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Intra- and inter-network functional alterations in Parkinson's disease with mild cognitive impairment

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Title: Intra- and inter-network functional alterations in Parkinson's disease with mild cognitive impairment

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Abstract

Mild cognitive impairment (MCI) is prevalent in 15-40% of Parkinson's disease (PD) patients at diagnosis. In this investigation, we study brain intra- and inter-network alterations in resting state functional magnetic resonance imaging (rs-fMRI) in recently-diagnosed PD patients and characterise them as either cognitive normal (PD-NC) or with MCI (PD-MCI). Patients were divided into two groups, PD-NC (N=62) and PD-MCI (N=37) and for comparison, healthy controls (HC, N=30) were also included. Intra- and inter-network connectivity were investigated from participants' rs-fMRIs in 26 resting state networks (RSNs). Intra-network differences were found between both patient groups and HCs for networks associated with motor control (motor cortex), spatial attention and visual perception. When comparing both PD-NC and PD-MCI, intra-network alterations were found in RSNs related to attention, executive function and motor control (cerebellum). The inter-network analysis revealed a hyper-synchronisation between the basal ganglia network and the motor cortex in PD-NC compared to HCs. When both patient groups were compared, intra-network alterations in RSNs related to attention, motor control, visual perception, and executive function were found. We also detected disease-driven negative synchronisations and synchronisation shifts from positive to negative and vice versa in both patient groups compared to HCs. The hyper-synchronisation between basal ganglia and motor cortical RSNs in PD and its synchronisation shift from negative to positive compared to HCs, suggest a compensatory response to basal dysfunction and altered basal-cortical motor control in the resting state brain of PD patients.

Introduction

Parkinson's disease dementia (PDD) is a frequent complication of PD with a cumulative incidence approaching 80% (Hely, et al., 2008). Mild cognitive impairment associated with PD (PD-MCI) is recognised as a clinical entity with a prevalence of 15-40% at PD diagnosis and is a risk factor for the subsequent development of PDD (Yarnall, et al., 2014). Both PD-MCI and PDD contribute to a poorer quality of life (Lawson, et al., 2014), with PDD resulting in increased falls, neuropsychiatric disturbance, increased carer burden, loss of independence and higher likelihood of nursing home placement (Yarnall, et al., 2013).

PD-MCI as defined by the Movement Disorders Society (MDS) Task Force (Litvan, et al., 2012) encompasses cognitive deficits of executive, attention, memory, language and visuospatial functions. The underlying pathology of the motor aspects of PD is relatively well understood with established evidence-based therapies (Fox, et al., 2011). However, the aetiology underlying cognitive deficit in PD is less well known. At the cellular level, there is high variability in Lewy body-related pathological load and in the distribution of the pathology in cortical and subcortical structures. Furthermore, there remains an undetermined role for additional concurrent pathologies such as neurofibrillary tangles, tau and amyloid-β deposition in PD, although these are associated cognitive decline (Halliday, et al., 2011; Irwin, et al., 2013) and dynamic changes in neurotransmitter pathways (Kehagia, et al., 2010) are also a major feature. Consequently, there is a high degree of heterogeneity in the type and severity of non-motor symptoms in patients with PD-MCI and PDD (Galvin, et al., 2006; Parkkinen, et al., 2008).

Though the cellular level pathologies are diverse and complex, they do result in brain-wide neural system dysfunctions which are more amenable to investigation (Gratwicke, et al., 2015). These macro level functional alterations have the potential to be biomarkers of disease progression, particularly when related to the cognitive changes seen in PD, and one methodology employed to measure this is resting state functional magnetic resonance

imaging (rs-fMRI). Independent component analysis (ICA), in particular, is a powerful data-driven method that decomposes rs-fMRI data into resting state networks (RSNs). These RSNs are associated with distinct brain functions (Baggio, et al., 2015a), and can show changes or functional alterations which are specific to a particular disease (Dipasquale, et al., 2015).

The most widely reported RSN, the default mode network (DMN), plays an active role in cognitive processing with previous studies showing impaired deactivation in PD-MCI (van Eimeren, et al., 2009) and 'over activity' in PD (Shine, et al., 2014; Shine, et al., 2011). It is dynamically linked to several other cognitive networks, including the dorsal attentional network (DAN) and ventral attention network (VAN), both of which also show connectivity changes in PD-MCI (Baggio, et al., 2015b; Peraza, et al., 2015a; Shine, et al., 2014). As a collective, the DMN, DAN and VAN, together with several other RSNs, underlie specific cognitive processes such as attention, executive and motor functions.

The aim of this study was to quantify changes in rs-fMRI intra and inter-network connectivity in early PD and PD-MCI using high dimensional ICA, dual regression and informative network connectivity measures (Filippini, et al., 2009). We hypothesised that participants with PD and, in particular, those with PD-MCI, would show functional connectivity alterations in the DMN, DAN and VAN, and that there would be changes in connectivity both within and between these networks.

Methods

Recruitment and clinical assessments

Participants with a new diagnosis of PD were recruited from clinics in Newcastle upon Tyne and Gateshead areas of the UK as part of the Incidence of Cognitive Impairment in Cohorts with Longitudinal Evaluation in PD (ICICLE-PD) study (Yarnall, et al., 2014). The diagnosis of PD was made by a movement disorder specialist utilising the United Kingdom Parkinson's Disease Society Brain Bank Criteria for idiopathic PD (Hughes, et al., 2002), with reconfirmation after 18 months from their first assessment. Exclusion criteria included Parkinsonism diagnosed before study launch, insufficient English proficiency to complete neuropsychological assessments, advanced cognitive impairment (Mini-Mental State Examination, MMSE < 24) or dementia at diagnosis (Emre, et al., 2007), and suggestive diagnosis of other brain conditions and Parkinsonian diseases such as dementia with Lewy bodies (DLB), progressive supranuclear palsy, and repeated strokes (Duncan, et al., 2015; Yarnall, et al., 2014). Additionally, unrelated healthy control participants (HCs) were enrolled from community sources in Newcastle/Gateshead to provide normative data. The study was approved by the Newcastle and North Tyneside Research Ethics Committee and all subjects provided written informed consent.

