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DOI:

[10.1212/WNL.0000000000004201](https://doi.org/10.1212/WNL.0000000000004201)

Document Version

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Citation for published version (APA):

Niccolini, F., Wilson, H., Pagano, G., Coello, C., Mehta, M. A., Searle, G. E., Gunn, R. N., Rabiner, E. A., Foltynie, T., & Politis, M. (2017). Loss of phosphodiesterase 4 in Parkinson disease: Relevance to cognitive deficits. *Neurology*, 89(6), 586-593. <https://doi.org/10.1212/WNL.0000000000004201>

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

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Study funding: Supported by Edmond J. Safra Foundation and the NIHR Mental Health Biomedical Research Centre at South London and Maudsley NHS Foundation.

Statistical analysis was performed at the Department of Basic & Clinical Neuroscience, King's College London.

Potential Conflicts of Interest: The authors declare no conflict of interest.

Word count: Title=83 characters with space; Abstract=250 words; Manuscript=2,994.

No of References: 40; No of Tables: 2; No of Figures: 3.

Supplemental data: Supplemental methods, Supplemental table 1, 2 and 3.

Keywords: Parkinson's disease; PDE4; PET; rolipram; working memory; cognitive impairment

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.

Author contributions: M.P. conceived the study, conceptualized the experimental design and acquired funding for the study. F.N., E.A.R., R.N.G., T.F. and M.A.M gave input to experimental design. F.N. and G.P performed the imaging and clinical assessments and acquired the data. F.N., G.P., E.A.R. and M.P. organised the study. F.N. and M.P. wrote the first draft and prepared the manuscript. F.N. and G.E.S. 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 subjects. 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 molecular imaging.

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 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, and it is estimated that up to 80% of PD patients will develop cognitive impairment over the course of their illness (1). Deficits in executive functions can occur early in *de-novo* patients with PD without a formal diagnosis of cognitive impairment or even before the development of overt motor symptoms (2).

Spatial working memory is an executive function that typically is impaired early in the course of PD (3, 4). 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 (5).

Phosphodiesterase 4 (PDE4) is an intracellular enzyme widely expressed in neurons and glial cells, where it hydrolyses cyclic adenosine monophosphate (cAMP) (6, 7). PDE4 regulates the cAMP–protein kinase A (PKA)–cAMP response element binding protein (CREB) pathway, and modulates the transcription of proteins involved in synaptic plasticity and memory process (8). Aberrant and sustained activation of cAMP/PKA signalling can lead to long-lasting plasticity and memory deficits (9). 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 (9).

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 (10), and impaired spatial working memory (11). Reduced PDE4 protein levels in the hippocampus along with hyperactivity of the PKA pathway has been associated with cognitive decline in a rodent model of Huntington's disease (12). In mice, intraperitoneal administration of the PDE-4 inhibitor rolipram enhanced spatial working memory consolidation in the Morris water maze task (13). In rodents, rolipram has been shown to improve working memory deficits caused by administration of scopolamine, a muscarinic receptor antagonist (14) and MK-801, a NMDA antagonist (15). Treatment with PDE4 inhibitors also ameliorated spatial working memory deficits in transgenic mouse model of Alzheimer's disease (16).

Here, we investigated *in vivo* the expression of PDE4 and its relevance to cognitive deficits in PD patients, using PET with [^{11}C]rolipram, which is a selective PDE4 radioligand for human use (17).

Age-related reduction of PDE4 has been previously reported *in vitro* in the cortical regions of rodents (18) and *in vivo* in the striatum and frontal cortex of non-human primates (19). However, in humans a [^{11}C]rolipram PET study has shown that age does not affect PDE4 expression in cortical and subcortical regions of healthy controls and patients with major depressive disorder between 18 to 55 years of age (20). As age-related reduction in PDE4 expression in healthy people 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 (21, 22).

