

Loss of phosphodiesterase 4 in Parkinson's disease: Relevance to cognitive deficits

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Abbreviations: LED=levodopa equivalent dose; PDE4=phosphodiesterase 4; PDQ-39=39-item Parkinson's disease Questionnaire; UPDRS= Unified Parkinson's Disease Rating Scale; V_T =volume of distribution.

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generated the figures. H.W., C.C and G.E.S analysed the data. F.N., M.P., and T.F. interpreted the data. F.N., T.F. and G.P. recruited the participants. All authors revised and gave input to the manuscript.

Abstract

Objective: To assess *in vivo* the expression of phosphodiesterase 4 (PDE4) and its relevance to cognitive symptoms in Parkinson's disease (PD) patients using [^{11}C]rolipram PET.

Methods: We studied 12 levodopa-treated PD patients with no concurrent diagnosis of mild cognitive impairment or dementia. Their data were compared to those from 12 healthy controls. All participants underwent neuropsychiatric and cognitive assessment using the Cambridge Neuropsychological Test Automated Battery (CANTAB[®]). Parametric images of [^{11}C]rolipram volume of distribution (V_T) values were determined with the Logan plot.

Results: PD patients performed worse than healthy controls in cognitive examinations assessing psychomotor speed, episodic memory, and spatial working memory and executive function. PD patients showed reductions in [^{11}C]rolipram V_T compared to healthy controls, in the caudate (28%), thalamus (23%), hypothalamus (32%) and in the cortex (16%). Within thalamic sub-regions [^{11}C]rolipram V_T values in PD patients were decreased by 12-32% with most marked decreases observed in prefrontal and temporal thalamic nuclei whereas motor nuclei were the less affected. Within the cortex [^{11}C]rolipram V_T values in PD patients were decreased by 11-20% with most marked decreases observed in posterior dorsolateral frontal cortex, medial frontal cortex and supplementary motor area, whereas orbitofrontal cortex was less

affected. Worse performance in spatial working memory correlated with lower [^{11}C]rolipram V_T values in posterior dorsolateral frontal cortex, medial frontal cortex, supplementary motor area, precentral gyrus, caudate, and prefrontal thalamic nuclei.

Conclusions: Our findings demonstrate loss of PDE4 expression in the striato-thalamo-cortical circuit, which is associated with deficits of spatial working memory in PD patients.

Introduction

Patients with Parkinson's disease (PD) carry a six-fold increased risk of developing dementia compared to the general population (1). Spatial working memory is an executive function that typically is impaired early in the course of PD (2, 3). The Parkinson Associated Risk Syndrome (PARS) study has shown impaired working memory in individuals at risk for developing PD suggesting that these subdomain cognitive deficits may be part of the PD premotor stage (4).

Phosphodiesterase 4 (PDE4) is an intracellular enzyme expressed in neurons and glial cells, where it hydrolyses cAMP (5). PDE4 regulates the cAMP–PKA–cAMP response element binding protein (CREB) pathway, and modulates the transcription of proteins involved in synaptic plasticity and memory process (6). Long-lasting plasticity and memory deficits can be caused by abnormal and sustained activation of cAMP/PKA signalling (7). Disinhibition of cAMP/PKA signalling occurs in the prefrontal cortex of aged rats and monkeys with working memory deficits suggesting a key role of cAMP/PKA pathways in the modulation of executive dysfunction (7).

Here, we investigated *in vivo* the expression of PDE4 and its relevance to cognitive deficits in PD patients, using PET with [¹¹C]rolipram, which is a selective PDE4 radioligand for human use (8). As age-related reduction in PDE4 expression in healthy individuals older than 55 years old has not been investigated, we also explored the influence of age in our cohort of healthy controls.

Our findings suggest loss of PDE4 expression in striato-thalamo-cortical network in patients with PD, which is associated with working memory deficits.

Materials and methods

Participants and clinical characteristics

Twelve patients with a diagnosis of idiopathic PD according to the Queen Square Brain Bank criteria were recruited from specialist Movement Disorders clinics at the National Hospital of Neurology & Neurosurgery, Queen Square, London (Table 1). None of these patients fulfilled the diagnostic criteria of PD mild cognitive impairment or PD dementia (9, 10).