Study participants completed a battery of neuropsychological tests: MMSE, the Montreal cognitive assessment (MoCA) (Nasreddine, et al., 2005), selected tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB: Tower of London, Paired Associates Learning (PAL), Spatial Recognition Memory (SRM), Pattern Recognition Memory (PRM), semantic fluency and phonetic fluency) (Robbins, et al., 1994). Power of attention (PoA) was estimated as a composite score of simple reaction time, choice reaction time and digit vigilance from the Cognitive Drug Research (CDR) battery. Motor disease severity was assessed with the revised Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III (Goetz, et al., 2008). The National Adult Reading Test (NART)

(Mathias, et al., 2007) was used to estimate premorbid levels of intelligence. Patients were assessed and underwent MRI scanning in the "ON" state; i.e. taking their usual dopaminergic medication, which was standardised to the Levodopa equivalent daily dose (LEDD) (Tomlinson, et al., 2010).

In agreement with previous studies (Duncan, et al., 2015; Mak, et al., 2015; Yarnall, et al., 2014), we applied recommended modified level II MDS criteria (Litvan, et al., 2012) for the diagnosis of PD-MCI with a threshold of 1.5 standard deviations (SD) below normative values (HCs). The level II MDS criteria establish that patients below this threshold in at least two neuropsychological tests assessing five cognitive domains (attention, execution, visuospatial, memory and language) would qualify for the diagnosis of PD-MCI. A complete description of the participants' clinical and neuropsychological assessments can be found in Yarnall, et al. (2014).

MRI acquisition and pre-processing

A total of 32 HC and 121 PD participants had brain resting state functional (rs-fMRI) and structural (MRI) taken with a 3 Tesla Philips Intera Achieva scanner. Structural images were obtained with a magnetisation prepared rapid gradient-echo sequence (MP-RAGE), sagittal acquisition, echo time 4.6 ms, repetition time 8.3 ms, inversion time 1250 ms, flip angle=8 degrees, sensitivity encoding factor=2, and in plane field of view of 240 x 240 mm with slice thickness of 1.0 mm. For the rs-fMRI, participants were asked to lie inside of the scanner with their eyes closed during the recording. Functional images were acquired with a gradient-echo planar imaging sequence with 25 contiguous axial slices, in-plane resolution = 2x2 mm, slice thickness of six mm, and repetition time of three seconds. A total of 128 fMRI volumes were obtained per participant.

Images were pre-processed using FSL (FMRIB Software Library version 5.0, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). Functional images were slice timing and motion corrected. Additionally, to further reduce artefactual influences, the six movement

parameters (rotations and translations), and the cerebrospinal fluid signal from the lateral ventricles were regressed out using the Resting-State fMRI Data Analysis Toolkit (REST) (Song, et al., 2011) in Matlab (MATLAB 7.14, The MathWorks Inc., Natick, MA, USA). Finally, functional and structural images were linearly coregistered to the MNI (Montreal Neurology Institute) standard space. Functional images were spatially resampled to 4x4x4 mm voxel size, high pass filtered (150 seconds) and spatially smoothed with a six mm FWHM (full width half maximum) filter.

In order to maximise data quality for this study, we applied a conservative exclusion criteria for movement within the MRI scanner; those participants that moved >2 mm translation or >1º rotation were excluded from the analysis (Liao, et al., 2010; Peraza, et al., 2015b).

Independent component analysis and dual regression

The multivariate exploratory linear optimized decomposition into independent components (MELODIC) algorithm within FSL was implemented to estimate canonical RSNs (Beckmann, et al., 2005). For this, a high dimensional group concatenated MELODIC was run in the HC group to obtain 70 independent component maps; this number of components is recommended in order to obtain accurate maps for subcortical brain regions (Abou Elseoud, et al., 2011; Dipasquale, et al., 2015). Component maps were visually analysed in their spatial and spectral content (Kelly Jr, et al., 2010) and 26 RSNs were identified as being of biological interest according to the previous literature (Agosta, et al., 2012; Beckmann, et al., 2005; Damoiseaux, et al., 2008), see Figure 1.

FSL-dual regression (Filippini, et al., 2009) was implemented to assess intra-RSN connectivity. Briefly, dual-regression spatially regresses the canonical RSNs on each participant's fMRI to extract a representative time series per map (first regression). Posteriorly, these time series are regressed again onto each participant's fMRI (second regression) in order to obtain the individual RSN maps, which are used for group comparisons. Prior to dual-regression, the 26 RSNs were concatenated in a single 4D file for

the dual-regression algorithm. In this way, the algorithm evaluates each RSN while the remaining 25 are used as spatial covariates.

Assessing inter-network connectivity

Inter-network connectivity was investigated using FSL-Nets (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets). This method takes the time series from the first regression in the dual-regression analysis to create inter-network connectivity matrices, which were estimated in this investigation using normalised covariance and transformed to Fisher-z scores as implemented in the FSL-Nets tool.

Statistical analysis

Group differences in age were assessed with a one-way ANOVA for the HC, PD-NC and PD-MCI groups. Gender differences were studied with a χ^2 test. Years of education and NART differences among the three groups were assessed with Kruskal-Wallis tests. Disease duration was assessed with a Mann-Whitney test between patient groups. LEDD, MDS-UPDRS III, MMSE, and MoCA were analysed for statistical comparisons with standard student t-tests between both patient groups. The remaining clinical variables were compared with Mann-Whitney tests when variables didn't show a Gaussian distribution.

Between-group comparisons for the dual-regressed maps were implemented using the general linear model (GLM) and significant differences were analysed with the FSL-randomise function (10,000 permutations). All results from FSL-randomise were corrected for multiple comparisons using threshold-free cluster enhancement (TFCE) and were considered significant at a p-value<0.05. Age, gender, and years of education were included as covariates of no interest in all GLM designs. For comparisons between patient groups, LEDD was also included as a covariate of no interest (Tahmasian, et al., 2015).

For the inter-network analysis with FSL-Nets, between group differences were also assessed with FSL-randomise (10,000 permutations) using the same strategy for the

covariates of no interest as in the dual-regression (see above). Inter-network connectivity differences were taken as significant at a p-value<0.05 FWE corrected for multiple comparisons.

Significant intra and inter-network between group differences were investigated for associations with clinical variables that contributed to the MCI diagnostic criteria. For the intra-network relations a voxelwise GLM was fitted using the fsl_glm function as follows; dual-regression result ~ clinical variable + age + gender + years of education + LEDD. For the results comparing against HCs only the patient groups were fitted to the GLM model and for the results comparing patient groups, the entire PD cohort was fitted to the model. Significance was assessed with nonparametric permutations (FSL-randomise, 1000 permutations) and TFCE correction for multiple voxel comparisons (p-value < 0.05 corrected). Similarly, the GLM was applied to investigate relationships between clinical variables and inter-network connectivity differences from FSL-Nets. The clinical variables investigated in were: MoCA, Power of Attention, Digit Vigilance Accuracy, PRM, SRM, UPDRS III, Tower of London, Animal naming, Language total score, and PAL (Yarnall, et al., 2014).