Twelve healthy individuals with no history of neurological or psychiatric disorders served as the 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 screened successfully to undertake PET and MRI scanning under scanning safety criteria (<http://www.mrisafety.com>; <https://www.gov.uk/government/publications/arsac-notes-for-guidance>), had no history of other neurological or psychiatric disorders, and were not under treatment

with substances with known actions in PDEs (e.g. apremilast, cilomilast, luteolin, piclamilast, roflumilast and ibudilast).

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 (23). 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, and also following a challenge with levodopa 250/carbidopa 25, with an observational ON-medication period of 150 min. 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 Cambridge Neuropsychological Test Automated Battery (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). CANTAB[®] neuropsychological tests have been previously described in detail (24) and shown to be highly sensitive to deterioration in executive function in PD (25). Quality of life was measured with the patient self-reported 39-item PD Questionnaire (PDQ-39).

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 healthy controls. We used the FreeSurfer image analysis suite (version 5.3.0 <http://surfer.nmr.mgh.harvard.edu>) to process individual MRI scans, to derive measures of cortical thickness and subcortical nuclei volumes (Supplemental methods).

[¹¹C]rolipram PET data analysis

Image processing and kinetic modelling was carried out using the Molecular Imaging and Kinetic Analysis Toolbox software package (MIAKATTM: www.miakat.org; 26), implemented in MATLAB[®] (The Mathworks, Natick, MA, USA). 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).

Appropriate and individualised brain extraction fractional intensity threshold was selected and applied to the individual isotopic MRI, using the FSL Brain Extraction Tool. The T1-weighted MR image was segmented into white matter, grey matter and cerebrospinal fluid. Normalisation of the T1 MR image into stereotaxic space enabled non-linear registration between template brain MRI [Montreal Neurological Institute (MNI)-152 template], stereotaxic neuroanatomical CIC atlas version 2.0 (27), and the individual subject.

Individual PET frames were corrected for head motion using frame-by-frame rigid registration using a frame with high signal-to-noise ratio as reference. PET images were co-registered to the corresponding MPRAGE MRI.

Parent in plasma input function were generated using the continuous and discrete blood samples. Parent fraction was modelled using the Hill model (28). Parametric images of [^{11}C]rolipram V_T values were calculated with the Logan plot (29) which provides more accurate [^{11}C]rolipram V_T values than those obtained with compartmental models (30). The anatomical CIC atlas version 2.0 (27) 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. Multivariate analysis of variance (MANOVA) was used to assess the main effects of regional [^{11}C]rolipram V_T between PD patients and healthy controls. If the overall multivariate test was

significant, 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 ($P_{uncor}=0.016$; $P_{adj}=0.031$)] (Supplemental Table 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 healthy controls (Supplemental Table 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 $P>0.10$; Supplemental Table 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 ($P=0.018$; Table 2; Figures 1A, 2 and 3) and frontal cortex regions ($P=0.029$; Table 2; Figures 1B, 2 and 3). Although the general trend was for lower [^{11}C]rolipram V_T 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 2; 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 2; 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 2; 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 $P > 0.10$).

Discussion

Using [^{11}C]rolipram PET molecular imaging *in vivo*, we report for the first time 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.

There are no previous clinical or preclinical studies investigating the expression of PDE4 in PD. Studies in PDE4 knockout mice have demonstrated impaired spatial working memory (11). 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 (12).

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 (31). The medial frontal cortex plays an important role in performance monitoring on subsequent trials and in the implementation of associated adjustments in cognitive control (32). Neuroimaging studies have shown a close interaction between the posterior medial frontal cortex and dorsolateral prefrontal cortex (32). 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 (32). This functional interplay is also mediated by the supplementary motor area, which plays a role in spatial rehearsal (33), 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 (34). Output from the basal ganglia projects to the thalamus which closes the circuit by projecting back to the dorsolateral frontal cortex (35). 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 (36). 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 subjects 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 (5). 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 (4, 37, 38). 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 (39). 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 (7). 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.

