Cerebral pathological and compensatory mechanisms in the premotor phase of leucine-rich repeat kinase 2 parkinsonism

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Compensatory cerebral mechanisms can delay motor symptom onset in Parkinson’s disease. We aim to characterize these compensatory mechanisms and early disease-related changes by quantifying movement-related cerebral function in subjects at significantly increased risk of developing Parkinson’s disease, namely carriers of a leucine-rich repeat kinase 2-G2019S mutation associated with dominantly inherited parkinsonism. Functional magnetic resonance imaging was used to examine cerebral activity evoked during internal selection of motor representations, a core motor deficit in clinically overt Parkinson’s disease. Thirty-nine healthy first-degree relatives of Ashkenazi Jewish patients with Parkinson’s disease, who carry the leucine-rich repeat kinase 2-G2019S mutation, participated in this study. Twenty-one carriers of the leucine-rich repeat kinase 2-G2019S mutation and 18 non-carriers of this mutation were engaged in a motor imagery task (laterality judgements of left or right hands) known to be sensitive to motor control parameters. Behavioural performance of both groups was matched. Mutation carriers and non-carriers were equally sensitive to the extent and biomechanical constraints of the imagined movements in relation to the current posture of the participants’ hands. Cerebral activity differed between groups, such that leucine-rich repeat kinase 2-G2019S carriers had reduced imagery-related activity in the right caudate nucleus and increased activity in the right dorsal premotor cortex. More severe striatal impairment was associated with stronger effective connectivity between the right dorsal premotor cortex and the right extrastriate body area. These findings suggest that altered movement-related activity in the caudate nuclei of leucine-rich repeat kinase 2-G2019S carriers might remain behaviourally latent by virtue of cortical...
compensatory mechanisms involving long-range connectivity between the dorsal premotor cortex and posterior sensory regions. These functional cerebral changes open the possibility to use a prospective study to test their relevance as early markers of Parkinson’s disease.

Keywords: Parkinson’s disease; compensation; premotor LRRK2 parkinsonism; motor imagery; functional MRI

Introduction

Clinical symptoms of Parkinson’s disease emerge when cerebral degenerative processes overcome compensatory mechanisms. Both phenomena start several years before clinical onset of Parkinson’s disease (Palop et al., 2006), but their exact cerebral correlates at the system level are unknown. Experimentally induced dopaminergic depletion in animals treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) provides a controlled window into the presymptomatic period of induced parkinsonism. These studies revealed sensory-driven compensatory mechanisms in cortico-thalamo-cortical circuits (Bezard et al., 2003; Escola et al., 2003; Pessiglione et al., 2003), but it remains unclear to what extent these animal models can be generalized to human Parkinson’s disease. More recently, neuroimaging studies in non-manifesting carriers of mutations known to cause mono-genetic forms of Parkinson’s disease, such as Parkin (PARK2) or PINK1 (PARK6) mutations (Broussolle et al., 2000; Hilker et al., 2001; Khan et al., 2002a, b, 2005b; Walter et al., 2004; Buhmann et al., 2005; Baumer et al., 2007; Binkofski et al., 2007; Hagenah et al., 2007, 2008; Schweitzer et al., 2007; van Nuenen et al., 2009a, b; Reetz et al., 2010; Saunders-Pullman et al., 2010), have been used to map cerebral changes occurring before the clinical onset of Parkinson’s disease (Farrer, 2006). Non-manifesting carriers of these mutations showed structural and functional alterations similar to those observed in idiopathic Parkinson’s disease, for example, nigrostriatal and premotor dysfunctions. However, it remains unclear whether the recessive genetic markers studied so far are representatives of classic Parkinson’s disease phenotypes, particularly for heterozygous carriers of mutations in recessive genes. For example, neuropathological examination of patients with Parkinson’s disease with homozygous and compound heterozygous parkin mutations has demonstrated absence of Lewy bodies, questioning whether the pathophysiological mechanism in parkin-related Parkinson’s disease might be different from idiopathic Parkinson’s disease (Pouloupolous et al., 2012). Also, there is imaging evidence to suggest that different genetic mutations lead to different cerebral phenotypes; non-manifesting parkin, PINK1 and ATP13A2 mutation carriers have increased grey matter volume in the putamen, whereas leucine-rich repeat kinase 2 (LRRK2) carriers have increased grey matter volume in the right caudate nucleus (Reetz et al., 2010).

Here, we study carriers of the LRRK2 mutation, the most frequent genetic cause of Parkinson’s disease worldwide (Farrer, 2006). This dominantly inherited risk factor might be associated with changes in brain activity reflecting pathological and compensatory mechanisms of Parkinson’s disease before symptoms become clinically evident. We characterize functional and structural markers of cortical changes associated with the LRRK2 G2019S mutation, comparing asymptomatic LRRK2 carriers with non-mutation carriers. We assess a core deficit of Parkinson’s disease: the internal selection of motor representations (Brown and Marsden, 1988; Helmich et al., 2009). To identify these markers, we use a validated motor imagery task in which subjects are asked to make laterality judgements (left or right) of hand pictures (de Lange et al., 2005; Helmich et al., 2009) while measuring behavioural performance (reaction times) and cerebral activity (using functional MRI). Previous studies have shown that subjects solve this task by mentally moving their own hand from its current position into the stimulus orientation for comparison (Parsons, 1987; de Lange et al., 2008a). This task allows subjects to internally select which hand is going to be mentally moved, according to hand-specific biomechanical constraints and the subjects’ own hand posture (de Lange et al., 2006). The validity of this task has been demonstrated in healthy subjects (Shenton et al., 2004; de Lange et al., 2006) and in patients with Parkinson’s disease (Dominey et al., 1995; Helmich et al., 2007, 2012). The known pathophysiology of LRRK2 suggests that latent dopaminergic deficits in LRRK2 mutation carriers might result in impaired activity in the striatum (Galter et al., 2006; Lin et al., 2011). We also predict that, similar to what has been observed in idiopathic patients with Parkinson’s disease (Helmich et al., 2007; van Nuenen et al., 2012), increased connectivity between posterior sensory areas and the cortical motor system might compensate for those striatal deficits.

