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Cortical disinhibition in Parkinson's disease

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In Parkinson's disease, striatal dopamine depletion produces profound alterations in the neural activity of the cortico-basal ganglia motor loop, leading to dysfunctional motor output and parkinsonism. A key regulator of motor output is the balance between excitation and inhibition in the primary motor cortex, which can be assessed in humans with transcranial magnetic stimulation techniques. Despite decades of research, the functional state of cortical inhibition in Parkinson's disease remains uncertain. Towards resolving this issue, we applied paired-pulse transcranial magnetic stimulation protocols in 166 patients with Parkinson's disease (57 levodopa-naïve, 50 non-dyskinetic, 59 dyskinetic) and 40 healthy controls (age-matched with the levodopa-naïve group). All patients were studied OFF medication. All analyses were performed with fully automatic procedures to avoid confirmation bias, and we systematically considered and excluded several potential confounding factors such as age, gender, resting motor threshold, EMG background activity and amplitude of the motor evoked potential elicited by the single-pulse test stimuli. Our results show that short-interval intracortical inhibition is decreased in Parkinson's disease compared to controls. This reduction of intracortical inhibition was obtained with relatively low-intensity conditioning stimuli (80% of the resting motor threshold) and was not associated with any significant increase in short-interval intracortical facilitation or intracortical facilitation with the same low-intensity conditioning stimuli, supporting the involvement of cortical inhibitory circuits. Short-interval intracortical inhibition was similarly reduced in levodopa-naïve, non-dyskinetic and dyskinetic patients. Importantly, intracortical inhibition was reduced compared to control subjects also on the less affected side ($n = 145$), even in *de novo* drug-naïve patients in whom the less affected side was minimally symptomatic (lateralized Unified Parkinson's Disease Rating Scale part III = 0 or 1, $n = 23$). These results suggest that cortical disinhibition is a very early, possibly prodromal feature of Parkinson's disease.

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Abbreviations: AUC = area under the curve; ICF = intracortical facilitation; ISI = inter-stimulus interval; MDS-UPDRS = Movement Disorder Society Unified Parkinson's Disease Rating Scale; MEP = motor evoked potential; RMT = resting motor threshold; ROC = receiver operating characteristic; SICF = short-interval intracortical facilitation; SICI = short-interval intracortical inhibition; TMS = transcranial magnetic stimulation

Introduction

The neuropathology of Parkinson's disease is primarily defined by the degeneration of dopaminergic nigrostriatal projection neurons (Fearnley and Lees, 1991; Kordower *et al.*, 2013), which generates profound alterations in the neural activity of cortico-basal ganglia motor loops, leading to a dysfunctional motor output (Obeso *et al.*, 2008). Motor deficits have been repeatedly associated with changes in the activity of the motor cortex, both in Parkinson's disease animal models (Doudet *et al.*, 1990; Goldberg *et al.*, 2002; Escola *et al.*, 2003; Guo *et al.*, 2015; Pasquereau *et al.*, 2016; Chen *et al.*, 2019; Hyland *et al.*, 2019) and in patients with Parkinson's disease (Lefaucheur 2005; Monchi *et al.*, 2007; Disbrow *et al.*, 2013). A key regulator of cortical motor output is the balance between excitation and inhibition, which can be assessed in humans with transcranial magnetic stimulation (TMS) techniques (Kujirai *et al.*, 1993; Chen, 2004). Despite decades of investigation, whether or not cortical inhibition is altered in Parkinson's disease remains unclear.

A common measure to explore inhibitory mechanisms is the short-interval intracortical inhibition (SICI) induced by paired-pulse TMS stimuli. This measure is modulated primarily—but not only—by drugs acting upon γ-aminobutyric acid type a (GABAa) receptors (Ziemann *et al.*, 2015). Several studies have described reduced SICI (i.e. less inhibition) in the motor cortex of patients with Parkinson's disease (Ridding *et al.*, 1995; Strafella *et al.*, 2000; Cunic *et al.*, 2002; Buhmann *et al.*, 2004; Fierro *et al.*, 2008; Barbin *et al.*, 2013; Kaçar *et al.*, 2013; Bologna *et al.*, 2018). However, high variability exists, at least partly due to small sample sizes, methodological differences among studies, and uncontrolled putative confounding factors (e.g. conditioning and unconditioned stimulus intensities, level of muscle contraction, etc.). Indeed, a considerable number of studies have failed to replicate the alteration of SICI (Berardelli *et al.*, 1996; MacKinnon *et al.*, 2005; Chu *et al.*, 2009; Vacherot *et al.*, 2010; Kojovic *et al.*, 2017; Ponzo *et al.*, 2017; Guerra *et al.*, 2019). Overall, the disagreement between studies raises serious doubts about the actual role of SICI in Parkinson's disease (Latorre *et al.*, 2019). Furthermore, even if SICI is altered in Parkinson's disease, it might be an indirect consequence of a shift in the excitation/inhibition balance towards excitation rather than a genuine loss of inhibition (MacKinnon *et al.*, 2005; Peurala *et al.*, 2008; Ni *et al.*, 2013; Shirota *et al.*, 2019). Finally, whether and how the possible loss of motor cortex inhibition, if any, may reflect the clinical state of patients and the evolution of the disease remains unclear (Strafella *et al.*, 2000; Bares *et al.*, 2003; Barbin *et al.*, 2013; Kojovic *et al.*, 2015; Shirota *et al.*, 2019).

To address these issues, here we studied a relatively large sample of patients with Parkinson's disease ($n = 166$), applying paired-pulse TMS protocols (Fig. 1) to their most affected side compared to healthy control subjects ($n = 40$). All patients were studied OFF medication. All data were analysed with fully automatic procedures to avoid possible confirmation bias, and several potential confounding factors—i.e. age, gender, resting motor threshold (RMT), background of EMG activity and amplitude of motor evoked potential (MEP) elicited by the single-pulse test stimuli—were systematically considered and excluded. To gain mechanistic insight about the cortical circuits involved, we

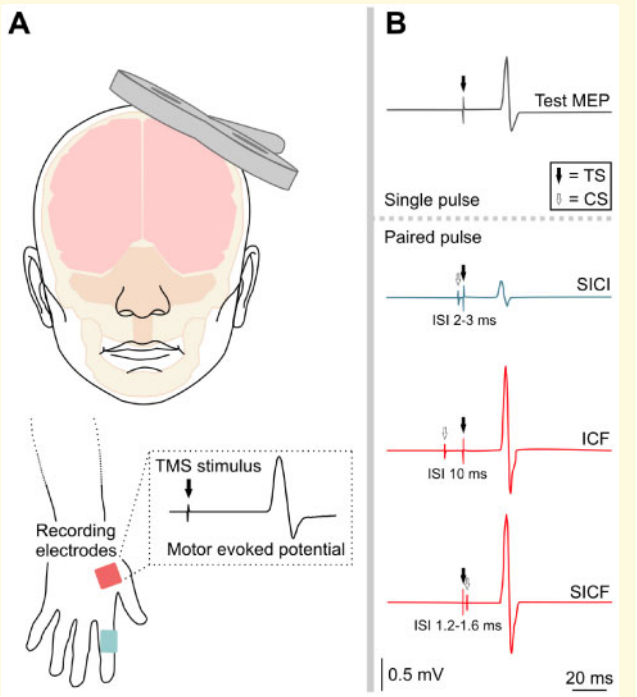

Figure 1 Experimental procedures. (**A**) Schematic experimental set-up using TMS on the primary motor cortex inducing MEPs in the contralateral first dorsal interosseous (FDI) muscle. MEPs were recorded through EMG from the relaxed FDI muscle using disposable surface electrodes. (**B**) Representative examples of MEPs induced by single-pulse and paired-pulse TMS techniques. CS = conditioning stimulus; TS = test stimulus.