Results

From the cohort of participants, two HCs and 12 PD patients were excluded due to artefacts and vascular infarcts in their functional and structural MRIs respectively. Additionally, 10 PD patients were also excluded due to the motion exclusion criteria (>2 mm translation). After exclusions, 30 HCs and 99 PD patients remained for further functional analysis. From the cohort of PD patients, 62 were diagnosed as PD with normal cognition (PD-NC) and 37 as PD-MCI according to the MDS level II (Lawson, et al., 2014).

The three groups were assessed for movement differences within the scanner. This test showed a significant difference in total translational movement (Kruskal Wallis: χ^2=7.42, p-value=0.024, df=2) but not in rotational movement. A post-hoc t-test revealed that this difference was driven by the PD-NC group which, on average, moved more than the HCs (p-value = 0.006) but not significantly more than the PD-MCI group (p-value = 0.83).

Statistical comparisons of demographic and clinical variables are shown in Table I. Those with PD-MCI were significantly older, had greater LEDD and had lower educational attainment compared to both the PD-NC and HC groups. As expected, the PD-MCI group performed worse in cognitive assessments when compared to PD-NC and HCs and were more medicated compared with the PD-NC.

Dual-regression and FSL-Nets

The 26 RSNs of interest were investigated by dual-regression for intra-network related changes; representative contrast maps are shown in Figure 2. Significant clusters are shown in Table II, labelled by RSN and cluster index for the three between-group comparisons. In general, when comparing patient groups to HCs, we found a decrease in functional connectivity in the patient groups. When comparing HC and PD-NC groups, lower functional synchronisations were found in the PD-NC group for the RFPN, DAN, MN2, and FPN. Conversely, increased synchronisations in the PD-NC group were found for the LFPN at the left subcallosal cortex when compared to HCs.

When comparing HCs with the PD-MCI group, lower functional synchronisations were found in PD-MCI for the VN2, DAN and TEMP networks. Comparisons between both patient groups, PD-NC and PD-MCI, showed significantly lower synchronisation in the MCI group for the VAN, and dmPFN. Higher synchronisation in the PD-MCI group when compared to PD-NC was found for cerebellar and insular networks; CBN2 and INSN (see Table II).

However, when analysing individual clusters, we noted that some of the significant decreases in functional synchronisation were, in actuality, negative synchronisations in functional connectivity or anticorrelations (Keller, et al., 2013), i.e. the absolute connectivity in these cases was higher in patients than in HCs; see for instance clusters FPN-20, DAN-08 or VN2-25 whose summarised values are shown in the boxplots in Figures 3A-B. Comparisons between both patient groups, PD-NC and PD-MCI, also showed increased anticorrelations for the PD-MCI patients, e.g. cluster VAN-29 in Figure 3C.

Inter-network brain connectivity was assessed with FSL-Nets, with the objective to determine differences in connectivity between the identified RSNs. Figures 4A-C show the within-group mean connectivity matrices. Between-group comparisons for the connectivity matrices revealed a significantly increased synchronisation in PD-NC group compared with HCs between the basal network BS1 and the motor network MN1 (p-value <0.05 , FWE corrected

for multiple comparisons). A post-hoc analysis of these inter-network values revealed that this increased synchronisation was a result of a shift from a negative to positive synchronisation in the PD-NC group (Figure 4D). In PD-MCI, this synchronisation change although also present, did not reach significance after correction for multiple comparisons when compared against HCs.

Several intra-network connectivity clusters (Table II) significantly related to the clinical variables, see Table III. Spatial and Pattern recognition memory (SRM, PRM), Power of Attention (PoA), paired associates learning (PAL), MoCA, and Tower of London test significantly correlated with dual-regressed results. The most significant relation occurred between cluster FPN-20 and the SRM variable. This cluster demonstrated a disconnection between the frontal pole network and the right middle frontal gyrus. For the only significant inter-network difference (BS1-MN1), we detected a significant positive relation between this inter-network connectivity and PAL (coefficient B_1 = 1.59, p-value = 0.045, R^2=0.25, SE=2.18).

Discussion

We studied functional connectivity in patients diagnosed with PD with normal cognition (PD-NC) and PD-MCI. In general, our dual regression analyses found lower functional synchronisations in the patient groups when compared to HCs. An analysis of the significant clusters revealed that some of the apparent synchronisation decreases in the patient groups were actually a reflection of increases in negative synchronisations which are commonly referred to as anticorrelations (Keller, et al., 2013). Our analysis examining connectivity between networks with FSL-Nets found increased synchronisation between one of the identified basal ganglia networks, BS1, and motor networks, MN1 in both patient groups compared to HCs.

Lower and higher functional synchronisations in PD-NC and PD-MCI

The PD-NC group demonstrated lower synchronisations for RSNs related to attention (RFPN, DAN), motor control (MN2), and memory recall and execution (FPN) when compared to HCs. The RFPN showed a decreased connectivity in the right supramarginal gyrus, a region related to space perception and limb location (de Schotten, et al., 2005). The DAN network showed significantly reduced connectivity in the right precentral gyrus that suggests a disconnection between this primarily attentional network and the right sensorimotor and frontal cortices. A similar finding was found in the PD-MCI group but with a smaller cluster in the right frontal pole.

For the motor network MN2, we found reduced connectivity with occipital and parietal cortices. Lower connectivity between this network and the occipital and parietal regions was also reported by Peraza, et al. (2014) in dementia with Lewy bodies (DLB). This dementia is also characterised by the presence of Parkinsonism and visuospatial and attentional impairments. **Specifically, significant regions from the MN2 related with PoA which**

assesses attention function. Similarly, the FPN-20 cluster significantly related with SRM at several brain regions such as the right middle frontal gyrus, which aligns again with expected attention and executive deficits (Talati and Hirsch, 2005).