Materials and methods

We included 62 first-degree asymptomatic first-degree relatives of Ashkenazi patients with Parkinson’s disease who are carriers of the G2019S mutation in the LRRK2 gene [age 47.2 ± 9.7 years, mean ± standard deviation (SD); 28 male subjects]. Given the dominant inheritance of this mutation, ~50% of the subjects also have the mutation and are at higher risk of developing Parkinson’s disease. All subjects were native Hebrew speakers and provided written informed consent after receiving full explanation on the nature of the study. The study was approved by the Tel Aviv Sourasky Medical Centre Institutional Review Boards. Before scanning, subjects underwent a complete neurological examination, including the Unified Parkinson’s Disease Rating Scale.

Genomic DNA was isolated from peripheral blood using standard protocols or from saliva according to manufacturer’s instructions (Oragene). To detect the 6055G_A (G2019S) mutation (rs34637584) in LRRK2 exon 41, we amplified a 171-bp fragment with the following primers: forward 5’ CCTGTGCATTTTCTGGCAGATA 3’ and reverse 5’ CCTCTGATGTTTTTATCCCCATTC 3’. PCR fragments were sequenced using the
BigDye® terminator chemistry (Applied Biosystems) and were analysed using an automated ABI Prism 3130xl Genetic Analyzer (Applied Biosystems). In addition, \textit{LRRK2} G2019S mutation was also detected using TaqMan® assay ID C\textsubscript{63498123}_10 in the StepOnePlus\textsuperscript{TM} Real-Time PCR System (Applied Biosystems). The participants did not know their genotype status.

Exclusion criteria were as follows: clinical diagnosis of Parkinson’s disease according to the Queen Square Brain Bank criteria (Gibb and Lees, 1988), other neurological diseases (such as severe head trauma, stroke or history of psychiatric disease treated with neuroleptics), general exclusion criteria for MRI scanning (such as claustrophobia, pace-maker and implanted metal parts) and failure to perform the motor imagery task during a training session outside the scanner (error rate >90\%) or inside the scanner (error rate >30\%). We also excluded subjects with severe head movements (Euclidean distance >4.0 mm). During data collection, examiners and participants were blinded for the mutation status.

Motor imagery experiment

We used line drawings of left and right hands, with either the back or palm of the hand in view (Fig. 1). The left and right hand drawings were identical mirror images. The hand drawing could be rotated in either a counter-clockwise or a clockwise orientation. For both orientations, four different rotations (45°, 75°, 105° and 135°) were used; this yielded eight different rotations. These stimuli were presented through a PC running Presentation software (Neurobehavioural Systems). Images were projected through an LCD projector (NEC, VT660K) onto a screen positioned in front of the subjects’ forehead and viewed through a tilted mirror. Responses were gathered with an MRI-compatible response box (HH-1 x 4L, Current Designs) and were saved on a log-file for further analysis. Before entering the functional MRI, all participants underwent a preparatory session during which adequate performance of the task was assured. Inside the scanner, participants were asked to report whether the drawing of the hand on display represented a left or a right hand (regardless of its rotation) by pressing one of the buttons that were located underneath their left and right big toes. During scanning, reaction times and error rates were measured for subsequent behavioural analysis. The imaging session consisted of 32 task blocks (duration 60 s per block) intermixed with 30 baseline periods (duration 10 s). Each block consisted of 12 trials, which started with a fixation cross, displayed for a variable interval (0.5–1.5 s), followed by the presentation of a drawing of a hand. When a response was provided, the stimulus was replaced by the fixation cross for a jittered period of 1.5–2.5 s, and then a subsequent drawing was shown. Subjects did not receive feedback. Rotation and laterality of the hand drawings were randomized from trial to trial. On the basis of previous studies (Helmich et al., 2007), the reaction time cut-off was set at 5.0 s. Subjects performed 384 trials. During the experiment, the posture of the patients’ left and right hand was manipulated. At the beginning of each block, a text instructed the patients to position their hands in one of four postures: (i) both palms up; (ii) the left palm up, right palm down; (iii) the left palm down, the right palm up; or (iv) both palms down. The period during which the text was displayed and the instruction for postural adjustment took place (duration 5 s) was followed by a baseline period during which a fixation cross was displayed on the screen. Each posture change was followed by a block of 12 trials. During the whole experiment, the patients were lying supine in the scanner, facing the bore of the magnet, unable to see their hands. Before the start of the scanning session, participants were trained until they could perform the task.