The PDE4 family is comprised of four isoforms: PDE4A, B, C and D which encode for distinct proteins (40). Preclinical studies have shown that distinct subtypes of PDE4 isoforms differentially modulate synaptic activity and have distinct neurological role (10, 11). PDE4B modulates long-term depression and loss of this isoform causes impaired spatial working memory (11), whereas PDE4D regulates long-term potentiation and loss of this isoform leads to impaired fear conditioning in mice (10). [^{11}C]rolipram binds with high affinity to all four PDE4 isoforms (17), 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 (18, 19). 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) (18), and [^{11}C]rolipram binding was decreased by 20-25% in the striatum and frontal cortex of aged monkeys (19). However, more recent work indicated that age does not affect [^{11}C]rolipram V_T in cortical and subcortical regions of healthy controls and patients with major depressive disorder aged between 18 to 55 years old (20). 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.

In conclusion, our findings provide evidence for a novel neurochemical change in PD, which is linked with working memory deficits. PDE4 could serve as a novel therapeutic target for manipulation with pharmacotherapy, and PDE4 modulating drugs could potentially have a therapeutic role in the alleviation of cognitive symptoms in PD.

Acknowledgements

We thank all participants and their families, the PET technicians and radiochemists, the MRI radiographers, and the clinical research nurses at Imanova Ltd for their cooperation and support with this study. This study represents independent research supported by the Edmond J. Safra Foundation and the National Institute for Health Research (NIHR) / Wellcome Trust King's Clinical Research Facility and the NIHR Biomedical Research Centre and Dementia Unit at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. Tom Foltynie's research is supported by the Brain Research Trust, UCL's NIHR Biomedical Research Unit, the European Union FP-7 scheme and the Michael J. Fox Foundation. Marios Politis research is supported by Parkinson's UK, Lily and Edmond J. Safra Foundation, Michael J Fox Foundation (MJFF) for Parkinson's research, and KCL's NIHR Biomedical Research Unit.

References

1. Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sørensen P. Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. *Arch Neurol*. 2003;60: 387-92.
2. Kehagia AA, Barker RA, Robbins TW. Neuropsychological and clinical heterogeneity of cognitive impairment and dementia in patients with Parkinson's disease. *The Lancet Neurology* 2010;9:1200-1213.
3. Owen AM, Iddon JL, Hodges JR, Summers BA, Robbins TW. Spatial and non-spatial working memory at different stages of Parkinson's disease. *Neuropsychologia* 1997;35:519-532.
4. Lewis SJ, Dove A, Robbins TW, Barker RA, Owen AM. Cognitive impairments in early Parkinson's disease are accompanied by reductions in activity in frontostriatal neural circuitry. *J Neurosci* 2003;23:6351-56.
5. Weintraub D, Chahine L, Hawkins K, et al. and the PARS Investigators. Cognitive impairment as a feature of prodromal Parkinson's disease: evidence from the PARS study. Abstract LB243 the 19th International of Parkinson's Disease and Movement Disorders in Congress, San Diego, USA 2015.
6. Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev*. 2006;58:488-520.
7. Lakics V, Karran EH, Boess FG. Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. *Neuropharmacology* 2010;59:367-74.
8. Izquierdo LA, Barros DM, Vianna MR, et al. Molecular pharmacological dissection of short- and long-term memory. *Cell Mol Neurobiol*. 2002;22:269-87.