The only network that showed higher connectivity in the PD-NC group compared to HCs was the LFPN network with the left subcallosal cortex. The LFPN is one of the attentional networks with the highest activity in the supramarginal gyrus which links this network to spatial attention. Its increased synchronisation with the subcallosal cortex is difficult to interpret in PD. **However, this region showed a positive relation with the PRM and Tower of London tests, the latter test being related to executive function. The higher connectivity in PD-NC patients and the positive relation with the clinical variables suggests a possible compensation mechanism in cognitively intact PD patients.**

For the PD-MCI group when comparing to HCs, differences were found for RSNs related to vision (VN2), attention (DAN), and hearing (TEMP). Both the VN2 and TEMP networks showed apparent lower connectivity with the primary motor cortices while the DAN had apparent lower connectivity with the right frontal pole, which a post-hoc analysis revealed to be a result of negative synchronisations. **Functional alterations in PD-MCI between the DAN and frontal regions have been reported before (Baggio, et al., 2015b) and would map onto attention/executive deficits in PD-MCI, although we did not find a significant relation with any clinical variables in the current cohort. The lower functional connectivity between auditory and visual related networks with the pre- and postcentral cortices, related negatively with the PAL test. The PAL test assesses new learning through a visual recognition task and this may map onto visual and memory impairments in PD-MCI.**

When we compared both patient groups, the RSN that showed the broadest differences was the dorso-medial pre-frontal network (dmPFN) with the left frontal cortex, mainly the middle frontal and precentral gyri. This network is part of the fronto-basal-ganglia system which

previous investigations related to motor control and cognition (Aron, et al., 2007; Aron and Poldrack, 2006). **This aligned with the positive relationship between dmPFN clusters in the pre- and postcentral gyri and the Tower of London test which assesses executive function and planning. The MoCA also related with the VAN-28 cluster at the right precentral and cingulate gyri which may reflect the cognitive deficits in PD.**

The PD-MCI group demonstrated increased synchronisations for the insula network INSN and cerebellar network CBN2 with the lateral occipital and subcallosal cortices, when compared with PD-NC patients. The insula network, also referred to as the salience network, is commonly associated with orienting attention to a stimulus (Seeley, et al., 2007). The insula has been reported as functionally impaired in the Lewy body diseases (Roquet, et al., 2016) and it has been associated with complex visual hallucinations (Blanc, et al., 2014) and thus despite the absence of visual hallucinations in our PD-MCI cohort early changes in the insula network may be an important pre-requisite to these phenomena particularly as cognitive symptoms progress.

Negative synchronisations in Parkinson's disease

The negative synchronisations observed in the present study (see Figure 3) revealed by our dual regression analyses show a characteristic that is commonly neglected in many functional studies: anticorrelations. Positive synchronisations can be interpreted as a function of direct communication between two brain regions working in synchrony and mediated by excitatory connections (Keller, et al., 2013). However, negative synchronisations or anticorrelations are not easily interpreted (Chai, et al., 2012) and shifts from positive to negative correlations in patient groups are even more challenging to understand. Nevertheless, recent evidences have shown that negative synchronisations in fMRI have a biological origin (Keller, et al., 2013; Liang, et al., 2012), with current hypotheses suggesting that they are an expression of inhibitory brain mechanisms between

two or more neuronal systems driven by allocation of functional resources (Coombs Iii, et al., 2014; Schafer, et al., 2012), or a dynamic self-regulatory process which optimises brain function in a constantly changing sensory environment, such as the dynamic interrelation between the anticorrelated DAN and DMN (Fox, et al., 2005).

Shifting from positive to negative synchronisations or vice versa in patient groups might be driven by compensatory mechanisms in the presence of the disease. Previous investigations have reported the spreading of RSN synchronisations in neurodegenerative patient groups, and have been interpreted as an attempt by the RSN in question to control resources of a secondary system altered by disease (Gorges, et al., 2015). Our observations of negative synchronisations or anticorrelations certainly demand further investigation in order to understand their role in neurodegenerative diseases.

Hyper cortico-basal network connectivity in PD-NC

We observed that the PD-NC group presented with higher mean connectivity than HCs and PD-MCI group. However, FSL-Nets only showed a significant increase in functional connectivity between the BS1 and MN1 networks in the PD-NC group compared with HCs and this significantly related positively with PAL test. Recent research suggests that in Lewy body diseases, increased connectivity can occur at an early stage in the disease process (Gorges, et al., 2015). This may reflect the recruitment of additional neuronal circuitry needed to compensate for the presence of neuropathology in the primary neural network responsible for a given function, as explained previously. Furthermore, we observed that the cortico-basal connectivity between BS1 and MN1 was significantly greater in PD-NC than the HC group, which corresponded to a shift from negative to positive connectivity, although connectivity in neither group was significantly different to zero. A similar finding was reported by Yu et al. in 2013 using a ROI analysis between the putamen and the SMA (Yu, et al., 2013). As discussed in the previous section, functional anticorrelations in a healthy brain might relate to self-regulatory interactions between neural networks. Following this logic, the shift from negative to positive synchronisation observed in this study between the basal ganglia and motor cortex in PD, may represent a negotiating mechanism of motor control that has become dysfunctional in the presence of the pathology.

Limitations

There are some limitations within our present study. First, our patients were on dopaminergic medication and scanned in their "ON" state, which might alter functional connectivity. However, previous investigations have shown that Levodopa replacement therapy tends to normalise functional activity towards HC levels (Tahmasian, et al., 2015); despite this, we still found significant functional connectivity alterations. Related to this, our patients were also assessed with a battery of neuropsychological tests which were used for the diagnosis of MCI. As demonstrated in previous reports on this cohort (Yarnall et al. 2014) and consistent with findings in other cohorts (Cholerton et al. 2014) there is substantial heterogeneity in cognitive subdomain deficits experienced by PD-MCI patients which raises difficulties in terms of interpretation. Study of the different MCI subtype profiles in our cohort will require a stratified statistical analysis with a larger cohort which is beyond the scope of the current study although it is a focus of our ongoing work.

Another concern is years of education, which differ between the patient groups especially PD-NC and PD-MCI, which might be an important confounder related to cognitive reserve and functional brain activity. We reduced the influence of years of education and medication (LEDD) by including these variables as covariates of no interest in our analyses. A third factor is motion within the scanner; total head movement index was higher in the PD-NC group than in HCs. However, our PD-NC group moved less than two mm within the scanner which was within our motion inclusion criteria, and correction for motion was applied by the FSL toolbox. Additionally, we regressed the six motion covariates from the functional images to further reduce its effects.