Behavioural analysis

First, we analysed the influence of the factors ‘rotation’ (eight levels: −135, −105, −75, −45, 45, 75, 105 and 135°), ‘hand’ (two levels: left or right) and ‘group’ (two levels: non-mutation carriers or non-manifesting \textit{LRRK2}-carriers) by means of repeated measures ANOVA on reaction times collected during scanning. Second, we tested the effects of biomechanical constraints and body posture on behavioural performance. The term ‘biomechanical constraints’ refers to the reaction time difference in mentally rotating a hand towards a lateral, as compared with a medial orientation with respect to the body axis. Lateral and medial orientations were coded as follows: counter-clockwise rotations (−135, −105, −75 and −45°) were averaged and recoded as a lateral orientation for left hands and a medial orientation for right hands; clockwise rotations (45, 75, 105 and 135°) were averaged and recoded as a medial orientation for left hands and as a lateral orientation for right hands. The term ‘hand posture’ refers to the posture of the participants’ hand (pronated or supinated) with respect to the posture of the hand drawing (irrespective of medio-lateral rotation). Trials were coded as matching (‘match’) or non-matching (‘non-match’) with respect to the subjects’ own hand posture. Thus, we tested the effects of hand factors (two levels: left or right), biomechanical constraints (two levels: medial or lateral), hand posture (two levels: match or non-match) and group (two levels: non-mutation carriers or non-manifesting \textit{LRRK2}-carriers) by means of repeated measures ANOVA on reaction times collected during scanning. The Greenhouse–Geisser method was used to correct for non-sphericity. $\alpha$-level was set at $P = 0.05$.

Functional magnetic resonance image acquisition and preprocessing

Imaging was performed on a GE 3T Signa HDxt scanner with a resonant gradient echoplanar imaging system. All images were acquired using a standard eight-channel head coil. Each subject received an anatomical scan using spoiled gradient (3D-SPGR) echo sequence with field of view 250 × 250 mm; matrix size 256 × 256 mm; voxel size 0.98 × 0.98 × 1 mm; repetition time = 9 ms; echo time = 3.6 ms.

Figure 1 Task set-up. An illustrative subset of the stimuli used in the motor imagery task (here shown only for the backs of right hands, across different orientations). After the presentation of the drawing of a hand, subjects had to report whether the stimulus was a left or a right hand, irrespective of its rotation. The stimulus could be orientated either laterally (upper row) or medially (lower row) with respect to the body midline.
and functional scans (64 x 64, field of view 20, 35 slices, 3.5 mm thickness, no gap, repetition time 2200 ms, REP 57). Functional data were preprocessed and analysed with SPM5 (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm). First, functional echo planar imaging images were spatially realigned using a least squares approach and a six parameter (rigid body) spatial transformation (Friston et al., 1995). Subsequently, the time series of each voxel was realigned temporally for acquisition of the first slice (slice time correction). Anatomical images were spatially co-registered to the mean of the functional images (Ashburner and Friston, 1997) and were segmented using a unified segmentation approach. The resulting transformation matrix was then used to normalize the anatomical and functional images. The normalized functional images were resampled at an isotropic voxel size of 2mm and smoothed with an isotropic 8mm full-width at half-maximum Gaussian kernel.

Analysis of task-related effects

First level analysis

The preprocessed functional MRI time series were analysed at the first-level using an event-related approach in the context of the general linear model. This model considered the biomechanical constraints (two levels: medial or lateral), hand posture (two levels: match or non-match), laterality (factor hand, two levels: right or left) and rotation (four levels: 45, 75, 105 and 135°). The effect of stimulus rotation on cerebral activity was separately modelled for each condition using a linear basis function (parametric modulation with four levels corresponding to 45, 75, 105 and 135°). In addition, our first-level model included separate regressors of no interest: one regressor modelling incorrect and miss trials, two regressors describing the signal intensity averaged on each scan over the segmented white matter and over a blank portion of the MRI (out of brain signal) and regressors describing head motion (linear, quadratic and cubic effects of the six movement parameters belonging to each volume and also first derivative of each of those regressors, to control for spin-history effects) (Lund et al., 2005). Parameter estimates for all regressors were obtained by maximum-likelihood estimation, while using a temporal high-pass filter (cut-off 128 s), and modelling temporal autocorrelation as a first-order auto-regressive process.

Random effects analysis

We tested two different imagery-related effects over the whole sample of subjects. First, we tested for rotation-related effects, entering the relative contrast image for each subject into a within-subjects one-sample t-test. Second, we tested for orientation-related effects, entering the relative contrast images (biomechanical constraints difficult, biomechanical constraints easy) for each subject into a within-subjects paired t-test. Crucially, we then compared rotation- and orientation-related effects between the two groups, performing two-sample t-tests on the relevant contrast images from each subject. Although gender and age distribution were not significantly different between groups, we added this information as a covariate to the second-level analysis, to account for possible gender- and/or age-related cerebral differences during motor imagery.