Twelve healthy individuals with no history of neurological or psychiatric disorders were recruited as control group. To assess the effect of age on PDE4 expression, we studied a younger (n=6; 5 males; mean age \pm SD=39.0 \pm 8.4; age range=26-52 years old) and an older (n=6; 5 males; mean age \pm SD= 66.3 \pm 3.6; age range=53-78 years old) subgroup of healthy controls.

All participants had no history of other neurological or psychiatric disorders, were not taking substances with known actions in PDEs (e.g. apremilast, cilomilast, luteolin,

piclamilast, roflumilast and ibudilast), and had no contraindications to undertake PET and MRI scanning according to scanning safety criteria (<http://www.mrisafety.com>; <https://www.gov.uk/government/publications/arsac-notes-for-guidance>).

PD patients were all on levodopa treatment. Daily and lifetime dopaminergic medication dose was calculated with a formula based on the theoretical equivalence to levodopa (11). Motor symptom severity was assessed with the Unified PD Rating Scale part-III (UPDRS-III) and according to Hoehn & Yahr stage. Motor assessments were performed OFF medication after overnight withdrawal of patient's dopaminergic medications. Neuropsychiatric symptoms were assessed with the Apathy Evaluation Scale, Beck Depression Inventory-II (BDI-II), and the Hamilton Depression Rating Scale (HDRS). Non-motor Symptoms Scale for PD was used to assess non-motor symptoms. The Mini Mental Status Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were used to assess general cognitive status. Further cognitive assessments were carried out using the CANTAB[®] and included assessments related to psychomotor speed (Reaction Time), attention (Rapid Visual Information Processing), episodic memory (Paired Associate Learning and Delayed Match to Sample), working memory and executive function (Spatial Working Memory; Supplemental Material). CANTAB[®] neuropsychological tests have been previously described in detail (12) and shown to be highly sensitive to deterioration in executive function in PD (13). Quality of life was measured with the patient self-reported 39-item PD Questionnaire (PDQ-39).

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the institutional review boards and the research ethics committee. Written informed consent was obtained from all study participants in accordance with the Declaration of Helsinki.

Imaging data analysis

MRI-based volumetric analysis

Since PDE4 is an intracellular enzyme, neuronal loss may affect PDE4 availability as measured with PET. We investigated volumetric changes in cortical and subcortical nuclei regions in our cohort of PD patients compared to all healthy controls. FreeSurfer image analysis suite (version 5.3.0 <http://surfer.nmr.mgh.harvard.edu>) was used to process individual MRI scans, to derive measures of cortical thickness and subcortical nuclei volumes (Supplemental methods).

[¹¹C]rolipram PET data analysis

The Molecular Imaging and Kinetic Analysis Toolbox software package (MIAKATTM; www.miakat.org; 14), implemented in MATLAB[®] (The Mathworks, Natick, MA, USA) was used to carry out image processing and kinetic modelling. MIAKATTM combines in-house code with wrappers for FMRIB Software Library (FSL, <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>) and Statistical Parametric Mapping (SPM, <http://www.fil.ion.ucl.ac.uk/spm/>) commands in order to provide state-of-the-art functionality within a coherent analysis framework. The MIAKATTM processing pipeline was followed, ensuring that all quality control steps were completed to generate both parametric images and regional estimates of [¹¹C]rolipram volume of distribution (V_T; Supplemental methods). The anatomical CIC atlas version 2.0 (15) was used to define regions of interest (ROIs).

Statistical analysis

Statistical analysis and graph illustration were performed with SPSS (version 20 Chicago, Illinois, USA) and GraphPad Prism (version 6.0c) for MAC OS X, respectively. For all variables, variance homogeneity and Gaussianity were tested with Bartlett and Kolmogorov-Smirnov tests. Two-tailed t-test (parametric variables) and Mann-Whitney U test (non-parametric variables) were used for between-group comparisons, as appropriate. P values for each variable were calculated following Benjamini-Hochberg multiple-comparisons test in order to reduce false discovery rate. We set the false discovery rate cut-off at 0.05. We interrogated correlations between PET and clinical data using Spearman's r correlation coefficient and we applied the Benjamini-Hochberg correction. All data are presented as mean \pm SD, and the level α was set for all comparisons at $P<0.05$, Benjamini-Hochberg corrected.