There are concerns about the impact of cortical atrophy in functional connectivity studies. Cortical density differences between groups were not broad in our participants due to their early disease stage and were only significant when comparing HCs and PD-MCI patients (HC > PD-MCI) and delimited within the insula cortices which agrees with previous structural

studies (Duncan, et al., 2015), see Supplementary Material. Previous investigations in rs-fMRI have proven that cortical density differences do not alter the position of the significant functional clusters, although their size may be slightly decreased (Damoiseaux, et al., 2012). Hence, we are confident that the small structural differences between HCs and PD-MCI patients did not affect our functional findings.

Finally in the present study we did not carry out any reliability testing of our probabilistic group ICA procedure which is a potential limitation (Abou-Elseoud, et al., 2010). There are, however, emerging reliability procedures (albeit it is not established by consensus which approach is better) which may be useful to apply in the future to our data and others to confirm the reliability of the group ICA maps.

Conclusion

We reported changes in functional connectivity in patients diagnosed with PD-NC and PD-MCI using the level II MDS criteria. Our findings suggest that functional alterations in RSNs are more evident in our PD-NC group than in our PD-MCI group when compared to each other and against HCs. Most of these alterations were in RSNs associated to attention, motor control, visual and executive functions.

Altered functional synchronisations were found between RSN activity and several brain regions in PD-NC. When assessing connectivity among RSNs with FSL-Nets, the PD-NC group showed higher synchronisation values for cortico-basal networks, which was also present in the PD-MCI group although not to a significant level. Finally, we observed shifts in the sign of the synchronisation in the PD groups which might be related to the disease stage and compensatory brain mechanisms. Longitudinal studies observing this transition will shed more light on the pathophysiological alterations in brain connectivity that are driven by PD.

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Figure 1. Resting state networks (RSNs) inferred from the HC group using group ICA (MELODIC). A total of 70 independent components were estimated and 26 of them identified as of biological interest according to the previous literature. Similarly RSN names were assigned following convention in previous literature when possible; otherwise names were given according to the highest positive region in the RSN map. Brain maps are shown in radiological convention.

Figure 2. Dual-regression results for between group comparisons: HC vs. PD-NC, HC vs. PD-MCI, and PD-NC vs. PD-MCI. Regions and MNI coordinates are described in Table II. HC – healthy controls, PD-NC – PD with normal cognition, PD-MCI – PD with mild cognitive impairment, DAN – dorsal attention network, RFPN – right fronto-parietal network, MN2 – motor network 2, FPN – fronto-polar network, LFPN – left fronto-parietal network, VN2 – visual network 2, TEMP – temporal network, dmPFN – dorso-medial prefrontal network, CBN2 – cerebellar network 2. Brain maps are presented in radiological convention, i.e. right is left hemisphere.

Figure 3. Cluster mean values from the significant differences shown in Figure 2 and Table II as boxplots. A) HC vs PD-NC, B) HC vs PD-MCI, and C) PD-NC vs PD-MCI. Note: although some results are reported as decreased synchronisations in the patient group, e.g. HC > PD-NC in panel B), some of the findings relate to correlations shifting from positive in the healthy control (HC) group to negative correlations in the disease group, for example cluster VN2-25 (visual network 2, cluster 25) in panel B. Resting state network acronyms and clusters codes are described in Figure 1 and Table II respectively. HC - healthy controls, PD-NC - PD with normal cognition, PD-MCI - PD with mild cognitive impairment. In each boxplot, the central mark is the sample median and the extremes are the 25th and 75th percentiles.

Figure 4. FSL-Net group connectivity matrices. Panels A to C show FSL-Net normalised covariance matrices from the three participant groups; healthy controls (HCs), PD with normal cognition (PD-NC) and with mild cognitive impairment (PD-MCI); for displaying purposes, matrices are not adjusted for confounding covariates. D) The PD-NC group showed significant higher connectivity between basal network BS1 and motor network MN1 with a shift from negative to positive synchronisation (*p-value < 0.05 FWE corrected for multiple comparisons, and corrected for age, gender, levodopa equivalent daily dose and years for education which were included as covariates of not interest).