Statistical inference

Statistical inference (P < 0.05) was performed at the cluster-level, correcting for multiple comparisons for the search volume (i.e. whole brain). Clusters were initially defined by using a voxel-level intensity threshold of P < 0.001 uncorrected. We also performed region of interest analyses, using a voxel-level statistical threshold of P < 0.01, family wise error corrected for multiple comparisons for the search volume (i.e. the region of interest). There were seven regions of interest. First, we searched for differential imagery-related activity in the dorsal premotor cortex because this region is known to be specifically involved in motor imagery of hand movements (de Lange et al., 2006; Helmich et al., 2007). We localized the left and right dorsal premotor cortex by testing for rotation-related activity across the whole group (i.e. in carriers and non-mutation carriers), correcting for multiple comparisons across the whole brain. This analysis isolated two maxima along the precentral gyrus: left dorsal premotor cortex (MNI coordinates −26, −2, +52) and right dorsal premotor cortex (MNI coordinates +34, −4, +48) (Table 2). Then we tested for group differences in a sphere of 10mm around these functionally defined dorsal premotor cortex coordinates. Second, we focused on the right extrastriate body area (MNI coordinates +46, −78, +6) because this region showed increased motor imagery-related activity in symptomatic Parkinson’s disease in a previous study (Helmich et al., 2007). Thus, we tested for group differences in a sphere of 10mm around these coordinates, defined on the basis of an independent study (Helmich et al., 2007). Third, we searched for between-groups differences in the anatomically defined bilateral putamen and caudate nucleus (as obtained from the Anatomical Automatic Labelling atlas) (Tzourio-Mazoyer et al., 2002).

Effective connectivity analysis

Having identified increased motor imagery-related activity in the right dorsal premotor cortex of LRRK2 carriers, we next hypothesized a change in connectivity between this premotor region and other brain regions involved in compensatory processes in Parkinson’s disease during motor imagery. More specifically, based on the previous finding of increased connectivity between dorsal premotor cortex and extrastriate body area in symptomatic Parkinson’s disease (Helmich et al., 2007), we hypothesized that LRRK2 carriers may also have increased connectivity between the dorsal premotor cortex and the extrastriate body area during motor imagery. As dorsal premotor cortex activity was dependent on the length of the imagined movement (factor: rotation), we hypothesized that altered connectivity of the dorsal premotor cortex should also increase with increasing rotation.

For connectivity analyses, we used the psychophysiological interaction method (Friston et al., 1997). A psychophysiological interaction analysis makes inferences about regionally specified responses caused by the interaction between a psychological factor and a physiological activity in a specified index area. The analysis was constructed to test for differences in the regression slope of the activity in all remaining brain areas on the activity in the index area (e.g. right dorsal premotor cortex), depending on the degree of rotation (45, 75, 105 and 135°). The index area was defined by the first eigen-time series of all voxels within a 6mm radius sphere centred on the regional maximum in the right dorsal premotor cortex that showed a relative increase in blood oxygen level-dependent signal during mental rotation of hands with increasing degree of rotation (45 > 75 > 105 > 135°; P < 0.05 uncorrected). In four non-mutation carriers and five LRRK2 carriers, no significant voxels were found for that contrast in the dorsal premotor cortex; therefore, their data could not be included in the analysis. First, we performed a psychophysiological interaction analysis for each subject at the first-level. Then, we entered the individual psychophysiological interaction contrast images in a two-sample t-test at the second-level (random effects analysis). Based on our a priori hypothesis, we considered only those voxels in our region of interest (right extrastriate body area).
Voxel-based morphometry

We considered the possibility that between-groups differences in functional activity could reflect between-groups anatomical differences. Therefore, we performed a voxel-based morphometry analysis. Voxel-based morphometry analyses were done in SPM8. We segmented the anatomical MRI scan of each subject into grey matter, white matter, CSF and extra-cerebral compartments (e.g. out-of-brain, skull and skin). We used the DARTEL toolbox (Ashburner, 2007) as implemented in SPM8 to create a study-specific anatomical template and register all individual grey matter images to this template. All images were subsequently normalized to MNI space while correcting for volume changes induced by normalization. Finally, we smoothed all grey matter images using a kernel of 10 mm full-width at half-maximum and performed a regression analysis on these smoothed images to test for differences in grey matter between the two groups (non-mutation carriers and non-manifesting LRRK2-carriers). We included age, gender and total grey matter as covariates, as these factors have been shown to have a great impact on grey matter volume (Good et al., 2001). To maximize the power of the voxel-based morphometry analysis, we also included the subjects who were not able to adequately perform the functional MRI motor imagery task. Therefore, the voxel-based morphometry analysis included 28 non-mutation carriers and 34 non-manifesting LRRK2-carriers. Besides whole-brain analysis, we focused the inferences on a set of regions of interest based on previous voxel-based morphometry studies on non-manifesting PARK-gene carriers (Binkofski et al., 2007; Reetz et al., 2010). We used an automated parcellation method (Anatomical Automatic Labelling atlas) (Tzourio-Mazoyer et al., 2002) as implemented in the WFU-Pickatlas SPM8 toolbox (Maldjian et al., 2003) to generate anatomical masks on putamen and caudate nuclei in each hemisphere for each subject.