9. Ramos BP, Birnbaum SG, Lindenmayer I, Newton SS, Duman RS, Arnsten AF. Dysregulation of protein kinase a signaling in the aged prefrontal cortex: new strategy for treating age-related cognitive decline. *Neuron*. 2003;40:835-45.
10. Rutten K, Misner DL, Works M, et al. Enhanced long-term potentiation and impaired learning in phosphodiesterase 4D-knockout (PDE4D) mice. *Eur J Neurosci*. 2008;28:625-32.
11. Rutten K, Wallace TL, Works M, et al. Enhanced long-term depression and impaired reversal learning in phosphodiesterase 4B-knockout (PDE4B^{-/-}) mice. *Neuropharmacology* 2011;61:138-47.
12. Giralt A, Saavedra A, Carretón O, Xifró X, Alberch J, Pérez-Navarro E. Increased PKA signaling disrupts recognition memory and spatial memory: role in Huntington's disease. *Hum Mol Genet*. 2011;20:4232-47.
13. Hosseini-Sharifabad A, Ghahremani MH, Sabzevari O, et al. Effects of protein kinase A and G inhibitors on hippocampal cholinergic markers expressions in rolipram- and sildenafil-induced spatial memory improvement. *Pharmacol Biochem Behav*. 2012;101:311-9.
14. Zhang HT, Crissman AM, Dorairaj NR, Chandler LJ, O'Donnell JM. Inhibition of cyclic AMP phosphodiesterase (PDE4) reverses memory deficits associated with NMDA receptor antagonism. *Neuropsychopharmacology* 2000;23:198-204.
15. Zhang HT, O'Donnell JM. Effects of rolipram on scopolamine-induced impairment of working and reference memory in the radial-arm maze tests in rats. *Psychopharmacology* 2000;150:311-316.

16. Sierksma AS, van den Hove DL, Pfau F, et al. Improvement of spatial memory function in APPswe/PS1dE9 mice after chronic inhibition of phosphodiesterase type 4D. *Neuropharmacology* 2014;77:120-30.
17. DaSilva JN, Lourenco CM, Meyer JH, Hussey D, Potter WZ, Houle S. Imaging cAMP-specific phosphodiesterase-4 in human brain with R-[¹¹C]rolipram and positron emission tomography. *Eur. J. Nucl. Med. Mol. Imaging* 2002;29:1680- 1683.
18. Tohda M, Murayama T, Nogiri S, Nomura Y. Influence of aging on rolipram-sensitive phosphodiesterase activity and [³H]rolipram binding in the rat brain. *Biol Pharm Bull* 1996;19:300-302.
19. Harada N, Nishiyama S, Ohba H, Sato K, Kakiuchi T, Tsukada H. Age differences in phosphodiesterase type-IV and its functional response to dopamine D1 receptor modulation in the living brain: a PET study in conscious monkeys. *Synapse*. 2002;44:139-45.
20. Fujita M, Hines CS, Zoghbi SS, et al. Downregulation of brain phosphodiesterase type IV measured with 11C-(R)-rolipram positron emission tomography in major depressive disorder. *Biol Psychiatry*. 2012;72:548-54.
21. Emre M, Aarsland D, Brown R, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov Disord*. 2007;22:1689-707.
22. Litvan I, Aarsland D, Adler CH, et al. MDS Task Force on mild cognitive impairment in Parkinson's disease: critical review of PD-MCI. *Mov Disord*. 2011;26:1814-24.
23. Politis M, Wu K, Loane C, et al. Staging of serotonergic dysfunction in Parkinson's disease: an in vivo 11C-DASB PET study. *Neurobiol Dis* 2010; 40: 216–21.