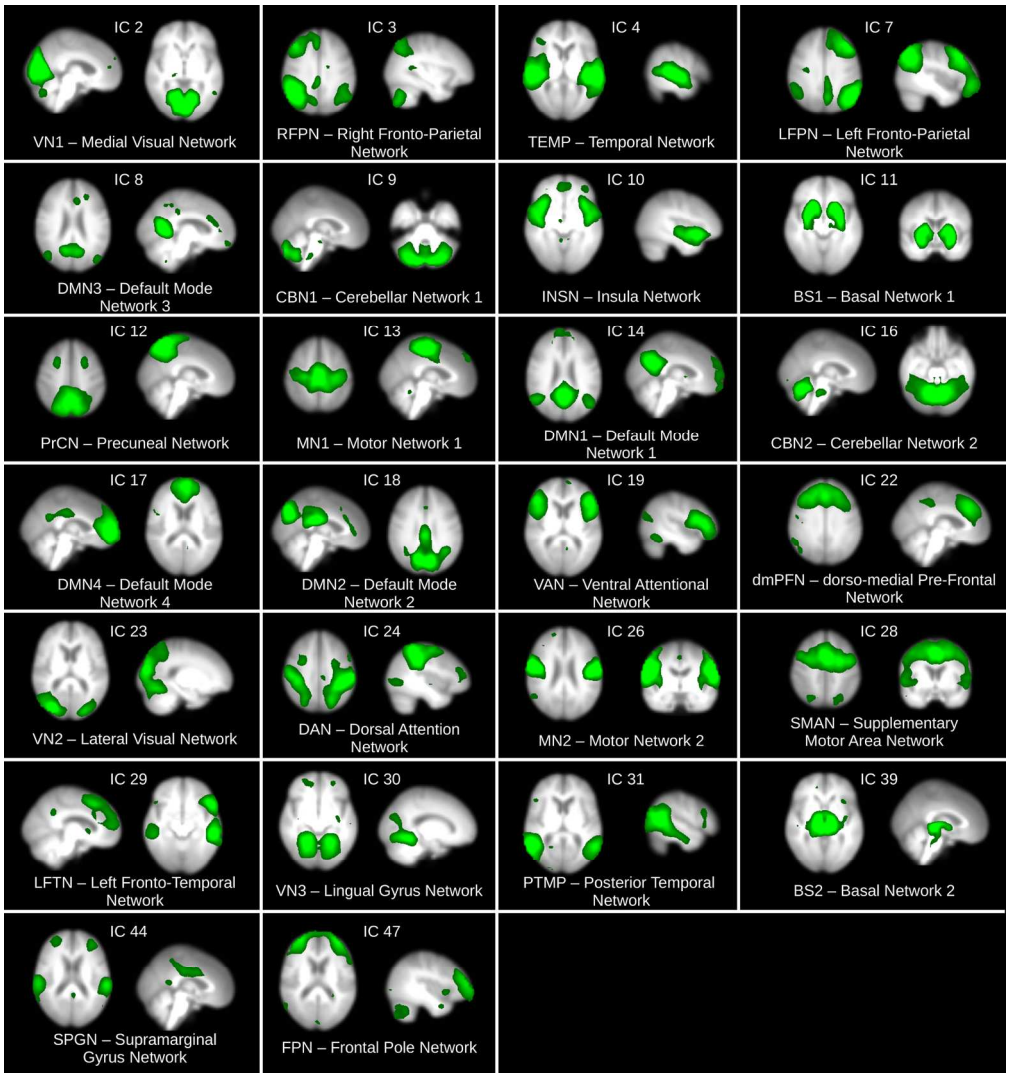

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Figure 1
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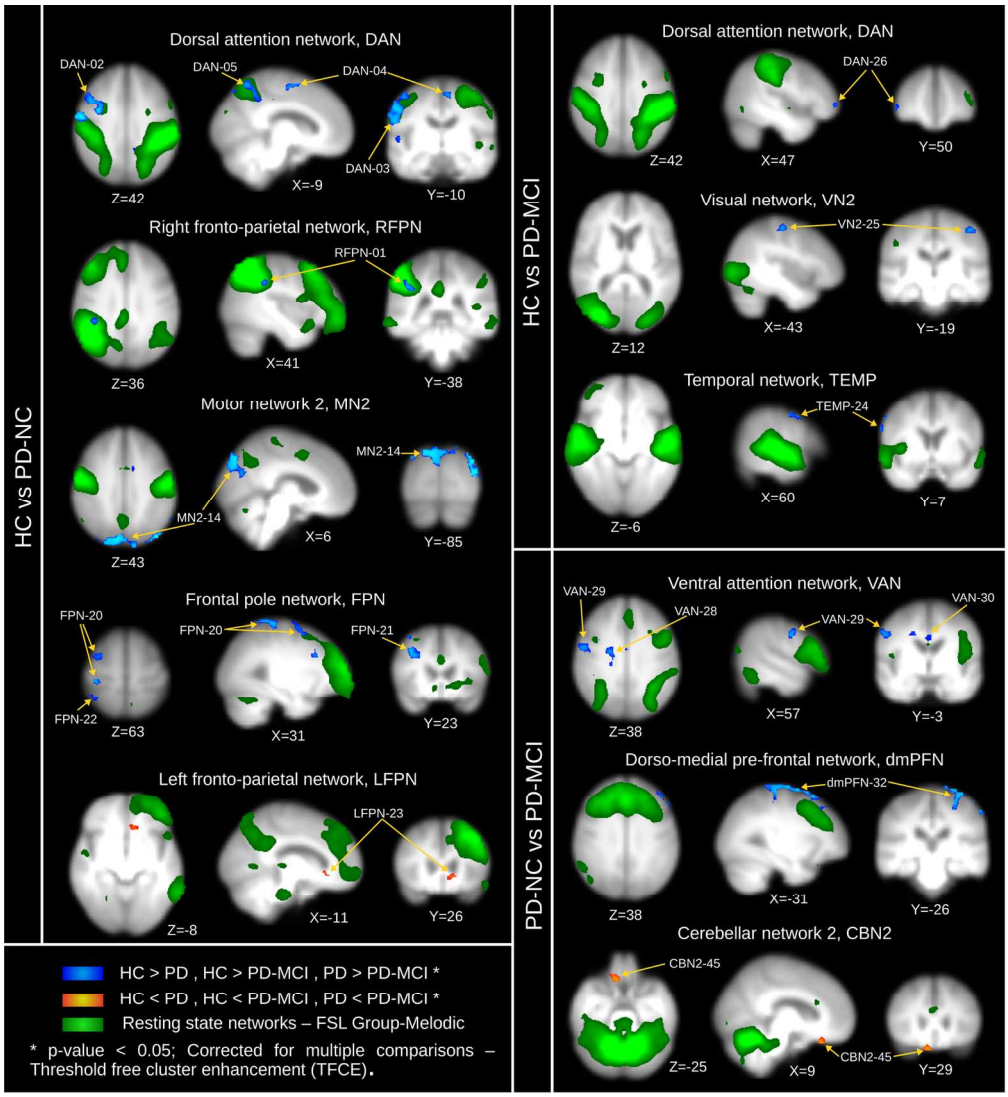

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Figure 2
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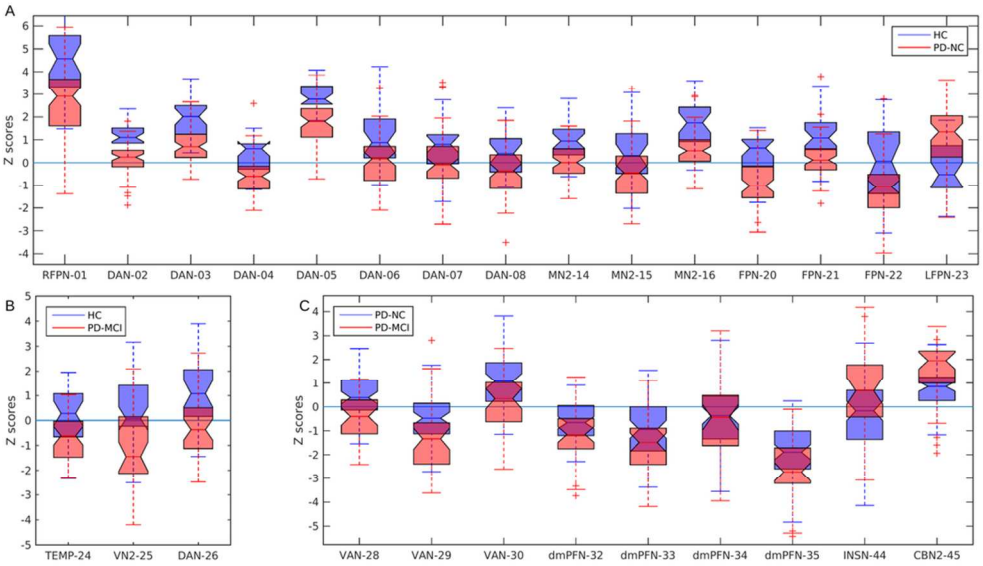