investigated SICI with conditioning stimuli of relatively low intensity (80% RMT), which maximizes inhibition (Kujirai *et al.*, 1993; Ibañez *et al.*, 2020), and we tested whether conditioning stimuli with equal low intensity affected short-interval intracortical facilitation (SICF) and intracortical facilitation (ICF) in the same patients. Finally, to investigate whether the alterations in the excitation/inhibition balance may change throughout the evolution of the disease, we grouped patients as levodopa-naïve ($n = 57$), non-dyskinetic ($n = 50$) or dyskinetic ($n = 59$). In a subset of patients ($n = 145$), we also examined the clinically less affected side. At clinical presentation, motor features are typically restricted to one side of the body. Accordingly, our dataset included a number of very early *de novo* drug-naïve patients with minimal parkinsonian features in the less affected side [lateralized Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS-III) = 0 or 1, $n = 23$]. We specifically investigated the minimally symptomatic side of those patients to gain insight into the prodromal stage of Parkinson's disease.

Materials and methods

Subjects

We studied 166 patients [57 females; age (mean ± standard deviation), 61.8 ± 10.7 years] with Parkinson's disease (Brain Bank criteria) and 40 healthy controls (21 females; age, 55.6 ± 11.1 years). Subjects were recruited and studied from November 2015 until July 2019 at CINAC, Hospital Universitario HM Puerta del Sur, Móstoles (Madrid, Spain). Major neuropsychiatric comorbidities were considered as an exclusion criterion. All patients were selected, evaluated and classified by the same team of specialized movement disorder neurologists. Patients were classified into three groups: levodopa-naïve patients ($n = 57$; 20 females; age, 56.7 ± 10.8 years), patients chronically treated with levodopa medication but without levodopa-induced dyskinesias (non-dyskinetic; $n = 50$; eight females; age, 62.0 ± 9.8 years), and patients with levodopa-induced dyskinesias (dyskinetic; $n = 59$; 30 females; age 66.4 ± 9.1 years). A subset of levodopa-naïve patients was fully drug-naïve. This subgroup was classified as *de novo* ($n = 42$). In levodopa-treated patients, presence or absence of dyskinesias was determined by both anamnesis (including specific questions about the occurrence of involuntary movements during the ON state to both patients and/or their relatives) and neurological examination ON medication. All patients were then studied neurophysiologically in the practically-defined OFF medication state, after withdrawal of levodopa (and other dopaminergic drugs) for at least 12 h (overnight), except a subset of 14 highly fluctuating patients in the dyskinetic group who did not tolerate the OFF state well and were studied after at least 1 h of wearing-off. Note that even though 12 h withdrawal is a standard protocol commonly applied in experimental and clinical trials in Parkinson's disease, the washout periods that would be necessary to fully eliminate the long-term effects of levodopa and dopamine agonists are much longer. Motor signs were assessed with the MDS-UPDRS-III. Lateralized MDS-UPDRS-III values were obtained by summing items 3.3b–3.8b and 3.15a–3.17d for the more affected and the less affected sides, respectively. Healthy control subjects presented a negative history of neurological or psychiatric conditions and were medication-free at the time of the study. These subjects were age-matched with the levodopa-naïve Parkinson's disease group. All subjects gave informed consent and all procedures were conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committee. Throughout the study, patients were seated comfortably and were instructed to refrain from speaking and to remain awake while in a calm, relaxed state.

EMG recordings

We recorded EMG activity from the relaxed first dorsal interosseous (FDI) using disposable surface electrodes. We studied the most affected FDI in all patients ($n = 166$) and also the less affected FDI in a subset of patients ($n = 145$). In healthy controls the non-dominant FDI was studied. EMG signals were band-pass filtered (2 Hz–2 kHz), notch filtered (50 Hz) in case of line noise contamination, and amplified ($\times 1000$; D360, Digitimer Ltd) and single trials were digitized (sampling rate 5 kHz) using a CED 1401 A/D converter and Signal 5 software (Cambridge Electronic Design). EMG signals were monitored via visual feedback on a computer screen.

Transcranial magnetic stimulation

We used a 70-mm figure-of-eight-shaped magnetic coil to perform monophasic TMS through a Magstim BiStim^2. The coil was held tangential to the scalp with the handle oriented backwards and 45° from the midline. The induced current presented a posterior-anterior (PA) direction activating preferentially I1 waves (Sakai *et al.*, 1997; Di Lazzaro *et al.*, 1998). Intensities were expressed as a percentage of the maximum stimulator output (%MSO). TMS was delivered over the FDI 'hot spot'. We measured the individual RMT, defined as the minimum TMS output intensity required to evoke a MEP peak-to-peak amplitude of $\geqslant 0.05$ mV in 5 of 10 trials in the resting FDI. Once the RMT was determined, we assessed SICI, SICF, and ICF. The SICI and ICF protocol consisted of a suprathreshold test stimulus preceded by a subthreshold conditioning stimulus (Kujirai *et al.*, 1993). We tested inter-stimulus intervals (ISIs) of 2 and 3 ms for SICI, and 10 ms for ICF. The SICF protocol consisted of a suprathreshold test stimulus followed by the subthreshold conditioning stimulus (Tokimura *et al.*, 1996; Ziemann *et al.*, 1998). To focus on the first peak of the I-wave interaction, we assessed SICF for ISIs of 1.2, 1.4, and 1.6 ms (Ziemann *et al.*, 1998; Dileone *et al.*, 2018). We randomized all ISIs with the test stimuli. Test stimulus intensity was adjusted to evoke an unconditioned MEP amplitude of ~1 mV. For the conditioning stimulus of SICI, ICF and SICF we used a relatively low intensity stimulus (80% of RMT), to maximize inhibition (Kujirai *et al.*, 1993; Ibañez *et al.*, 2020) and minimize contamination of SICI by SICF (Ilic *et al.*, 2002; Peurala *et al.*, 2008; Ni *et al.*, 2013). We delivered a median of six to seven trials per ISI and 12–14 trials for the test stimulus, with 6 s ± 10% intertrial interval. Experiments were carried out without neuronavigation, knowing that for the primary motor cortex the supposed advantage of navigated-TMS remains debatable (Gugino *et al.*, 2001; Sparing *et al.*, 2008; Julkunen *et al.*, 2009; Jung *et al.*, 2010). Note that healthy controls were studied only on the non-

dominant hemisphere, as there is no clear evidence supporting hemispheric laterality of SICI (Cahn *et al.*, 2003; Ilic *et al.*, 2004; Bäumer *et al.*, 2007), particularly using conditioning stimuli at 80% (Hammond *et al.*, 2004).

