neuropsychiatric Inventory hallucinations subscale; PDD = Parkinson disease dementia; UPDRS = Unified Parkinson’s Disease Rating Scale.

Values denote mean ± 1 SD.

Figure 1  
**Muscarinic M1/M4 spatial covariance pattern in Parkinson disease dementia (PDD)**

Disease-related M1/M4 spatial covariance pattern in PDD projected onto orthogonal (A) and rendered (B) displays of the QNB template. Distribution of subject scaling factor (SSF$_{QNB}$) scores across groups (C). Ant = anterior; Pos = posterior; Sup = superior.
prefrontal areas with relative increases (red) in cerebellum, brainstem, striatothalamic, and motor regions. Detailed description of specific regions participating in the rCBF disease-related pattern is provided (table e-2).

Relationships between SCP expressions and age, MMSE, CAMCOG, CAMCOG_memory, CAMCOG_exec, total NPI, NPI hallucinations subscale, and Unified Parkinson's Disease Rating Scale III were investigated in PDD. No correlations were found between SSF_QNB and these measures ($|r| \leq 0.23$, $p \geq 0.14$). For the rCBF pattern expression, total NPI correlated with SSF_rCBF ($p = 0.62$, $p = 0.006$), which was not observed for the other variables ($|r| \leq 0.28$, $p \geq 0.09$). An exploratory examination of NPI subscores did not yield any specific relationships with SCP expressions.

Summary data for the donepezil-treated group are shown (table 2). During the observation period, differences in MMSE were identified between baseline and 12-week scores ($p < 0.001$). The pattern consists of concurrent decreases in M1/M4 binding (blue) in fusiform, anterior cingulate, lingual gyrus, and precentral areas with concurrent preserved or increases (red) in pre/medial/orbitofrontal, parietal, and posterior cingulate regions.

**Table 2** Summary data of patients with PDD treated with donepezil

<table>
<thead>
<tr>
<th>PDDdonepezil</th>
<th>Statistic, $p$ value</th>
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<tbody>
<tr>
<td>No.</td>
<td>18</td>
</tr>
<tr>
<td>M/F</td>
<td>11:7</td>
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<tr>
<td>Age</td>
<td>71.6 ± 4.6</td>
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<tr>
<td>MMSE&lt;sub&gt;b&lt;/sub&gt;</td>
<td>18.1 ± 4.3</td>
</tr>
<tr>
<td>MMSE&lt;sub&gt;12w&lt;/sub&gt;</td>
<td>22.4 ± 4.7</td>
</tr>
<tr>
<td>(MMSE&lt;sub&gt;12w&lt;/sub&gt; − MMSE&lt;sub&gt;b&lt;/sub&gt;)</td>
<td>4.3 ± 3.8, $t_{17} = 4.8, &lt;0.001$</td>
</tr>
<tr>
<td>$\Delta$MMSE&lt;sub&gt;rel,b&lt;/sub&gt; (%)</td>
<td>26.8 ± 24.6</td>
</tr>
</tbody>
</table>

Abbreviations: 12w – at 12 weeks; b – at baseline; MMSE – Mini-Mental State Examination; PDD – Parkinson disease dementia. Values denote mean ± 1 SD.

$\Delta$MMSE<sub>rel,b</sub> (%) = ((MMSE<sub>12w</sub> − MMSE<sub>b</sub>)/MMSE<sub>b</sub>) × 100%.
Details of specific regions participating in the pattern are supplied (table e-3).

DISCUSSION We undertook a multivariate network perspective of (R, R) $^{123}$I-QNB SPECT, a M1/M4 receptor ligand in ChEI-naive patients with PDD. We derived a disease-related M1/M4 pattern of spatial covariance that appears largely distinct from rCBF, which implies the presence of several dysfunctional cholinergic networks in PDD. We also identified a clear M1/M4 covariance pattern that was associated with an improvement in MMSE; this network had distinctive spatial elements suggesting certain cortical regions and their associated cholinergic innervation may have a more preeminent role in cognitive amelioration by cholinergic treatments. Relevant to the present study, this spatial covariance technique has extensively and successfully been utilized in perfusion SPECT and glucose metabolism PET data for the investigation of disease progression and symptomatology in PD.21–22