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Figure 3
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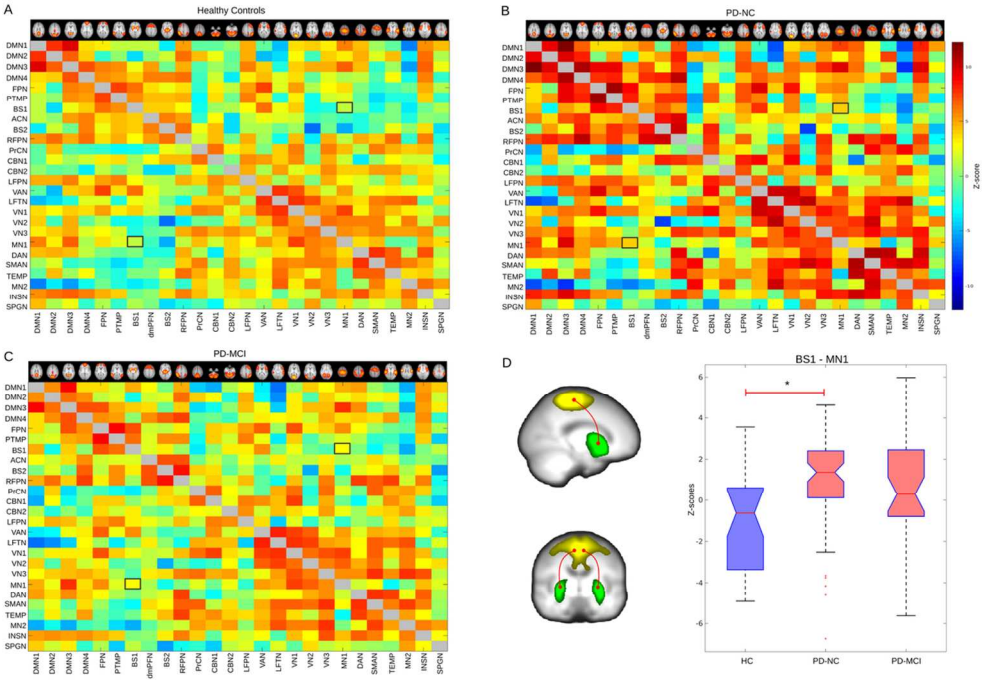

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Figure 4

113x78mm (300 x 300 DPI)

Table I. Demographics and clinical variables

	Healthy Controls	PD-NC; Mean (SD)	PD-MCI; Mean (SD)	Significance, p-value
Number of participants	N=30	N=62	N=37	
Age	64.05 (7.92)	62.77 (10.83)	70.40 (9.13)	F(2,126)=7.39, p=0.001†
Male/Female	17/13	37/25	28/9	X^2=3.36, p=0.186
Education (years)	14.13 (3.65)	14.32 (3.9)	10.97 (3.24)	p<0.001 *
NART	118.6(6.96)	117.47(9.6), N=61	112.52(9.5), N=36	p=0.008 *
Disease duration (months).	NA	6.43 (4.76)	5.81 (4.51)	p=0.458 ǂ
LEDD	NA	147.06 (112.0)	201.59 (155.8)	t_{97}=2.01, p=0.046
MDS-UPDRS III	NA	24.59 (10.39)	28.86 (10.97)	t_{97}=1.93, p=0.056
MMSE	29.36 (0.88)	29.01 (0.93)	28.18 (1.48)	t_{97}=3.4, p=0.001
MoCA	28.2 (1.78), N=29	26.92 (2.30), N=53	22.97 (3.64), N=34	t_{85}=6.2, p<0.001
Power of Attention	1217.46 (101.175)	1289.36 (126.98), N=61	1400.65 (172.04)	t_{96}=3.6, p<0.001
Digit Vigilance Accuracy	98.0 (2.5)	97.59 (3.91), N=61	83.9 (17.27)	p<0.001 ǂ
PRM	21.89 (1.61), N=29	21.15 (2.2), N=58	18.02 (2.8), N=36	p<0.001 ǂ
SRM	17.27 (1.22), N=29	16.08 (1.7) N=58	14.11 (2.5), N=36	p<0.001 ǂ
Tower of London	17.44 (1.76), N=29	15.68 (2.25), N=58	12.88 (4.1), N=36	p<0.001 ǂ
Animal naming	NA	23.9 (5.58), N=60	17.57 (6.6), N=35	t_{93}=4.97, p=<0.001
Language total score	NA	24.45 (7.29), N=50	19.29 (6.6), N=32	t_{80}=3.24, p=0.002
PAL	NA	1.70 (0.43), N=58	2.42 (0.83), N=35	p<0.001 ǂ

NART, national adult reading test; LEDD, Levodopa equivalent daily dose; MDS-UPDRS III, Movement Disorder Society – unified Parkinson's disease rating scale part III; MMSE, mini-mental state examination; MoCA, Montreal cognitive assessment; NA, not available; PRM, pattern recognition memory; SRM, spatial recognition memory; PAL, paired associates learning; SD, standard deviation.
†ANOVA: healthy controls, PD-NC, and PD-MCI
X^2 test Healthy controls, PD-NC, and PD-MCI
*Kruskal Wallis test, Healthy controls, PD-NC, PD-MCI; Post-hoc Mann-Whitney U: Age, PD-NC < PD-MCI p-value <0.001; NART, PD-NC > PD-MCI p-value =0.005.
ǂMann-Whitney U test; PD-NC and PD-MCI
Student t-tests: PD-NC and PD-MCI

Table II. Dual-regression results from the between group comparisons; corrected for multiple comparisons with threshold-free cluster enhancement (TFCE, p-value<0.05). L/R, right and left hemisphere.