Results

Cognitive and neuropsychiatric function

PD patients performed worse than healthy controls in the assessments of psychomotor speed [five choice median reaction time ($P_{uncor}=0.014$; $P_{adj}=0.012$) and median movement time ($P_{uncor}<0.001$; $P_{adj}<0.001$)], episodic memory [delayed match to sample % correct ($P_{uncor}=0.003$; $P_{adj}=0.006$)], and working memory and executive function [spatial working memory between errors ($P_{uncor}=0.016$; $P_{adj}=0.031$)] (Table e-1).

Neuropsychiatric assessments showed significant differences between healthy controls and PD patients (BDI-II: $P < 0.05$; HDRS: $P < 0.05$); however, the neuropsychiatric burden in our cohort of PD patients was minimal and did not meet the cut-off score for depression.

Volumetric analysis

Freesurfer analysis showed no volumetric differences in cortical and subcortical nuclei ROIs between the groups of PD patients and all healthy controls (Table e-2). Moreover, no significant associations were observed between cortical and subcortical volumes and [^{11}C]rolipram V_T for the whole brain analysis in the group of healthy controls ($P > 0.10$ for all brain regions).

Effect of age on PDE4 expression

We found no significant differences in cortical and subcortical nuclei [^{11}C]rolipram V_T between the group of younger and older healthy control subgroups (all $P > 0.10$; Table e-3). Moreover, in the group of healthy controls there were no significant associations between age and [^{11}C]rolipram V_T for any brain region ($P > 0.18$ for all brain regions).

Effect of medication on PDE4 expression

We did not find any interactions between daily and lifetime levodopa equivalent dose measured in total or for levodopa or dopamine agonist medications separately, and [^{11}C]rolipram V_T in all the regions of interested examined ($P > 0.10$).

PDE4 expression in Parkinson's patients

We found 5-32% decreases in [^{11}C]rolipram V_T in PD patients compared to the group of healthy controls. PD patients had significantly lower mean [^{11}C]rolipram V_T in subcortical nuclei (Table [e-4](#); Figures 1A, 2 and 3) and frontal cortex regions (Table [e-4](#); Figures 1B, 2 and 3). Although [^{11}C]rolipram V_T was lower in all cortical regions, there were no significant differences in [^{11}C]rolipram V_T between the patients with PD and healthy controls in parietal, temporal and occipital cortices (all $P>0.10$).

PD patients had significantly lower mean [^{11}C]rolipram V_T in the caudate, accumbens, thalamus and hypothalamus compared to healthy controls, with no significant changes observed in putamen, globus pallidus and substantia nigra (Table [e-4](#); Figures 1A, 2 and 3). Within the thalamus, loss of [^{11}C]rolipram V_T was driven by prefrontal, temporal, posterior parietal and primary sensory thalamic nuclei, with no significant changes in motor thalamic nuclei in PD patients compared to healthy controls (Table [e-4](#); Figures 1A, 2 and 3).

In the frontal cortex, [^{11}C]rolipram V_T was decreased in the precentral gyrus, dorsolateral frontal cortex, posterior dorsolateral frontal cortex, medial frontal cortex including anterior medial frontal cortex and posterior medial frontal cortex, central and frontal operculum and supplementary motor area (Table [e-4](#); Figures 1B, 2 and 3).