We derived a voxel cholinergic SCP from $^{123}$I-QNB images that differentiated PDD from controls. The disease-related pattern comprised decreased and preserved/increased M1/M4 uptake in a number of concomitant brain regions or networks. The covariant negative-weighted pattern mainly converged on limbic/paralimbic regions. Notably, this cholinergic receptor network mapped onto previously described resting-state networks, including anterior insula and the anterior cingulate, key nodes of the salience network (SN), which is important for initiation of cognitive control and switching between networks to aid access to working memory and attention resources.23,24 Networks involving the insula have also been shown to play a role in episodic memory,25 while hippocampus, parahippocampus, and amygdala are known to be involved in memory storage and retrieval.26 As such, this pattern would align with a cognitive network deficit implicating the basal forebrain and these structures, i.e., a cholinergic limbic-paralimbic/SN dysfunction. The disease-related pattern also encompassed regions implicated in dorsal (occipital → parietal) and ventral (occipital → temporal → limbic) visual streams,27 providing indirect evidence for the role of distinct cholinergic networks in visual function in PDD, which would be in keeping with known visuoperceptual deficits and predisposition...
to visual hallucinations, symptoms that both show good response to cholinesterase inhibitors. The associated rCBF pattern largely comprised relative decreases in temporoparietal and prefrontal areas along with relative increases in cerebellum, brainstem, striatothalamic, and motor regions that implicate a number of functional networks in PDD. Regions that were concomitantly reduced appear to involve hubs of the frontoparietal attention (inferior parietal, dorsolateral prefrontal cortex) and default mode networks (DMNs) (medial prefrontal, posterior cingulate, ventral precuneus, inferior parietal), which is of interest since, respectively, attention deficits are one of the most disabling cognitive symptoms in PDD, while network theories have strongly implicated the DMN in contributing to cognitive decline. Our previous studies revealed modulation of the frontoparietal network in PDD that was similar to patients with dementia with Lewy bodies (DLB), while also demonstrating, albeit in DLB, its relationship with severity and frequency of cognitive fluctuations. Other investigations have reported decreases in DMN connectivity in PDD and its association with cognitive dysfunction in PD. These and our rCBF findings appear to provide further evidence that implicate the DMN and frontoparietal networks in the pathogenesis of symptoms in PDD, in particular cognitive. Moreover, perhaps not unexpectedly, the rCBF pattern seemed to represent an extended topography of the PD-related motor and cognitive patterns, which have been previously reported from PET studies using similar network approaches, thus indirectly validating the analytic methodology used in the present study.

We failed to detect any correlations between the M1/M4 pattern expressions and neuropsychological and neuropsychiatric measures in PDD. For rCBF pattern expression, only total NPI score was found to correlate. Thus, patients with more global severe neuropsychiatric symptoms, a marker of greater disease severity, were more likely to express the perfusion SCP characteristic of PDD. The lack of correlations may be explained by either the notion that each spatial covariance pattern is likely to characterize a number of overlapping and convergent brain networks and thus fails to project on specific cognitive and clinical parameters or that patterns derived from combined (control–dementia) cohorts are less sensitive. Isolating key networks from these patterns could increase sensitivity, but this is methodologically challenging.

We found a clear M1/M4 covariance pattern that correlated with a change in MMSE that could indicate a positive treatment response. This pattern showed relative decreases in fusiform, striatum, anterior cingulate, lingual gyrus, and precentral areas with relative preservation or increase in prefrontal, parietal, and posterior cingulate regions. From a network perspective, there was covariant preservation/upregulation in regions overlapping key nodes of the DMN and frontoparietal networks that could imply that a relative cholinergic maintenance of these networks is prerequisite for ChEI treatment response in PDD, and more generally may point toward the potential relevance of these networks and their cholinergic innervation and its associated cognitive symptoms. Notably, a recent study showed that cholinergic and serotonergic antagonists can impair DMN-like network in mice similarly, suggesting that both neurotransmitter systems are involved in maintaining the integrity of the DMN-like networks. Hence, this pattern appears to provide some evidence that supports the cholinergic DMN maintenance hypothesis, and its potential significance as a predictor of positive treatment response in PDD and perhaps in other neurodegenerative disorders.

Author Contributions
Dr. Colloby: co-designed the study, conducted all image and data analyses, and wrote the manuscript. Professor McKeith: reviewed the manuscript and secured project funding. Professor Burn: reviewed the manuscript and secured project funding. Professor Wyper: reviewed the manuscript and secured project funding. Professor O’Brien: reviewed
the manuscript and secured project funding. Dr. Taylor: co-designed the study and cowrote the manuscript.

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DISCLOSURE
S. Colloby reports no disclosures relevant to the manuscript. I. McKeith has been a consultant for GE Healthcare, Bayer Health care, and Nutricia. D. Burn has received an honorarium from Acadia. D. Wryper reports no disclosures relevant to the manuscript. J. O'Brien has been a consultant for GE Healthcare, Lilly, Bayer Healthcare, TauRx, and Nutricia and has received honoraria for talks from GE Healthcare, Lilly, and Novartis. J. Taylor has been a consultant of Lundbeck and received honoraria for talks from GE Healthcare and Flynn Pharmaceuticals. Go to Neurology.org for full disclosures.

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