Data analysis

All analyses were performed with fully automatic procedures in MATLAB (MathWorks, Natick, MA). Single-trial MEP amplitude was estimated as the peak-to-peak EMG response in a 10–60 ms window after the test stimulus. Single-subject MEP amplitude was estimated by aggregating single trial MEP amplitudes across trials and within ISIs, using either (i) the mean of single-trial MEP amplitudes (default); (ii) the median; or (iii) the exponential of the mean of log-transformed single-trial MEP amplitudes. When paired-pulse measures were pooled across ISIs, single-subject MEP amplitudes were averaged across ISIs, weighted by the available trials per ISI. Single-trial EMG background was estimated as the peak-to-peak EMG activity from 30 ms before to 18 ms after the test stimulus, excluding stimulus artefacts. Single-subject EMG background was estimated by averaging across trials the single-trial EMG background. SICI was estimated with either (i) all trials (default); (ii) only one trial (i.e. the very first test stimulus and the very first conditioning stimulus at 2 ms); (iii) only trials with EMG background within 2 standard deviations (SD) above the mean; or (iv) only trials with EMG background <0.05 mV. In the latter case, we considered only subjects that retained at least 80% of trials, and we refer to them as 'EMG-clean subjects'. Paired-pulse measures (i.e. SICI, SICF and ICF) were expressed as per cent change of single-subject MEP amplitudes for the conditioned stimulus (CS) compared to the test stimulus alone (TS), i.e. $100 \times [(MEP_{CS} - MEP_{TS})/MEP_{TS}]$. Throughout the text, we refer to the absolute value of this measure when mentioning the amount of paired-pulse inhibition (negative % change) or facilitation (positive % change). For example, –20% indicates less inhibition than –80%.

Statistical analysis

Comparisons between healthy controls and patients were performed with either (i) two-tailed unpaired *t*-tests; (ii) one-way independent-measures ANOVA; or (iii) one-way independent-measures analysis of covariance (ANCOVA), including age, gender, test MEP amplitude, EMG background and RMT as covariates, followed by Tukey's *post hoc* tests. Note that even though MEP ratios are typically not normally distributed, the normality of residuals assumed by parametric methods is respected when the sample sizes are sufficiently large due to the central limit theorem. Nevertheless, we also confirmed the main findings with the following non-parametric methods: (i) the area under the curve (AUC) of receiver operating characteristic (ROC) analysis, which is proportional to the Mann-Whitney U-statistic and provides a direct measure of discriminability; and (ii) the Kruskal-Wallis test as a non-parametric equivalent of one-way ANOVA. Comparisons for binary variables (e.g. gender) were also performed with *t*-tests (for between-subjects comparisons of binary variables, the *t*-test is completely equivalent to the chi-squared test, see D'agostino *et al.*, 1988; Andrés *et al.*, 1995). Comparisons between more affected and less affected side were performed with either (i) two-tailed paired *t*-tests; (ii) one-way repeated-measures ANCOVA, including side differences of test MEP amplitude, EMG background and RMT as covariates; or (iii) two-way ANCOVA, with side as within-subjects factor, group as between-subjects factor (i.e. levodopa-naïve, non-dyskinetic, dyskinetic) and covariates as above. In case of missing values, the subject was excluded. Correlations were assessed with Pearson's correlation coefficient R, after averaging SICI across groups of 10 subjects sorted by increasing values of the neurophysiological or clinical variable of interest. The overall sample size ($n = 166$ patients) was chosen to be one order of magnitude larger compared to the typical sample size of previous studies [$n = 13.9 \pm 4.5$ patients (mean ± SD) in 14 studies included in the review by Latorre *et al.*, 2019]. The level of significance was thus conservatively set at $P < 0.01$, whereas $P < 0.05$ was considered as tendency and $P < 0.1$ as mild tendency. Results are reported as mean ± SD in the text and with notched box plots in the figures [horizontal lines: median (Q2), first quartile (Q1) and third quartile (Q3); whiskers: minimum and maximum value excluding outliers; outliers: points larger than $Q3 + 1.5(Q3-Q1)$ or smaller than $Q1-1.5(Q3-Q1)$; notch: $Q2 \pm 1.57(Q3-Q1)/\sqrt{(n)}$]. Outliers reduce the probability of type I errors and increase the probability of type II errors, so eliminating outliers increases power at the expenses of inflating type I error rates (Bakker and Wicherts, 2014). All statistical analyses were thus performed without excluding outliers, and were also repeated after excluding the outliers (Supplementary Table 1).

Data availability

The data that support the findings of this study are available upon reasonable request.

Results

Demographics and clinical data

We studied the excitability of the motor cortex contralateral to the more affected side of the body in 166 patients with Parkinson's disease (57 levodopa-naïve, 50 non-dyskinetic, 59 dyskinetic) compared to 40 healthy controls. Clinical characteristics are provided in Table 1. Clinical differences between levodopa-naïve, non-dyskinetic and dyskinetic groups were consistent with disease progression. Note, however, that MDS-UPDRS-III scores were significantly different [one-way ANOVA, $F(2,158) = 24.5$, $P < 0.001$] between levodopa-naïve and both non-dyskinetic (Tukey, $P < 0.001$) and dyskinetic patients ($P < 0.001$), but not between non-dyskinetic and dyskinetic patients ($P = 0.996$).

SICI is reduced in Parkinson's disease

The grand-average MEPs for SICI using all available trials from all subjects showed that MEP amplitudes elicited by paired-pulse stimuli were similar when conditioning stimuli were delivered 2 or 3 ms before the test stimulus, both in healthy controls (paired *t*-test, $P = 0.61$, $n = 40$; Fig. 2A) and in patients with Parkinson's disease ($P = 0.19$, $n = 166$;

Table 1 Demographic and clinical characteristics

	Controls $n = 40$	*De novo* $n = 42$	Levodopa-naïve $n = 57$	Non-dyskinetic $n = 50$	Dyskinetic $n = 59$	All patients $n = 166$
Gender, % females	52.5	35.7	35.1	16.0	49.2	34.3
P-value	–	–	0.28	**0.002**	0.99	0.034
Age, years ± SD	55.6 ± 11.1	54.7 ± 10.4	56.7 ± 10.8	62.0 ± 9.8	66.4 ± 9.1	61.8 ± 10.7
P-value	–	–	0.96	0.018	**< 0.001**	**0.001**
Most affected hemisphere, % left	–	40.5	40.4	42.0	66.1	50.0
Disease duration, years ± SD	–	1.1 ± 0.7	1.6 ± 1.5	6.8 ± 3.4	10.6 ± 4.5	6.4 ± 5.0
LEDD, mg	–	–	56.85 ± 122.0	754.9 ± 420.2	1013.1 ± 334.7	610.4 ± 516.5
MDS-UPDRS-III OFF	–	19.4 ± 9.5	20.6 ± 9.7	32.1 ± 10.2	32.3 ± 9.9	28.1 ± 11.4
MDS-UPDRS-III MA	–	11.3 ± 4.6	11.4 ± 4.5	15.6 ± 5.4	13.3 ± 3.7	13.2 ± 4.7
MDS-UPDRS-III LA	–	2.6 ± 3.5	3.4 ± 3.8	6.6 ± 4.0	6.6 ± 3.3	5.4 ± 4.1

Note that the *de novo* drug-naïve group ($n = 42$) is a subset of patients from the levodopa-naïve group ($n = 57$). LEDD = levodopa-equivalent daily dose; LA = less affected side; MA = more affected side (both OFF medication). The *P*-values in the comparison between controls and all patients represent unpaired *t*-tests. The *P*-values in the comparison between controls, levodopa-naïve, non-dyskinetic and dyskinetic patients represent Tukey *post hoc* tests against controls after one-way ANOVA [gender: $F(3,202) = 6.1$ $P = 0.001$; age: $F(3,200) = 12.8$ $P < 0.001$].

Fig. 2B). We thus pooled 2–3-ms SICI for subsequent analyses. SICI was significantly lower (i.e. less inhibition) in patients with Parkinson's disease (–34.4 ± 41.2%) compared to healthy controls (–69.3 ± 20.6%; unpaired *t*-test: $P < 0.001$; Fig. 2C). This result was essentially the same when SICI was estimated calculating single-subject MEP amplitudes using either (i) means; (ii) medians; or (iii) exponential means of logarithms, and using either (i) all subjects and all trials; (ii) all subjects and only trials with EMG within 2 SD from the mean; (iii) all subjects and only one trial per subject (i.e. the first test MEP and the first MEP conditioned at 2 ms); or (iv) all controls ($n = 40$) compared with patients in whom the more affected side corresponded with the non-dominant hand ($n = 81$) (Supplementary Table 2).