Results

Participants

Of the 62 included candidates, 34 (55%) had the LRRK2 G2019S mutation. Thirteen subjects had an error rate >30% and were excluded from further analysis. It remains unclear why these participants had a poor task performance, but it seems unlikely to be related to their genetic status and any ongoing cerebral alteration. First, although a larger number of mutation carriers (n = 8) were excluded than non-mutation carriers (n = 5), the proportion of excluded subjects is similar across the two groups (\(\chi^2 = 2, P = 0.157\)). Second, previous work (Helmich et al., 2007; van Nuenen et al., 2012) clearly indicates that even patients at advanced stages of Parkinson’s disease (mean Hoehn and Yahr 2.1 and 1.4, respectively; range 1–3) can effectively perform the imagery task used in this study. However, in the aforementioned studies, participants and patients were trained before scanning to perform with an error rate <10%, whereas in this study, participants had a much shorter training. The reason for limited training in this study is that these participants performed an extensive battery of tasks [pen and paper questionnaires: Montreal Cognitive Assessment, University of Pennsylvania Smell Identification Test, State-Trait Anxiety Inventory, Blessed Dementia Scale, Trail Making Test, Unified Parkinson’s Disease Rating Scale, Category

Table 1 Groups characteristics

<table>
<thead>
<tr>
<th></th>
<th>Non-mutation carriers</th>
<th>Asymptomatic LRRK2-carriers</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Number (n)</td>
<td>18</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.7 ± 9.0</td>
<td>47.6 ± 9.1</td>
<td>0.23</td>
</tr>
<tr>
<td>Gender</td>
<td>44.4</td>
<td>57.1</td>
<td></td>
</tr>
<tr>
<td>(% of male subjects)</td>
<td>17:1</td>
<td>20:1</td>
<td></td>
</tr>
<tr>
<td>Handedness (right:left)</td>
<td>1.8 ± 1.3</td>
<td>1.9 ± 1.7</td>
<td>0.96</td>
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</table>

Baseline characteristics of subjects included in the functional MRI experiment. Values indicate mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Non-mutation carriers</th>
<th>Asymptomatic LRRK2-carriers</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPDRS III</td>
<td>1.8 ± 1.3</td>
<td>1.9 ± 1.7</td>
<td></td>
</tr>
</tbody>
</table>

Baseline characteristics of subjects included in the functional MRI experiment. Values indicate mean ± SD.

UPDRS = Unified Parkinson’s Disease Rating Scale.

Verbal Fluency Test, The Scale for Outcomes in Parkinson’s disease for Autonomic Symptoms, Digit Span Test, The Stroop colour-word test, Finger tapping; Computerized test: Neurotrax—a computerized cognitive program (Thaler et al., 2012); Motor test: gait performance across four different walking velocities. In the magnetic resonance scanner, the following tasks and imaging procedures were acquired: motor imagery task (functional MRI, reported in this manuscript), diffusion tensor imaging scan, resting state (functional MRI), observation of two video clips (functional MRI), Stroop task (functional MRI), n-back task (functional MRI), Domino task (functional MRI) and structural scan (data used in this manuscript) lasting >3 h.

One subject was excluded because of claustrophobia, three subjects were excluded because of technical problems and five subjects were excluded because of movement artefacts. One subject had a mutation in the glucocerebrosidase gene (GBA) and was excluded, as we were specifically interested in LRRK2 mutation carriers because they have an increased risk for developing Parkinson’s disease. GBA may or may not influence the risk for developing Parkinson’s disease. To keep our comparison between LRRK2 carriers and non-carriers as clear as possible, we decided to exclude the subject with both LRRK2 and GBA mutations. Of the remaining 39 subjects, the LRRK2 G2019S mutation screening revealed a mutation in 21 participants [54%; 11 male subjects; 48 ± 9 years (mean ± SD)] and no LRRK2 G2019S mutation in 18 participants (46%; eight male subjects; 45 ± 9 years). There were no significant baseline differences between the two groups (Table 1).

Behavioural results

The mean error rate was comparable between groups [non-mutation carriers: 10 ± 2%; LRRK2 carriers: 10 ± 2%; t(37) = −0.123, P = 0.913; mean ± standard error of the mean (SEM)] as were the mean reaction times [non-mutation carriers: 1418 ± 86 ms; LRRK2 carriers: 1443 ± 96 ms; t(37) = −0.192, P = 0.849]. The reaction times increased with increasing rotation of the hand drawing [main effect of rotation: F(3,46) = 53.15; P < 0.001]. This effect was not influenced by the mutation carrier status [rotation × group interaction: F(7,59) = 1.93; P = 0.124] or by the hand used to perform motor imagery [hand × rotation × group interaction: F(7,64) = 0.56; P = 0.683; Fig. 2].
We then tested for the effects of stimulus orientation (biomechanical complexity, biomechanical constraints: difficult or easy) and the effect of the subjects’ own body posture (matching or non-matching with respect to the stimulus) on reaction times. Reaction times were longer for stimuli in a biomechanically difficult than easy orientation \(F(1,17) = 62.21; P < 0.001\), and they were longer for stimuli that did not match the posture of the subjects’ own hand [posture: \(F(1,17) = 15.99; P = 0.001\)]. The subjects were faster when the stimuli were right hands \(F(1,17) = 9.35; P = 0.007\), and the behavioural slowing induced by the biomechanical complexity of the stimulus was larger for right than for left hands [hand \times biomechanical constraints: \(F(1,17) = 6.25; P = 0.023\)]. Importantly, there were no significant interactions with the factor group (all \(P > 0.2\)). These results strongly suggest that both groups used first-person motor imagery to solve the task (i.e., they mentally rotated their own hands, taking into account their current posture and biomechanical constraints of the imagined movements), rather than visual imagery. Having used a task that LRRK2 carriers and non-mutation carriers can solve, it becomes meaningful to compare cerebral responses between groups during performance of correct trials (Price and Friston, 1999).