24. Robbins TW, James M, Owen AM, Sahakian BJ, McInnes L, Rabbitt P. Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. *Dementia*. 1994;5:266-81.
25. Robbins TW, Cools R. Cognitive deficits in Parkinson's disease: a cognitive neuroscience perspective. *Mov Disord*. 2014;29:597-607.
26. Searle G, Coello C, Gunn R. Realising the binding potential directly and indirectly. *Journal of Cerebral Blood Flow and Metabolism Brain PET supplement 64 Brain* 2015;0791:19-20.
27. Tziortzi AC, Searle GE, Tzimopoulou S, et al. Imaging dopamine receptors in humans with [^{11}C](+)-PHNO: dissection of D3 signal and anatomy. *Neuroimage* 2011;54:264-277.
28. Tonietto M, Veronese M, Rizzo G, et al. Improved models for plasma radiometabolite correction and their impact on kinetic quantification in PET studies. *J Cereb Blood Flow Metab*. 2015;35:1462-9.
29. Logan J, Fowler JS, Volkow ND, et al. Graphical analysis of reversible radioligand binding from time-activity measurements applied to [$\text{N-}^{11}\text{C}$ -methyl]-(-)-cocaine PET studies in human subjects. *J Cereb Blood Flow Metab* 1990, 10:740-747.
30. Zanotti-Fregonara P, Zoghbi SS, Liow JS, et al. Kinetic analysis in human brain of [^{11}C](R)-rolipram, a positron emission tomographic radioligand to image phosphodiesterase 4: a retest study and use of an image-derived input function. *Neuroimage*. 2011;54(3):1903-9.

31. Owen AM, McMillan KM, Laird AR, Bullmore E. "Nback working memory paradigm: a meta-analysis of normative functional neuroimaging studies," *Human Brain Mapping* 2005;25:46-59.
32. Ridderinkhof KR, Ullsperger M, Crone EA, Nieuwenhuis S. The role of the medial frontal cortex in cognitive control. *Science* 2004;306:443-447.
33. Ullsperger M, von Cramon DY. Error monitoring using external feedback: specific roles of the habenular complex, the reward system, and the cingulate motor area revealed by functional magnetic resonance imaging. *J Neurosci.* 2003;23:4308-14.
34. Postle BR, D'Esposito M. Dissociation of human caudate nucleus activity in spatial and nonspatial working memory: an event-related fMRI study. *Brain Res Cogn Brain Res.* 1999;8:107-15.
35. Parent A, Hazrati LN. Functional anatomy of the basal ganglia, I: the corticobasal ganglia-thalamo-cortical loop. *Brain Res Brain Res Rev.* 1995;20:91-127.
36. Foltynie T, Brayne CE, Robbins TW, Barker RA. The cognitive ability of an incident cohort of Parkinson's patients in the UK: the CamPaIGN study. *Brain* 2004;127:550-560.
37. Sawamoto N, Honda M, Hanakawa T, et al. Cognitive slowing in Parkinson disease is accompanied by hypofunctioning of the striatum. *Neurology* 2007;68:1062-8.
38. Ekman U, Eriksson J, Forsgren L, Jakobson Mo S, Riklund K, Nyberg L. Association of mild cognitive impairment in Parkinson's disease to altered frontostriatal functional brain activity: a population-based cohort study. *Lancet Neurol* 2012;11:679-687.

39. Niccolini F, Foltynie T, Reis Marques T, et al. Loss of phosphodiesterase 10A expression is associated with progression and severity in Parkinson's disease. *Brain*. 2015;138:3003-15.
40. Zhang HT. Cyclic AMP-specific phosphodiesterase-4 as a target for the development of antidepressant drugs. *Curr. Pharm. Des.* 2009;15:1688-98.

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.

TABLES

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.

Table 2 Subcortical nuclei and frontal cortices [^{11}C]rolipram V_T in the groups of Parkinson's disease patients and healthy controls.