Dual-regression, cluster index	Number of Voxels	Min p-value	MNI coordinate	Brain regions – Harvard-Oxford atlas
HC > PD-NC				
RFPN-01	10	0.024	42,-38,36	R supramarginal gyrus
DAN-02	158	0.009	38,-2,40	R precentral gyrus, R middle frontal gyrus
DAN-03	144	0.005	58,-6,44	R precentral gyrus, R postcentral gyrus
DAN-04	43	0.017	-6,-14,60	L supplementary motor area, L precentral gyrus.
DAN-05	34	0.022	-10,-62,64	L lateral occipital cortex, L precuneus cortex
DAN-06	20	0.022	50,50,4	R frontal pole
DAN-07	18	0.036	18,-6,64	R superior frontal gyrus
DAN-08	17	0.038	62,-14,8	R planum temporale
DAN-09	8	0.04	-14,-46,76	L postcentral gyrus
DAN-10	5	0.038	-10,-78,52	L lateral occipital cortex, superior division
DAN-11	3	0.03	-66,-50,36	L supramarginal gyrus, posterior division
DAN-12	2	0.048	-18,-78,52	L lateral occipital cortex, superior division
DAN-13	1	0.048	42,-82,24	R lateral occipital cortex, superior division
MN2-14	601	0.001	-42,-78,40	L lateral occipital cortex, R cuneal cortex, R occipital cortex, L precentral gyrus, R lateral occipital cortex
MN2-15	73	0.026	26,-54,72	R superior parietal lobule
MN2-16	32	0.013	34,26,16	R inferior frontal gyrus, pars opercularis
MN2-17	5	0.042	-10,-18,36	L cingulate gyrus, posterior division
MN2-18	3	0.044	62,6,-4	R superior temporal gyrus, anterior division
MN2-19	2	0.05	2,-62,36	R precuneus cortex
FPN-20	192	0.013	38,-26,64	R postcentral gyrus, R middle frontal gyrus, R superior parietal lobule, R precentral gyrus.
FPN-21	25	0.014	38,22,32	R middle frontal gyrus
FPN-22	17	0.039	46,-46,60	R superior parietal lobule
HC < PD-NC				
LFPN-23	9	0.031	-10,26,-4	L subcallosal cortex.
HC > PD-MCI				
TEMP-24	30	0.02	62,6,40	R precentral gyrus.
VN2-25	22	0.019	-42,-18,56	L precentral gyrus.
DAN-26	8	0.031	50,50,0	R frontal pole.
PD-NC > PD-MCI				
RFPN-27	1	0.043	-2,-62,36	L precuneus cortex.
VAN-28	22	0.025	18,-10,40	R precentral gyrus, R cingulate gyrus.
VAN-29	21	0.018	58,-2,40	R precentral gyrus.
VAN-30	9	0.046	2,-6,32	R cingulate gyrus, anterior division.
VAN-31	2	0.05	-2,22,64	L superior frontal gyrus.

dmPFN-32	738	0.007	-26,6,68	L middle frontal gyrus, L precentral gyrus, L postcentral gyrus, R supplementary motor cortex.
dmPFN--33	56	0.017	14,62,-8	R frontal pole, L frontal pole.
dmPFN--34	25	0.033	18,-54,76	R superior parietal lobule.
dmPFN--35	9	0.031	-26,50,36	L frontal pole.
dmPFN--36	6	0.043	34,54,-16	R frontal pole.
dmPFN--37	4	0.046	42,10,60	R middle frontal gyrus.
dmPFN--38	4	0.04	-6,54,44	L frontal pole.
dmPFN--39	2	0.04	6,66,24	R frontal pole.
dmPFN--40	2	0.046	-22,66,16	L frontal pole.
dmPFN--41	1	0.05	46,26,48	R middle frontal gyrus.
dmPFN--42	1	0.05	54,18,44	R middle frontal gyrus.
dmPFN--43	1	0.049	62,2,40	R precentral gyrus.
PD-NC < PD-MCI				
INSN-44	5	0.034	-46,-70,48	L lateral occipital cortex, superior division.
CBN2-45	25	0.016	6,30,-28	R subcallosal cortex, R frontal pole.

Table III. Multiple regression results between significant cluster from the dual-regression analysis and clinical variables from the MCI diagnostic criteria. Cluster significance was assessed with FSL-randomise with threshold free cluster enhancement (TFCE) correction for multiple comparisons. YoE, years of education; LEDD, Levodopa equivalent daily dose; GLM, general linear model; PoA, power of attention; ToL, Tower of London; R/L, right and left hemisphere.

Dual-regression	TFCE GLM corrected clusters		GLM: Voxel ~ B_1*variable + age + gender + YoE + LEDD			
HC vs PD-NC	Variable	Number of voxels	B_1	Min p-value	MNI coordinate	Brain region
LFPN-23	PRM	6	0.23716	0.0028	-6,30,-8	L subcallosal cortex
LFPN-23	ToL	1	0.18869	0.0074	-6,26,-4	L subcallosal cortex
MN2-14	PoA	7	0.005	0.0012	-30,-94,24	L occipital pole
MN2-14	PoA	3	0.0059	0.00053	34,-86,40	R cerebral cortex
MN2-14	PoA	1	0.00591	0.00063	-46,-82,28	L cerebral cortex
FPN-20	SRM	4	0.47865	9.10E-005	38,18,56	R middle frontal gyrus
FPN-20	SRM	3	0.41244	0.0011	18,-46,76	R superior parietal lobule
FPN-20	SRM	2	0.40269	0.0029	38,-2,68	R precentral gyrus
FPN-20	SRM	1	0.36416	0.0023	22,-26,80	R postcentral gyrus
FPN-22	UPDRS	2	-0.073	0.00046	42,-46,68	R superior parietal lobule
FPN-22	UPDRS	2	-0.08	0.00053	42,-50,68	R superior Parietal lobule
HC vs PD-MCI						
LFPN-23	SRM	1	-0.2610	0.0025	-30,38,24	L frontal pole
TEMP-24	PAL	1	-0.7557	0.0021	62,6,36	R precentral gyrus
VN2-25	PAL	3	-0.9386	0.0088	-46,-18,52	L postcentral gyrus
PD-NC vs PD-MCI						
VAN-28	MoCA	2	0.13936	0.002277	26,-10,40	R precentral gyrus
VAN-28	MoCA	1	0.11588	0.0042	18,-18,40	R cingulate gyrus, posterior division
VAN-28	PRM	10	0.16192	0.0002	18,-14,40	R cingulate gyrus, anterior division
dmPFN-32	ToL	13	0.0036	0.00064	-34,-10,76	L precentral gyrus
dmPFN-32	ToL	6	0.0037	0.0022	-30,-38,72	L postcentral gyrus