To quantify the ability of SICI to discriminate between patients with Parkinson's disease ($n = 166$) and control subjects ($n = 40$), we performed a ROC analysis. With all subjects and all trials, the AUC was 0.80, with a sensitivity of 0.77 and a specificity of 0.75 (critical SICI –62.9%), suggesting good discriminative ability. Similar AUC values were obtained with the different estimates of SICI (Supplementary Table 2). Unless otherwise specified, all subsequent analyses were thus performed with SICI estimated from means of trials, with all available trials and all available subjects.

Addressing potential confounding factors

Demographic and neurophysiological differences between patients with Parkinson's disease and controls represent potential confounding factors for the main result that SICI is decreased in Parkinson's disease. We thus carried out additional analyses to exclude these confounding factors.

The first potential confounding factor is gender. There was a tendency towards a smaller percentage of females in the Parkinson's disease sample (34.3%) compared to the control group (52.5%; *t*-test: $P = 0.034$). However, there were no differences in SICI between males and females in patients with Parkinson's disease (*t*-test: $P = 0.63$) or in the control group (*t*-test: $P = 0.12$). Furthermore, SICI was significantly decreased in patients compared to controls separately for both males ($P = 0.002$) and females ($P < 0.001$).

The second potential confounding factor is age. The control group was age-matched to the levodopa-naïve group (Table 1), so patients with Parkinson's disease overall were slightly but significantly older (61.8 ± 10.7 years) than controls (55.6 ± 11.1 years; $P = 0.001$). However, SICI did not correlate with age in our patients ($R = 0.08$, $P = 0.77$; Fig. 3A). Furthermore, SICI was significantly decreased in levodopa-naïve patients (–36.5 ± 44.9%; $n = 57$) compared to controls (*t*-test: $P < 0.001$; AUC = 0.77). The same result was obtained in *de novo* patients (–31.7 ± 46.6%; $n = 42$) compared to controls (*t*-test: $P < 0.001$; AUC = 0.80; Supplementary Fig. 1A).

The third potential confounding factor is test MEP amplitude, which tended to be higher in patients (1.54 ± 0.81 mV) compared to controls (1.21 ± 0.62 mV; $P = 0.018$), probably due to the higher EMG background in patients (see below). However, SICI showed a tendency to correlate with test MEP amplitude in patients with Parkinson's disease, with greater amplitude corresponding to greater inhibition ($R = –0.53$, $P = 0.035$; Fig. 3B). This suggests that we might have overestimated SICI in patients, which is conservative for our main result. When we selected only subjects with a test MEP amplitude range of 0.5–1.5 mV (healthy controls: 1.02 ± 0.27 mV, $n = 26$; Parkinson's disease: 1.03 ± 0.27 mV, $n = 82$; $P = 0.84$) the difference in SICI was confirmed (healthy controls: –72.7 ± 18.4%, Parkinson's disease: –33.8 ± 39.3%; $P < 0.001$; AUC = 0.84).

The fourth potential confounding factor is EMG background, which also tended to be higher in patients (0.068 ± 0.059 mV) compared to controls (0.045 ± 0.032 mV; *t*-test: $P = 0.018$). Crucially, SICI was significantly correlated with EMG background (i.e. greater EMG

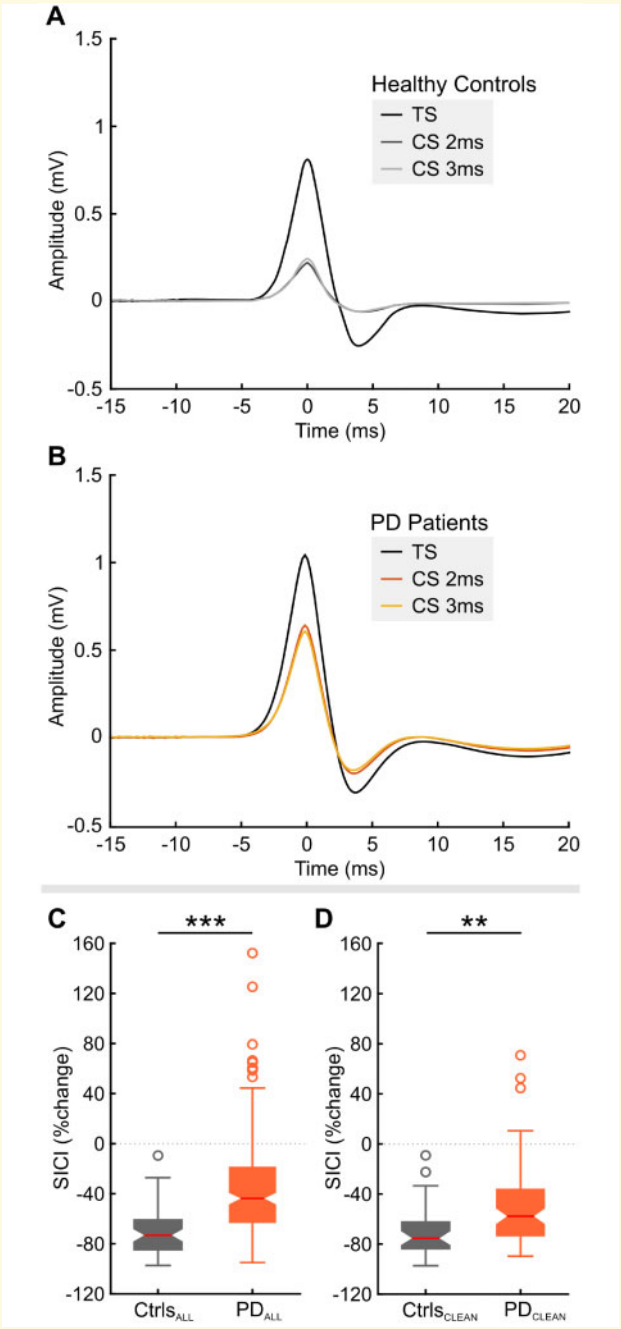

Figure 2 Reduction of SICI in Parkinson's disease. (**A**) Grand-average MEPs for SICI of healthy controls obtained by averaging all single-pulse and paired-pulse MEP trials after temporal realignment of each trial to the MEP peak. (**B**) Same as in **A** for more affected side of patients with Parkinson's disease. Note that in both **A** and **B**, conditioning stimuli delivered at 2 or 3 ms before the test stimulus induced similar inhibitory effects, so the two intervals were pooled for subsequent analyses. (**C**) SICI of the more affected side of patients with Parkinson's disease (PD_{ALL}, $n = 166$) compared to the healthy control group ($Ctrls_{ALL}$, $n = 40$) using all MEP trials. *T*-test or ANCOVA, $^{***}P < 0.001$. (**D**) SICI after conservatively eliminating all trials with EMG background >0.05 mV, presenting 'EMG-clean subjects' that retained at least 80% of trials after cleaning (PD_{CLEAN}, $n = 65$; $Ctrls_{CLEAN}$, $n = 26$). *T*-test or ANCOVA, $^{**}P < 0.01$. CS = conditioned stimulus; TS = test stimulus.

background, smaller inhibition) in patients with Parkinson's disease (R = 0.64, $P = 0.008$; Fig. 3C). To fully exclude this potential confound, we thus performed a conservative analysis eliminating all trials with EMG background >0.05 mV, using only subjects that retained at least 80% of trials ($n = 65$ patients, $n = 26$ controls). In these 'EMG-clean subjects', there were no differences in EMG background ($P = 0.20$) or in test MEP amplitude ($P = 0.22$) between patients and controls. Again, SICI was significantly reduced in Parkinson's disease (–48.5 ± 33.6%) compared to controls (–70.8 ± 21.7%, $P = 0.002$; AUC = 0.74; Fig. 2D).