### Imaging data

#### Rotation-related effects

First, we identified regions where activity increased with increasing stimulus rotation. We found a bilateral parieto-premotor network, confirming its involvement in mental rotation of hands (Table 2) (Johnson et al., 2002; de Lange et al., 2006; Helmich et al., 2007). Second, we tested for differential rotation-related changes in cerebral activity between LRRK2 carriers and non-mutation carriers (group \times rotation interaction). The head of the left caudate showed increased activity during increasing stimulus rotation in non-mutation carriers compared with LRRK2 carriers (MNI coordinates \(-16, -2, +20\); \(Z = 4.36; P = 0.006\) corrected), and we observed a trend for the same effect in head of the right caudate (MNI coordinates \(+18, +2, +18\); \(Z = 3.66; P = 0.063\) corrected). The right dorsal premotor cortex showed increased activity during increasing stimulus rotation in LRRK2 carriers compared with non-mutation carriers (MNI coordinates \(34, -12, +46\); \(Z = 3.24; P = 0.040\) corrected; Fig. 3A).

#### Biomechanical constraints and posture

As described earlier in the text, reaction times were longer when the imagery task involved laterally oriented hands. Cerebral activity following the same pattern was found in the bilateral insula, posterior parietal cortex and middle occipital gyrus (Table 3). There were no between-group differences in cerebral activity related to biomechanical complexity. At the conservative statistical threshold used in this study, we did not find significant posture-related cerebral activity, and no posture-related differences between groups.

#### Effective connectivity

From the contrasts aforementioned, it emerged that the right dorsal premotor cortex was more active during increasing stimulus orientation in LRRK2 carriers than non-mutation carriers. Building on recent observations in patients with symptomatic Parkinson’s disease, obtained during performance of the same task used in this study, we hypothesized that the increased dorsal premotor cortex activity might relate to increased connectivity with the extrastriate body area (Helmich et al., 2007). We tested this hypothesis with psychophysiological interaction, a tool designed to assess changes in effective connectivity between cerebral regions (Friston et al., 1997). Using the right dorsal premotor cortex as the seed region and the right extrastriate body area as the target region, we found...
that functional connectivity between these regions increased with stimulus rotation in LRRK2 carriers but not in non-mutation carriers (effect of group: MNI coordinates +40, −78, +4; \( t = 3.71; P = 0.032 \) corrected; Fig. 3B). In a whole brain analysis without the right extrastriate body area as a target region, no brain area reached significance (\( P < 0.05 \), corrected). Next, we reasoned that if this increased connectivity compensates for latent dopaminergic dysfunction in the striatum, then connectivity should increase as task-related activity in the right caudate decreases. To test this, we correlated task-related activity in the right caudate (\( \beta \)-values at +18, +2, +18 from rotation-related contrast image) with dorsal premotor cortex-extrastriate body area connectivity (\( \beta \)-values at +40, −78, +4 from psychophysiological interaction contrast image) separately for LRRK2 carriers and non-mutation carriers. In LRRK2 carriers, reduced activity in the right caudate predicted increased dorsal premotor cortex-extrastriate body area connectivity (\( r = -0.580; P = 0.018 \)). There was no significant correlation for the non-mutation carriers (\( r = 0.101; P = 0.731 \); Fig. 3C).

**Voxel-based morphometry**

There were no significant differences in grey matter volume between groups, even when lowering the statistical threshold to a lenient threshold of \( P < 0.01 \) uncorrected, and even when restricting our search to the striatum or to brain areas showing differential imagery-related activity. This indicates that the functional MRI findings we report are not a by-product of substantial structural changes in LRRK2 carriers.

**Discussion**

We studied a large group of asymptomatic LRRK2-G2019S mutation carriers to identify potential preclinical cerebral reorganization during a motor imagery task requiring internal selection of motor representations (de Lange et al., 2006; Helmich et al., 2007). This task was selected because it captures a core dysfunction in clinical stages of Parkinson’s disease (Brown and Marsden, 1988; Helmich et al., 2009). There are three main results. First, LRRK2-G2019S mutation carriers and non-carriers were equally sensitive to the extent and biomechanical constraints of the imagined movements, in relation to the current posture of the participants’ hands. This result confirms that this motor imagery task relies on internal selection of motor representations (de Lange et al., 2008a). This result also indicates that the observed cerebral effects were not driven by between-groups differences in performance. Second, asymptomatic LRRK2-G2019S mutation carriers had reduced imagery-related activity in the right caudate nucleus. This result

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**Table 3 Cerebral activity related to biomechanical constraints**