Correlations: PDE4 expression and Spatial Working Memory

In PD patients, higher number of errors in the spatial working memory test was associated with lower [^{11}C]rolipram V_T in the precentral gyrus ($r_s=-0.59$; $P_{uncor}=0.035$; $P_{adj}=0.045$), posterior dorsolateral frontal cortex ($r_s=-0.62$;

$P_{uncor}=0.032$; $P_{adj}=0.045$), medial frontal cortex ($r_s=-0.63$; $P_{uncor}=0.030$; $P_{adj}=0.045$), posterior medial frontal cortex ($r_s=-0.74$; $P_{uncor}=0.006$; $P_{adj}=0.036$), supplementary motor area ($r_s=-0.69$; $P_{uncor}=0.012$; $P_{adj}=0.036$), caudate ($r_s=-0.60$; $P_{uncor}=0.035$; $P_{adj}=0.045$), precommissural caudate ($r_s=-0.70$; $P_{uncor}=0.011$; $P_{adj}=0.036$), and prefrontal thalamic nuclei ($r_s=-0.60$; $P_{uncor}=0.039$; $P_{adj}=0.045$) (Figure 1C). We did not find any correlations between frontal cortex nor subcortical nuclei [^{11}C]rolipram V_T and motor, neuropsychiatric and cognitive measures of psychomotor speed and episodic memory (all $P>0.10$). We also did not find any associations between [^{11}C]rolipram V_T in all regions examined and disease duration (all $P>0.05$).

Discussion

Using [^{11}C]rolipram PET molecular imaging *in vivo*, we report loss of PDE4 expression within the striato-thalamo-cortical brain circuitry of PD patients, which is associated with spatial working memory deficits. Our findings demonstrate 16-32% loss of PDE4 expression in the striatum, thalamus, hypothalamus and frontal cortex of PD patients who had episodic and working memory deficits, were on dopamine-replacement therapy, but had no significant brain atrophy in the regions of interest examined.

PDE4 expression was decreased by 12-32% within the thalamus with most marked decreases observed in prefrontal and temporal lobe-projecting thalamic nuclei, whereas motor nuclei were less affected. Within the cortex PDE4 expression in PD patients were decreased by 11-20% with most marked decreases observed in the posterior dorsolateral frontal cortex, medial frontal cortex and supplementary motor area, whereas the orbitofrontal cortex and frontal operculum were the least affected.

We found lower PDE4 expression also in occipital regions but this did not reach the statistical significance probably due to the high inter-subject variability in [¹¹C]rolipram V_T observed in these cortical regions.

We found no differences in cortical and subcortical volumetric measures between PD patients and healthy controls. It is possible that the group comparisons were insufficiently powered to detect significant differences in cortical and subcortical volumetric measures between the PD patients and the healthy controls. Moreover, our group of PD patients included early and advanced stages of the disease with some PD patients showing motor complications such as levodopa-induced dyskinesias (LIDs). Although cortical thinning has been associated with longer PD duration (16), PD patients with LIDs exhibited increased cortical thickness in the frontal cortex (17) and subcortical nuclei (18). This could have also contributed to the lack of significant volumetric changes observed in our cohort of PD patients. We also acknowledge as limitation of this study that ROIs [¹¹C]rolipram PET analysis was carried out using the anatomical CIC atlas whereas cortical and subcortical volumetric changes were assessed with Freesurfer, which could have contributed to some differences in terms of regions.

There are no previous clinical or preclinical studies investigating the expression of PDE4 in PD. Different lines of evidence in experimental animals confirm a clear role for PDE4 in modulating cognition, including working memory. PDE4 knockout mice show enhanced long-term depression and impaired long-term memory in a fear-conditioning paradigm (19), and impaired spatial working memory (20). In animal models of Huntington's disease, decreased PDE4 protein levels and PKA hyper-activation in the hippocampus were associated with spatial memory deficits,

suggesting that PDE4-dependent regulation of cAMP/PKA signalling cascade may be one molecular mechanism underlying cognitive decline (21). In mice, intraperitoneal administration of the PDE-4 inhibitor rolipram enhanced spatial working memory consolidation in the Morris water maze task (22). In rodents, rolipram has been shown to improve working memory deficits caused by administration of scopolamine, a muscarinic receptor antagonist (23) and MK-801, a NMDA antagonist (24). Treatment with PDE4 inhibitors also ameliorated spatial working memory deficits in transgenic mouse model of Alzheimer's disease (25). PDE4 inhibitors related-increases in cAMP signals lead to memory enhancements, whereas increases in basal cAMP levels due to PDE4 loss lead to memory impairments (26). This might explain why PDE4 inhibitors are beneficial in improving cognitive deficits in rodents.