An additional potential confounding factor is RMT. However, we observed only a mild tendency for RMT to be lower in patients with Parkinson's disease compared to controls, both using the entire sample ($P = 0.096$; Table 2) or only the 'EMG-clean subjects' ($P = 0.089$). Furthermore, there was no significant correlation between SICI and RMT in patients with Parkinson's disease (R = 0.05, $P = 0.85$; Fig. 3D).

Finally, the decrease of SICI in patients with Parkinson's disease was confirmed when correcting for all potential confounding factors (age, gender, test MEP amplitude, EMG background, RMT), both in the entire sample [ANCOVA, $F(1,197) = 24.1$, $P < 0.001$] and in the 'EMG-clean subjects' [$F(1,83) = 7.9$, $P = 0.006$]. Overall, these results strongly support that SICI is reduced in Parkinson's disease.

SICI is similarly reduced in patients at different disease stages

To investigate whether the reduction of SICI in the more affected side evolves with the progression of the disease, we separated patients into levodopa-naïve ($n = 57$), non-dyskinetic ($n = 50$), and dyskinetic ($n = 59$) groups, and reanalysed the data considering these different disease stages and correcting for all potential confounding factors (age, gender, test MEP amplitude, EMG background, RMT). SICI was decreased compared to controls at all disease stages [ANCOVA, $F(3,195) = 8.3$, $P < 0.001$; Tukey, $P < 0.001$], with no significant differences between levodopa-naïve (–36.5 ± 44.9%), non-dyskinetic (–30.2 ± 44.2%) and dyskinetic patients (–35.9 ± 35.0%; Tukey $P > 0.78$, Fig. 4A). Additionally, we separated patients with Parkinson's disease ($n = 166$) into two subgroups based on their SICI being lower or higher than the median (–43.9%). In the group with SICI values below the median (–5.2 ± 39.4%, $n = 83$) the proportion of levodopa-naïve, non-dyskinetic and dyskinetic patients was 32.5%, 31.3% and 36.1%, respectively. In the subgroup with SICI values above the median (–63.5 ± 12.4%, $n = 83$) the proportion of levodopa-naïve, non-dyskinetic and dyskinetic patients was 36.1%, 28.9% and 34.9%, respectively. There were no significant differences in the proportion of levodopa-naïve, non-dyskinetic and dyskinetic patients between these two subgroups [$\chi^2(2) = 0.25$, $P = 0.88$]. These results suggest that the reduction of SICI is similar in patients at different disease stages.

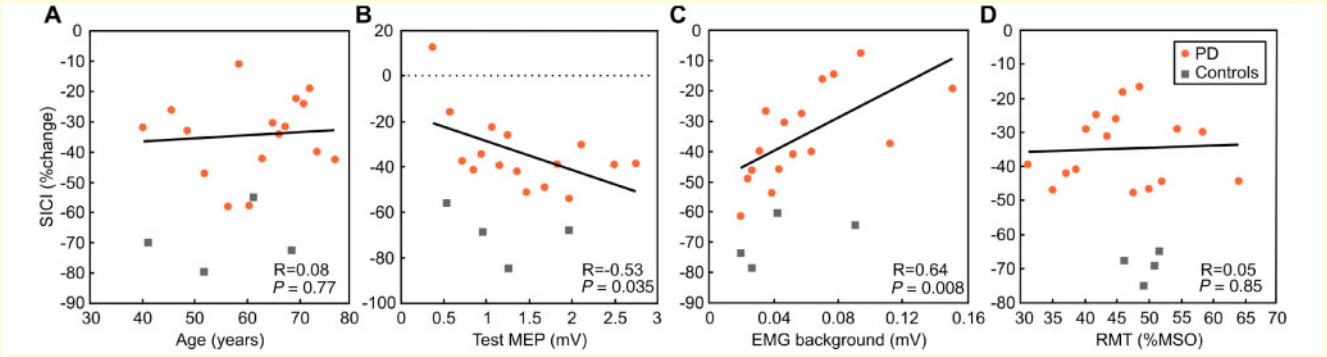

Figure 3 Correlations between SICI and potential confounding factors. (**A**–**D**) Each circle and square represents the average SICI of 10 subjects [patients with Parkinson's disease (PD) or healthy controls] grouped by increasing values of age (**A**), test MEP amplitude (**B**), EMG background (**C**), and RMT (**D**).

Table 2 Neurophysiological characteristics

	Controls	*De novo*	Levodopa-naïve	Non-dyskinetic	Dyskinetic	All patients
RMT, %MSO	49.3 ± 8.8	48.0 ±9.7	48.3 ± 10.3	44.7 ± 9.2	46.7 ± 9.3	46.6 ± 9.7
P-value	–		0.95	0.10	0.54	0.096
1 mV, %MSO	60.2 ± 11.2	60.2 ± 12.7	60.2 ± 12.9	55.8 ± 11.6	60.9 ± 13.0	59.1 ± 12.6
P-value	–	–	1.00	0.33	0.99	0.60
Test MEP (mV)	1.21 ± 0.62	1.53 ± 0.80	1.49 ± 0.74	1.63 ± 0.96	1.51 ± 0.76	1.54 ± 0.81
P-value	–		0.32	0.063	0.24	0.018
EMG background (mV)	0.04 ± 0.03	0.06 ± 0.04	0.06 ± 0.04	0.08 ± 0.08	0.07 ± 0.05	0.07 ± 0.06
P-value	–	–	0.53	0.015	0.26	0.018

Note that the *de novo* drug-naïve group ($n = 42$) is a subset of patients from the levodopa-naïve group ($n = 57$). MSO = maximum stimulator output. The *P*-values in the comparison between controls and all patients represent unpaired *t*-tests. The *P*-values in the comparison between controls, levodopa-naïve, non-dyskinetic and dyskinetic patients represent Tukey *post hoc* tests against controls after one-way ANOVA [RMT: $F(3,202) = 2.1$, $P = 0.10$; 1 mV: $F(3,200) = 1.9$, $P = 0.14$; Test MEP: $F(3,202) = 2.2$, $P = 0.090$; EMG background: $F(3,202) = 3.1$, $P = 0.027$].

SICI is also reduced in the less affected side

In most patients ($n = 145$), we also evaluated the excitability of the motor cortex contralateral to the less affected side of the body. In the overall group of patients, we observed a mild tendency for SICI to be slightly higher (i.e. more inhibition) in the less affected side (–42.7 ± 40.5%) compared to the more affected side (–35.0 ± 42.0%; paired *t*-test: $P = 0.050$), which gained significance after correcting for all potential confounding factors [side differences in test MEP amplitude, EMG background and RMT; ANCOVA, $F(1,141) = 7.0$, $P = 0.009$]. When patients were separated in groups as levodopa-naïve ($n = 55$), non-dyskinetic ($n = 42$) and dyskinetic ($n = 48$), we observed that the difference in SICI between more affected and less affected side depended on disease stage [two-way mixed ANCOVA, interaction group × side: $F(2,139) = 3.6$, $P = 0.029$]. Specifically, SICI tended to be higher (i.e. more inhibition) in the less affected side compared to the more affected side in the levodopa-naïve group (Tukey, $P = 0.034$), whereas no significant side differences were observed in the non-dyskinetic and dyskinetic groups ($P > 0.79$).