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>Functional region</th>
<th>Hemisphere</th>
<th>Cluster size</th>
<th>P-value</th>
<th>Local maximum</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle occipital gyrus</td>
<td>V2</td>
<td>L</td>
<td>557</td>
<td>&lt;0.001</td>
<td>−30 −80</td>
<td>32</td>
</tr>
<tr>
<td>Dorsal intraparietal sulcus</td>
<td>PPC</td>
<td>L</td>
<td>313</td>
<td>0.001</td>
<td>−16 −72</td>
<td>42</td>
</tr>
<tr>
<td>Middle occipital gyrus</td>
<td>V2</td>
<td>R</td>
<td>327</td>
<td>0.001</td>
<td>−30 −20</td>
<td>32</td>
</tr>
<tr>
<td>Dorsal intraparietal sulcus</td>
<td>PPC</td>
<td>R</td>
<td>238</td>
<td>0.004</td>
<td>−36 −22</td>
<td>6</td>
</tr>
<tr>
<td>Insular cortex</td>
<td>Insula</td>
<td>L</td>
<td>136</td>
<td>0.021</td>
<td>−36 −48</td>
<td>38</td>
</tr>
<tr>
<td>Inferior parietal lobe</td>
<td>L</td>
<td>L</td>
<td>93</td>
<td>0.050</td>
<td>−48 −64</td>
<td>6</td>
</tr>
</tbody>
</table>

MNI stereotactic coordinates of the local maxima of regions showing activity that increased as a function of biomechanical constraints (hand stimuli in lateral orientation evoked stronger responses than hand stimuli in a medial orientation) after presentation of left and right hands. For large clusters spanning several anatomical regions, more than one local maxima are given. Cluster size is given in number of voxels. Statistical inference (\( P < 0.05 \)) was performed at cluster level, correcting for multiple comparisons for the search volume (i.e. the whole brain). The intensity threshold necessary to determine the cluster-level threshold was set at \( P < 0.001 \) uncorrected at cluster level.

L = left; PPC = posterior parietal cortex; R = right; V2 = visual area 2.
indicates that asymptomatic LRRK2-G2019S mutation carriers have a functional impairment in the striatum. Furthermore, in contrast to the structural alterations seen in the ventrolateral striatum of patients with idiopathic Parkinson’s disease and non-manifesting parkin and PINK1 mutations (Buhmann et al., 2005; Binkofski et al., 2007; Reetz et al., 2009), the striatal impairment in asymptomatic LRRK2 carriers arose in the caudate nucleus. Third, asymptomatic LRRK2-G2019S mutation carriers had increased imagery-related activity in the right dorsal premotor cortex. Effective connectivity between this region and the right extrastriate body area increased in proportion to the caudate alteration. This suggests that long-range connectivity between the dorsal premotor cortex and posterior sensory regions might compensate for striatal impairments, a mechanism recently observed in patients with idiopathic Parkinson’s disease (Helmich et al., 2007; van Nuenen et al., 2012).

Altered caudate functionality in the premotor phase of LRRK2 parkinsonism

The motor imagery task evoked largely overlapping cerebral responses across LRRK2 mutation carriers and non-mutation carriers, namely activity in parieto-frontal regions previously associated with performance of this task (de Lange et al., 2006; Helmich et al., 2007). This result indicates that the two groups used similar cerebral circuits for solving the motor imagery task. A notable exception was found for the head of the caudate nucleus, showing less activity in non-manifesting LRRK2 mutation carriers. Given that the premotor phase of LRRK2 parkinsonism is also accompanied by nigrostriatal dopaminergic deficits (Khan et al., 2005a; Bruggemann et al., 2011), the imagery-related difference observed in the caudate might constitute a functional correlate of that dopaminergic deficit. Yet, this observation does not
fit with the known consequences of dopaminergic denervation in patients with symptomatic Parkinson’s disease, namely earlier and stronger alterations of the putamen than the caudate nucleus (Kish et al., 1988). However, if non-manifesting LRRK2 carriers have a similar upregulation of the nigrostriatal dopamine system as observed in non-manifesting MPTP-treated monkeys (Mounayar et al., 2007), then dopamine levels of non-manifesting LRRK2 carriers could be normalized in the putamen, but over-dosed in the relatively less depleted caudate. Given the restricted physiological range of dopaminergic modulation (Goldman-Rakic et al., 2000), an excess of dopamine in the caudate could lead to increased liability of the motor representations relevant for the imagery task (Cools and D’Esposito, 2011). The reduced caudate contributions observed in non-manifesting LRRK2 mutation carriers could then reflect a reduced ability of this striatal structure to support temporally sustained movement representations as required by the current motor imagery task. Given the known involvement of the head of the caudate in executive functions (Alexander et al., 1986; Marklund et al., 2009), the present finding would predict that LRRK2 carriers are characterized by cognitive alterations early in the disease, although this effect might also be driven by non-dopaminergic alterations (Alcalay et al., 2012).

A compensatory role for the dorsal premotor cortex?

Non-manifesting LRRK2 mutation carriers and non-mutation carriers solved the motor imagery task equally well, but the former group had stronger activity in the right dorsal premotor cortex. This effect is clearly located within the probabilistic cytoarchitectonic borders of Brodmann area 6 (Eickhoff et al., 2005), and more precisely within the dorsal premotor cortex (Mayka et al., 2006; Tomassini et al., 2007). On the basis of the guidelines offered by Picard and Strick (2001), we infer that the current effect is located in the caudal sector of the dorsal premotor cortex, being ~8 mm anterior to the primary motor cortex (as inferred on the basis of Mayka et al., 2006). This portion of the dorsal premotor cortex is typically associated with movement-related phenomena (Picard and Strick, 2001). In contrast, the local maximum reflecting imagery-related activity in mutation and non-mutation carriers (Table 2) is ~16 mm anterior to the primary motor cortex, most likely in the pre-dorsal premotor cortex according to Picard and Strick (2001).