In this study we found significant associations between loss of PDE4 expression in the caudate, thalamic nuclei and frontal cortices, and spatial working memory deficits in PD patients. The striato-thalamo-cortical circuit has been closely linked to spatial working memory. The dorsolateral prefrontal cortex is part of the network mediating spatial working memory processes (27). The medial frontal cortex plays an important role in performance monitoring on subsequent trials and in the implementation of associated adjustments in cognitive control (28). Neuroimaging studies have shown a close interaction between the posterior medial frontal cortex and dorsolateral prefrontal cortex (28). While the posterior medial frontal cortex controls for errors and sends the signal for adjustments, the dorsolateral prefrontal cortex in turn implements the necessary top-down control (28). This functional interplay is also mediated by the supplementary motor area, which plays a role in spatial rehearsal (29), and other subcortical structures such as the caudate and thalamic nuclei. Within

the caudate, the precommisural dorsal caudate is the area of the striatum that receives the largest corticostriatal projections from the dorsolateral prefrontal cortex and is involved in spatial memory processes (30). Output from the basal ganglia projects to the thalamus which closes the circuit by projecting back to the dorsolateral frontal cortex (31). Our findings show that, within the thalamus, loss of PDE4 expression only in the subregions connected to the prefrontal cortex is associated with spatial working memory deficits in patients with PD.

Cognitive impairment is a very common feature of PD, affecting up to 57% of patients within the first 3–5 years after PD diagnosis, and adds significantly to patients and carers' burden (32). Spatial working memory is affected early in the course of the disease and may represent one of the pre-motor symptoms of PD. Recent evidence has shown that healthy people over the age of 50 who are at risk of developing PD (exhibiting hyposmia and dopamine transporter reduction) performed worse at cognitive tests assessing spatial working memory (4). Previous functional MR and H₂¹⁵O PET molecular imaging studies have shown that blood flow is reduced in the fronto-striatal circuit while performing working memory tasks in PD patients with and without cognitive impairment (3, 33, 34). In agreement with these findings, we found significant correlations between worse performance in the spatial working memory tests and decreases in PDE4 expression in precentral gyrus, dorsolateral prefrontal cortex, medial frontal cortex and precommisural dorsal caudate.

We have recently shown that another PDE, PDE10A, is decreased by 14–28% in the striatum and pallidum of moderate/advanced PD patients and loss of PDE10A expression is associated with the severity of motor symptoms and complications (35). In comparison with PDE10A, which is mainly expressed in the basal ganglia, PDE4 is

widely expressed in the cortex and subcortical regions intimately involved in cognitive processes (5). Hereby, it is not surprising that we did not find significant decreases in PDE4 expression in subcortical nuclei specifically involved in the control of movement such as putamen and pallidum. Taking together our previous and recent findings, it is likely that while PDE10A plays a role in the control of movement, PDE4 is involved in the regulation of cognitive processes and both enzymes are decreased in PD patients leading to the manifestation of motor and cognitive symptoms.

We did not find correlations between PDE4 expression and disease duration. This may be explained by the wide cross-section in disease duration ranging from three to 25 years of PD duration and the small number of participants. Further longitudinal studies investigating PDE4 expression in a larger cohort of PD patients spanning from the *de novo* to advanced stages are needed to elucidate the role of this enzyme in the course of the disease.

The PDE4 family is comprised of four isoforms: PDE4A, B, C and D which encode for distinct proteins (8). Preclinical studies have shown that distinct subtypes of PDE4 isoforms differentially modulate synaptic activity and have distinct neurological role (19, 20). PDE4B modulates long-term depression and loss of this isoform causes impaired spatial working memory (20), whereas PDE4D regulates long-term potentiation and loss of this isoform leads to impaired fear conditioning in mice (19). [¹¹C]rolipram binds with high affinity to all four PDE4 isoforms (8), thus it was not possible to investigate in humans the different role of PDE4 subtypes.