Importantly, SICI in the less affected side was still significantly reduced compared to controls [*t*-test, $P < 0.001$; AUC = 73; ANCOVA, $F(1,176) = 13.1$, $P < 0.001$]. The reduced SICI on the less affected side compared to controls was also significant in the levodopa-naïve group alone [*t*-test, $P = 0.004$; AUC = 0.66; ANCOVA, $F(1,86) = 8.5$, $P = 0.005$; Fig. 4B], in the subset of fully drug-naïve *de novo* patients [*t*-test, $P = 0.002$; AUC = 0.69; ANCOVA, $F(1,74) = 11.8$, $P < 0.001$], and even in a subset of *de novo* patients with a minimally symptomatic side [$n = 23$: lateralized MDS-UPDRS-III = 0 or 1; *t*-test, $P = 0.002$; AUC = 0.73; ANCOVA, $F(1,55) = 7.6$, $P = 0.008$] (Fig. 4C). These results suggest that SICI is reduced early in the evolution of the disease and reduced SICI could be a prodromal feature of Parkinson's disease.

Clinical correlations

If the reduction of SICI develops before the onset of motor features, remains essentially consistent across disease stages and displays only minor differences between the more affected and less affected side in the early stages, then it is unlikely to primarily reflect disease severity. Accordingly,

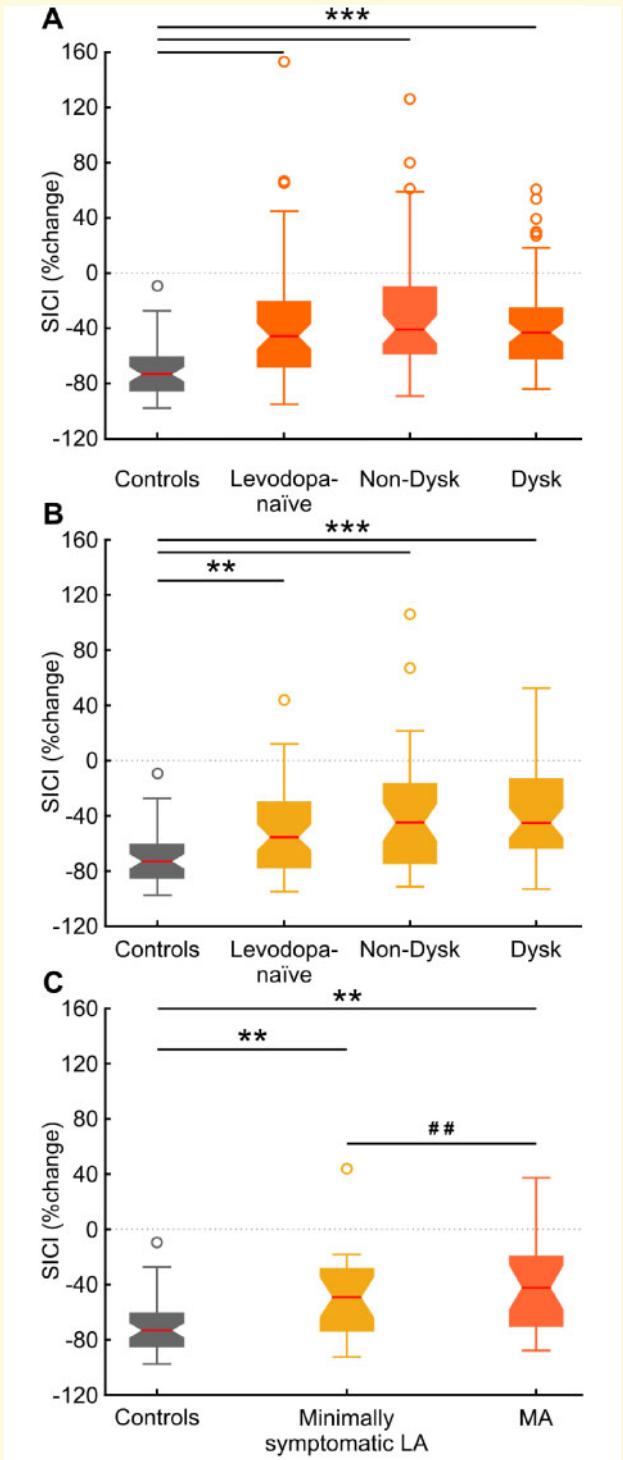

Figure 4 Decreased SICI at all disease stages (**A**) SICI in the more affected side of levodopa-naïve ($n = 57$), non-dyskinetic ($n = 50$), and dyskinetic ($n = 59$) patients with Parkinson's disease compared to healthy controls ($n = 40$). Tukey *post hoc* tests, $^{***}P < 0.001$. (**B**) SICI in the less affected side of levodopa-naïve ($n = 55$), non-dyskinetic ($n = 42$), and dyskinetic ($n = 48$) patients with Parkinson's disease compared to healthy controls ($n = 40$). Tukey *post hoc* tests, $^{**}P < 0.01$; $^{***}P < 0.001$. Outlier at 196.8% in the dyskinetic group not shown. (**C**) SICI of a subset of highly-asymmetric *de novo* patients with a minimally symptomatic less affected (LA) side (lateralized MDS-UPDRS-III = 0 or 1, $n = 23$) and SICI of corresponding more affected side (MA, lateralized MDS-UPDRS-III = 8.6 ± 3.6, $n = 23$) compared to healthy controls ($n = 40$). Independent-measures ANCOVA, $^{**}P < 0.01$; repeated-measures ANCOVA $^{\#\#}P < 0.01$.

SICI did not correlate with the MDS-UPDRS-III (R = 0.06, $P = 0.83$) or with disease duration (R = –0.17, $P = 0.53$).

Reduced SICI is not associated with increased SICF or ICF

To clarify whether reduced SICI reflected an alteration of genuine inhibition or was secondary to an increase of excitation, we also measured SICF and ICF using conditioning stimuli at the same intensity used for SICI (80% RMT). The grand-average MEPs for SICF using all available trials from all subjects showed that conditioning stimuli delivered 1.2 ms, 1.4 ms or 1.6 ms after the test stimulus induced similar facilitatory effects (Fig. 5A and B). The three ISIs of SICF were thus pooled for subsequent analyses. Correcting for all potential confounding factors (age, gender, test MEP amplitude, EMG background, RMT) we observed no significant differences in SICF between patients with Parkinson's disease and controls, for either the entire sample [ANCOVA: $F(1,190) = 0.63$, $P = 0.43$; Fig. 5C], only the 'EMG-clean subjects' (Fig. 5D), or considering the *de novo* group alone (Supplementary Fig. 1B), and no differences across disease stages [$F(3,188) = 2.0$, $P = 0.12$; Fig. 5E]. We also found no differences between patients and controls for ICF [$F(1,190) = 2.3$, $P = 0.13$; Supplementary Fig. 1C] and no differences across disease stages [$F(3,188) = 1.3$, $P = 0.28$; Supplementary Fig. 2]. Correcting also for SICF and ICF, the difference in SICI between patients with Parkinson's disease and controls remained highly significant [$F(1,185) = 19.6$, $P < 0.001$].