Increases in dorsal premotor cortex activity have been observed in non-manifesting parkin and PINK1 mutation carriers (Buhmann et al., 2005; van Nuenen et al., 2009b), and patients with idiopathic Parkinson’s disease also show increased dorsal premotor cortex activity (Sabatini et al., 2000; Wu and Hallett, 2005). As different tasks, disease states and mutation types seem to lead to increased dorsal premotor cortex activity, it might be argued that the dorsal premotor cortex effect reported in this study is non-specific, and that the human motor system deals with the consequences of different mutations by using a single mechanism centred on the dorsal premotor cortex (van Nuenen et al., 2009b). In fact, close comparison between the current findings, Buhmann et al. (2005) and van Nuenen et al. (2009b), suggests that different portions of the premotor cortex are recruited during performance of motor-related tasks in carriers of recessive mutations (parkin, PINK1) or dominant mutations (LRRK2). For instance, the internal selection of right thumb movements, studied by Buhmann et al. (2005), evoked stronger responses in two frontal clusters of parkin carriers. One cluster was centred on the medial frontal cortex (right rostral cingulate motor area, rostral supplementary motor area, extending into the adjacent dorsal premotor cortex). Another cluster was covering the left dorsal premotor cortex, with two local maxima at −39, +6, +42, and −24, −12, +51. Even ignoring the obvious difference in the hemispheric location of the effects found in Buhmann et al. (2005) and in the present study, the local maxima of the dorsal premotor cortex cluster of Buhmann et al. (2005) area 21 are 21 mm and 11 mm apart from the local maximum reported in this study (+34, −14, +46). The study of van Nuenen et al. (2009b), comparing the effects of internal selection of finger to thumb movements evoked in parkin and PINK1 mutation carriers, reports a premotor effect (+20, +6, +64) even further away to the effect found in the present study. These data indicate that the premotor effect found in the present study occurs at a different location than those found in parkin and PINK1 carriers. Taken together with the anatomical differences in striatal impairments observed between carriers of recessive mutations (parkin, PINK1) and dominant mutations (LRRK2), these findings suggest that different genetic sources of nigrostriatal dopaminergic dysfunction lead to increased activity in different fronto-striatal circuits. However, given that the motor tasks used in these studies (i.e. Buhmann et al., 2005; van Nuenen et al., 2009b; present study) also differ in procedures and effectors, it remains to be tested whether different portions of the dorsal premotor cortex, in different hemispheres, would be recruited when carriers with different mutations perform exactly the same task.

The increased caudal dorsal premotor cortex activity found in LRRK2 carriers might reflect a reduced ability of this region to specify the motor commands required to mentally match the current hand configuration of the subject with the target hand configuration shown on the screen. In this scenario, the increased influence that the right extrastriate body area was found to exert on dorsal premotor cortex in LRRK2 carriers might be interpreted as a compensatory mechanism for the dorsal premotor cortex alteration. Extrastriate body area is a cortical region originally defined in relation to the visual perception of body parts (Downing et al., 2001). More recent work has also highlighted its involvement in planning voluntary manual actions (Astafiev et al., 2004; Kühn et al., 2011), namely specifying the goal posture of a planned action (Zimmerman et al., 2011). The increased coupling between extrastriate body area and dorsal premotor cortex in LRRK2 carriers could reflect increased reliance on visual predictions of the action outcome during the specification of the motor plan evoked by the imagery task. Furthermore, the increased coupling between extrastriate body area and dorsal premotor cortex in LRRK2 carriers scaled as a function of task demands (hand orientation), after correcting for dorsal premotor cortex activity, and with an inverse relation to task-related activity in the caudate. Given the matched behavioural performance between groups, these observations suggest that increased dorsal
premotor cortex-extrastriate body area coupling can be interpreted as compensating the reduced caudate activity observed in LRRK2 carriers. This finding extends and qualifies previous reports on the relevance of compensation for a (latent) nigrostriatal dopaminergic dysfunction during motor execution in patients with symptomatic Parkinson’s disease (Samuel et al., 1997; Haslinger et al., 2001) and non-manifesting mutation carriers (Buhmann et al., 2005; van Nuenen et al., 2009b).

Conclusion
We have characterized cerebral alterations and potential compensatory mechanisms in asymptomatic LRRK2-G2019S mutation carriers. We show that this mutation leads to movement-related alterations in the caudate nucleus, a feature that distinguishes LRRK2 carriers from the endophenotype of other known genetic subtypes. LRRK2 carriers also show potentially compensatory activity implemented through long-range connectivity between the dorsal premotor cortex and posterior sensory regions, similar to what has been recently observed in idiopathic Parkinson’s disease. These findings might capture mechanisms compensating progressive neurodegeneration at the pre-motor stage of Parkinson’s disease. Alternatively, these findings might reflect congenital/developmental abnormalities associated with LRRK2, only superficially related to clinical Parkinson’s disease by virtue of a common compensatory mechanism deployed across different cerebral phenotypes. A prospective study, following these LRRK2-G2019S mutation carriers, could test the clinical significance of the current findings and their relevance as early markers of Parkinson’s disease.

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References

Appendix I

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