In our study we also investigated the effect of age on PDE4 expression in a cohort of healthy controls. Preclinical studies have shown age-related reduction of PDE4 expression in the striatum and cortex of rodents and non-human primates (36, 37). In the striatal and cortical brain tissue of aged rats (100 week old), [³H]rolipram binding was decreased by 58-69% compared to young rats (10 week old) (36), and [¹¹C]rolipram binding was decreased by 20-25% in the striatum and frontal cortex of aged monkeys (37). However, more recent work indicated that age does not affect [¹¹C]rolipram V_T in cortical and subcortical regions of healthy controls and patients with major depressive disorder aged between 18 to 55 years old (38). Our findings are in agreement with the previous study in humans, and we report no age-related reduction in cortical and subcortical PDE4 expression in healthy controls spanning between 26 to 78 years of age. However, it is possible that the small number of healthy controls in each group studied could not reach statistical significance and the PDE4 age-related changes observed in rodents might be explained by heterogeneity of PDE4 isoforms in different species. Further studies evaluating PD4 expression in larger cohort of healthy controls spanning from 20 to 80 years of age are needed to clarify the effect of age on PDE4 expression.

Our findings show *in vivo* a novel neurochemical change in PD, which is associated with working memory deficits. Novel PDE4 modulating drugs could potentially improve cognitive symptoms in PD.

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FIGURE LEGENDS

Fig. 1 PDE4 expression in the groups of Parkinson's disease patients and healthy controls. Column bar graphs showing mean ^{11}C rolipram volume of distribution (V_T) in (A) subcortical and (B) cortical brain regions between PD patients and healthy controls. (C) Correlations between loss of PDE4 expression and spatial working memory deficits in patients with PD. Higher number of errors at the spatial working memory test was associated with lower ^{11}C rolipram V_T in precentral gyrus, posterior dorsolateral frontal cortex, medial frontal cortex, posterior medial frontal cortex, supplementary motor area, caudate, precommissural caudate, and prefrontal thalamic nuclei. Colour bar reflects range of ^{11}C rolipram V_T intensity. Error bars represent mean \pm SD. * $P < 0.05$. All P values are Benjamini-Hochberg corrected for multiple comparisons.

Fig. 2 Mean cortical and subcortical loss of PDE4 expression in Parkinson's disease patients. Axial, coronal and sagittal (MNI co-ordinates: $x = 19$, $y = -8$, $z = 4$) mean summed ^{11}C rolipram PET images derived from (A) 12 healthy controls and (B) 12 PD patients in stereotaxic space showing significant loss of ^{11}C rolipram volume of

distribution (V_T) in the PD patients. Colour bar reflects range of [^{11}C]rolipram V_T intensity.

Fig. 3 Cortical and subcortical loss of PDE4 expression in a Parkinson's disease patient. Axial summed [^{11}C]rolipram PET images for (A) a 69 year-old healthy female (MMSE: 30; MoCA: 30; SWM errors: 8) and (B) a 72 year-old female with a seven years history of PD (H&Y: 2; UPDRS-III: 37; MMSE: 27; MoCA: 29; SWM errors: 26). Colour bar reflects range of [^{11}C]rolipram volume of distribution (V_T) intensity.

TABLE

Table 1 Clinical characteristics of Parkinson's disease patients

Subject	Sex	Age (years)	Disease duration ¹	PD medication duration (years)	Daily LED (mg) ²	Hoehn and Yahr	PDQ- 39
1	M	73.0	9	7.0	605	3	56
2	F	68.8	7	0.9	560	3	18
3	F	58.5	3	12.0	150	1	6
4	M	76.8	7	5.0	150	2	14
5	M	73.3	10	9.0	400	3	36
6	M	76.5	9	7.0	1229	2	15
7	F	80.0	25	21.0	1383	4	75
8	F	72.6	7	5.0	640	2	55

9	M	73.4	17	13.0	4236	4	37
10	M	74.6	13	11.0	730	2	9
11	M	70.5	10	9.0	4149.5	4	44
12	F	54.1	18	15.0	797	2.5	48
Mean	7M/ 5F	71.0	11.25	9.58	1252.46	2.71	34.42
±SD		(±7.5)	(±6.1)	(±5.3)	(±1421)	(±1.0)	(±22)

¹From time of first appearance of PD motor symptoms; ²LED: Levodopa Equivalent Dose.