Discussion

The present study shows that SICI is decreased in Parkinson's disease. From a mechanistic perspective, this reduction of SICI was obtained with relatively low-intensity conditioning stimuli (80% RMT) and was not associated with any significant increase in SICF (or ICF) with the same low-intensity conditioning stimuli, supporting a direct involvement of cortical inhibitory circuits. From a clinical perspective, the reduction of SICI was similar in levodopa-naïve, non-dyskinetic and dyskinetic patients. Importantly, SICI was reduced compared to controls also in the less affected side, even in very early *de novo* patients showing minimal motor signs. These results suggest that motor cortex disinhibition develops very early in the evolution of Parkinson's disease, possibly in the prodromal stage before the onset of motor features.

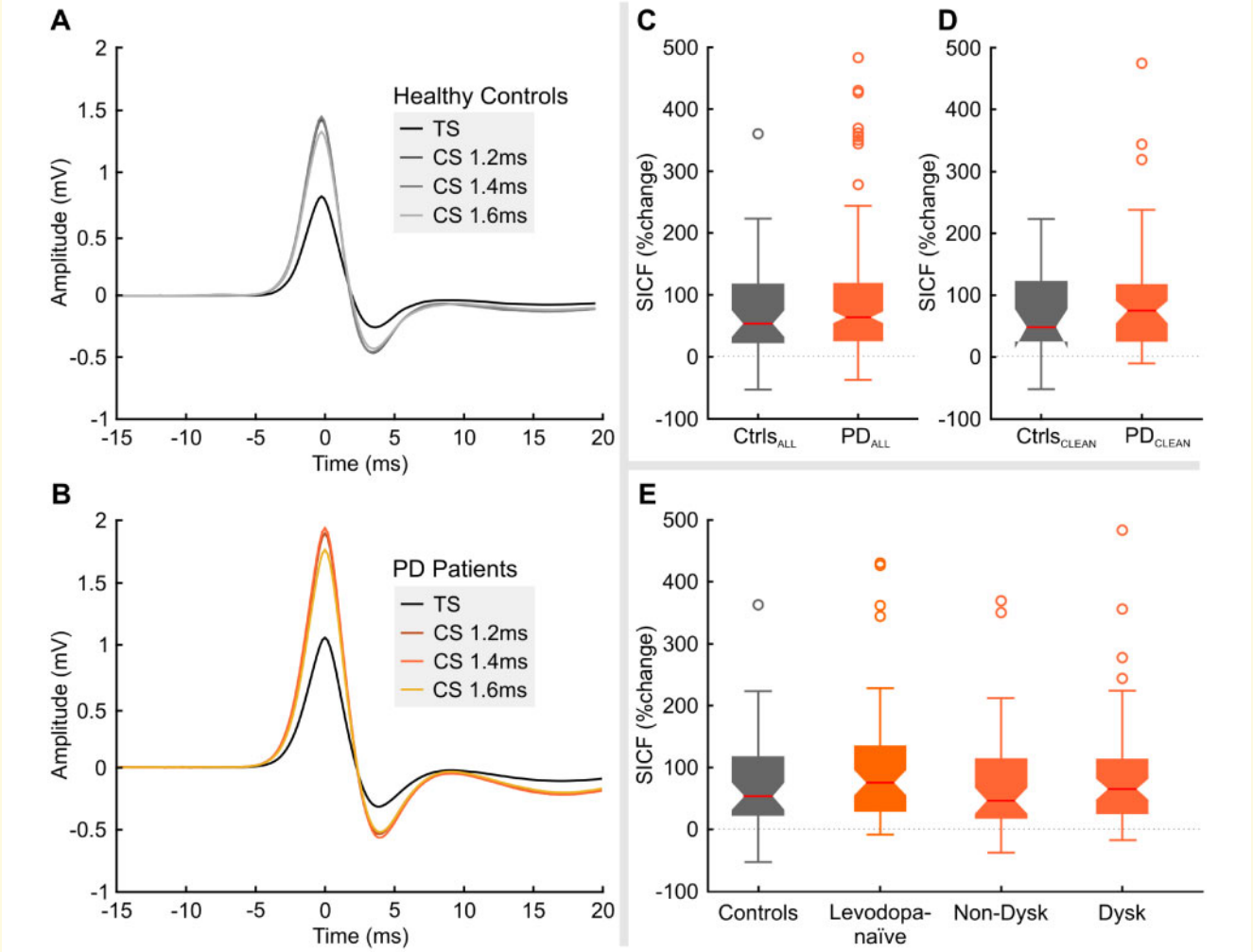

Figure 5 Reduced SICI is not due to increased SICF. (**A**) Grand-average MEPs for SICF (at 80% RMT) of healthy controls obtained by averaging all single-pulse and paired-pulse MEP trials after temporal realignment of each trial to the MEP peak. (**B**) Same as in **A** for more affected side of patients with Parkinson's disease. Note that in both **A** and **B** conditioning stimuli delivered at 1.2 ms, 1.4 ms or 1.6 ms after the test stimulus induced similar facilitatory effects, so the three intervals were pooled for subsequent analyses. (**C**) SICF from more affected side of patients with Parkinson's disease (PD_{ALL}) compared to healthy control group ($Ctrls_{ALL}$) using all MEP trials. (**D**) SICF after conservatively eliminating all trials with EMG background >0.05 mV, presenting 'EMG-clean subjects' that retained at least 80% of trials after cleaning (PD_{CLEAN}, $n = 62$; $Ctrls_{CLEAN}$, $n = 26$). (**E**) SICF in the more affected side of levodopa-naïve ($n = 57$), non-dyskinetic ($n = 46$), and dyskinetic ($n = 56$) patients with Parkinson's disease compared to healthy controls ($n = 40$). CS = conditioned stimulus; TS = test stimulus.

SICI is reduced in Parkinson's disease

The first aim of the present study was to clarify whether or not SICI is altered in Parkinson's disease, which had remained uncertain owing to variable if not conflicting results in the literature (Latorre *et al.*, 2019). From a methodological point of view, our work has four main strengths: (i) we studied a number of patients ($n = 166$), which is one order of magnitude higher than most previous studies; (ii) all analyses were performed with fully automatic procedures to exclude possible confirmation bias; (iii) we systematically investigated—and if necessary excluded—several possible confounding factors, such as gender, age, test MEP amplitude, EMG background, and RMT; and finally, (iv) we delivered conditioning stimuli at relatively low intensity (80% RMT) to maximize inhibition (Kujirai *et al.*, 1993; Ibañez *et al.*, 2020) and minimize contamination of SICI by SICF (Ilic *et al.*, 2002; Peurala *et al.*, 2008; Ni *et al.*, 2013)

In our patients with Parkinson's disease, SICI did not correlate with gender, age, or RMT, while it did correlate with test MEP amplitude and EMG background. The absence of correlation between SICI and gender may seem to contrast with a recent study showing stronger SICI (i.e. more inhibition) in female compared to male patients with Parkinson's disease (Kolmancic *et al.*, 2019). However, this discrepancy between studies is likely due to the use of different conditioning stimulus intensities: 90% RMT (Kolmancic *et al.*,

2019) versus 80% RMT (present study). In fact, at higher intensities of the conditioning stimulus, facilitatory processes start contributing to SICI measurements (Ilic *et al.*, 2002; Peurala *et al.*, 2008). Excitatory rather than inhibitory cortical circuits may thus underlie the possible gender-related differences of motor cortex excitability in Parkinson's disease.