A-ROIs¹	Healthy Controls	PD patients	<i>P</i> value uncorr	<i>P</i> value*	% change in PD
Subcortical					
Striatum (mean±SD)	0.85 (±0.10)	0.70 (±0.18)	0.0194	0.035	−17.7%
Caudate (mean±SD)	0.71 (±0.07)	0.51 (±0.20)	0.0036	0.020	−27.8%
Precommissural Caudate (mean±SD)	0.74 (±0.08)	0.54 (±0.19)	0.0029	0.020	−27.2%
Postcommissural Caudate (mean±SD)	0.65 (±0.08)	0.49 (±0.20)	0.0185	0.035	−24.8%
Putamen (mean±SD)	0.92 (±0.12)	0.82 (±0.19)	>0.10	>0.10	−11.3%
Precommissural Putamen (mean±SD)	0.97 (±0.13)	0.85 (±0.21)	>0.10	>0.10	−11.9%
Postcommissural Putamen (mean±SD)	0.90 (±0.12)	0.79 (±0.19)	>0.10	>0.10	−12.2%
Accumbens (mean±SD)	0.89 (±0.13)	0.73 (±0.17)	0.0187	0.035	−17.5%
Globus Pallidus (mean±SD)	0.87 (±0.15)	0.76 (±0.18)	>0.10	>0.10	−12.5%
Thalamus (mean±SD)	0.78 (±0.09)	0.60 (±0.16)	0.0037	0.013	−22.8%
Thalamus primary motor (mean±SD)	0.85 (±0.16)	0.72 (±0.16)	>0.10	>0.10	−12.3%

Thalamus primary sensory (mean±SD)	0.84 (±0.08)	0.72 (±0.16)	0.0257	0.043	−15.2%
Thalamus prefrontal (mean±SD)	0.81 (±0.11)	0.63 (±0.17)	0.005	0.013	−22.4%
Thalamus posterior parietal (mean±SD)	0.82 (±0.09)	0.69 (±0.18)	0.0272	0.043	−16.5%
Thalamus temporal (mean±SD)	0.68 (±0.08)	0.46 (±0.18)	0.0008	0.006	−32.0%
Thalamus occipital (mean±SD)	0.80 (±0.08)	0.65 (±0.19)	>0.10	>0.10	−19.2%
Hypothalamus (mean±SD)	0.77 (±0.26)	0.53 (±0.17)	0.0192	0.035	−31.8%
Substantia Nigra (mean±SD)	0.67 (±0.23)	0.52 (±0.15)	>0.10	>0.10	−14.6%
Frontal cortices					
Precentral gyrus (mean±SD)	0.74 (±0.10)	0.62 (±0.15)	0.026	0.043	−16.4%
DLFC (mean±SD)	0.77 (±0.09)	0.64 (±0.15)	0.026	0.043	−15.9%
Anterior DLFC (mean±SD)	0.77 (±0.09)	0.66 (±0.15)	>0.10	>0.10	−14.4%
Posterior DLFC (mean±SD)	0.78 (±0.10)	0.63 (±0.17)	0.019	0.043	−20.0%
MFC (mean±SD)	0.77 (±0.10)	0.62 (±0.15)	0.012	0.043	−18.7%

Anterior MFC (mean±SD)	0.76 (±0.10)	0.63 (±0.14)	0.015	0.043	−11.9%
Posterior MFC (mean±SD)	0.78 (±0.10)	0.63 (±0.17)	0.012	0.043	−20.0%
Frontal Operculum (mean±SD)	0.84 (±0.12)	0.70 (±0.16)	0.029	0.043	−13.6%
Central Operculum (mean±SD)	0.83 (±0.11)	0.69 (±0.16)	0.021	0.043	−17.1%
OFC (mean±SD)	0.85 (±0.11)	0.74 (±0.16)	0.067	>0.10	−12.9%
Medial OFC (mean±SD)	0.86 (±0.11)	0.77 (±0.17)	>0.10	>0.10	−10.5%
Lateral OFC (mean±SD)	0.84 (±0.12)	0.72 (±0.16)	>0.10	>0.10	−14.5%
SMA	0.76 (±0.11)	0.63 (±0.15)	0.023	0.023	−17.6%

¹A-ROIs: anatomical regions of interest. **P* values are Benjamini-Hochberg corrected for multiple comparisons. DLFC=dorsolateral frontal cortex; MFC=medial frontal cortex; OFC=orbitofrontal cortex; SMA=supplementary motor area.

A **B** **C**

A

$[^{11}\text{C}]$ rolipram V_T values

B

A

$[^{11}\text{C}]$ rolipram V_T values

B