On the other hand, the absence of correlation between SICI and RMT is important to support the consistency of the paired-pulse inhibitory protocol across subjects, while the absence of correlation with age is in line with a previous meta-analysis showing no significant age-related differences for SICI in healthy subjects (Bhandari *et al.*, 2016), at least in the resting muscle (Opie and Semmler, 2014). The correlation between SICI and test MEP amplitude (i.e. greater test MEP amplitude, more inhibition) is also consistent with previous studies in healthy subjects (Sanger *et al.*, 2001; Wagle-Shukla *et al.*, 2009; Udupa *et al.*, 2014; Miyaguchi *et al.*, 2017) and, since test MEP amplitude tended to be higher in our patients with Parkinson's disease, this is conservative for our main result of reduced SICI in Parkinson's disease. Finally, the correlation between SICI and EMG background (i.e. more muscle activation, less inhibition) was also expected (Ortu *et al.*, 2008), representing a crucial possible confounder. Even though SICI was reduced in Parkinson's disease compared to controls at all levels of EMG background, we fully excluded this possible confounder by performing very conservative sub-analyses eliminating all trials with background peak-to-peak EMG activity >0.05 mV. After correcting for all confounders, the reduction of SICI remained highly significant.

Several limitations of our study should also be considered. First, control subjects were studied only on their non-dominant side. There is no clear evidence supporting hemispheric laterality of SICI (Cahn *et al.*, 2003; Ilic *et al.*, 2004; Bäumer *et al.*, 2007), particularly with conditioning stimuli at 80% RMT (Hammond *et al.*, 2004). Nevertheless, we corroborated that SICI was significantly reduced compared to controls even considering only patients whose more affected side corresponded to the non-dominant hand. Second, we used a relatively low number of trials per ISI (six to seven; but note that in pooling the two ISIs for SICI we used 12–14 trials). However, our high number of subjects should have enabled us to largely overcome the possible loss of statistical power. To support this point, we showed that the reduction of SICI in Parkinson's disease compared to controls remained highly significant even using as low as one trial per subject. Indeed, increasing the number of trials would increase the reliability at the single-subject level, and therefore may enhance the ability of SICI to discriminate between patients and healthy controls. In this regard, it should be taken into account that reduced SICI is not specific to Parkinson's disease and has been reported in several other disorders, such as dystonia (Quartarone and Hallett, 2013), major depression (Lefaucheur *et al.*, 2008; Levinson *et al.*, 2010), obsessive-compulsive disorder (Greenberg *et al.*, 1998) and amyotrophic lateral sclerosis (Shibuya *et al.*, 2016) among others. Finally, we focused on SICI without considering other measures of cortical inhibition, such as cortical silent period (SP), long-interval intracortical inhibition (LICI), or short-afferent inhibition (SAI), which have previously been assessed in Parkinson's disease with mixed results (Dubbioso *et al.*, 2019; Latorre *et al.*, 2019). Future work should thus clarify to what extent different measures and mechanisms of cortical inhibition may be altered in Parkinson's disease.

Reduced SICI likely reflects alteration of cortical inhibitory circuits in Parkinson's disease

Our second aim was to clarify whether the reduction of SICI in Parkinson's disease may reflect solely a shift in the excitation/inhibition balance towards excitation or a genuine loss of cortical inhibition. The excitatory interpretation was originally proposed in an elegant study by MacKinnon *et al.* (2005), who found that the reduction of SICI in patients with Parkinson's disease OFF medication compared to healthy controls was not significant when conditioning stimuli were delivered at 80% RMT (more specific to inhibitory circuits, Kujirai *et al.*, 1993), but it became significant at 90–100% RMT (activating both inhibitory and excitatory circuits). Our results suggest that their negative result at 80% RMT was likely due to the small sample size ($n = 12$). More recently, several studies have shown that SICF is indeed increased in patients with Parkinson's disease compared to controls (Ni *et al.*, 2013; Guerra *et al.*, 2019; Shirota *et al.*, 2019). In the original study by Ni *et al.* (2013), increased SICF likely accounted for the reduction of SICI when the conditioning stimuli were delivered at SICF peaks (≈1.5 ms, ≈3 ms and ≈4.5 ms). However, SICI also decreased in patients with Parkinson's disease compared to controls when the conditioning stimuli were delivered at 1 ms and at the first SICF trough (2–2.5 ms), suggesting reduction of genuine inhibition (Ni *et al.*, 2013). Therefore, alterations of both inhibitory and excitatory cortical circuits likely coexist in Parkinson's disease.

We tested SICI at 2 and 3 ms, but we did not directly test SICF at 2 and 3 ms, so we cannot completely exclude a possible contamination of SICF in the reduced SICI in our patients with Parkinson's disease. However, several arguments make this possibility unlikely, and support the view that the reduced SICI reflects alteration of cortical inhibitory circuits. *A priori*, the intensity of our conditioning stimuli (80% RMT) was specifically chosen because of two reasons: (i) 80% RMT seems to maximize the activation of inhibitory circuits (Kujirai *et al.*, 1993; Ibañez *et al.*, 2020); and (ii) SICF at that intensity does not differ between Parkinson's disease and controls at either the first trough (~2–2.5 ms) or the second peak (~3 ms) of the I-wave interaction (Ni *et al.*, 2013). *A posteriori*, SICI was almost identical at 2 ms and 3 ms in both patients and controls, confirming that SICF was unlikely to have contaminated substantially our SICI

measurements. Finally, we found that the same conditioning stimulus intensity (80% RMT) did not induce any significant increase in SICF at 1.2–1.6 ms (first peak) in our patients with Parkinson's disease compared to controls. Importantly, the latter result might seem to contrast with the increase of SICF in Parkinson's disease previously discussed (Ni *et al.*, 2013; Guerra *et al.*, 2019; Shirota *et al.*, 2019). However, even though our SICF protocol did induce significant paired-pulse facilitation, it was purposely chosen as a control for SICI and not to maximize the activation of excitatory circuits. Future work should explore the entire curve of conditioning stimulus intensities within patients. Nevertheless, the overall picture seems to be is that both high-threshold (⩾90% RMT) excitatory intra-cortical circuits (Ni *et al.*, 2013; Guerra *et al.*, 2019; Shirota *et al.*, 2019) and low-threshold (80% RMT) inhibitory intra-cortical circuits are altered in Parkinson's disease.

Several possible pathophysiological mechanisms might contribute to the alteration of cortical inhibitory circuits. A classical explanation is that cortical disinhibition might be an indirect consequence of dopamine depletion due to altered thalamic inputs to the motor cortex (Nambu *et al.*, 1988; Inase and Tanji, 1995) in response to dysfunctional basal ganglia output (McGregor and Nelson, 2019). Interestingly, modern studies in rodents—building upon earlier anatomical works in cats and rats (Reinoso-Suárez *et al.*, 1982; Van der Kooy and Kolb, 1985; Ingham *et al.*, 1988)—characterized a direct pallidocortical pathway targeting primarily cortical GABAergic interneurons (Chen *et al.*, 2015; Saunders *et al.*, 2015). Decreased efferent inhibitory activity of the globus pallidus externus (GPe) due to dopamine depletion (Pan and Walters, 1988; Filion and Tremblay, 1991; Boraud *et al.*, 1998) might thus monosynaptically lead to cortical disinhibition. In addition, direct loss of dopaminergic innervation to the motor cortex (Goldman-Rakic *et al.*, 1992; Williams and Goldman-Rakic, 1993; Guo *et al.*, 2015) and other non-dopaminergic mechanisms might also contribute to cortical disinhibition. Whatever the exact mechanism(s), our results support that not only cortical excitatory circuits, but also cortical inhibitory circuits are altered in Parkinson's disease (Chu *et al.*, 2009; Ni *et al.*, 2013).

Reduced SICI is a very early, possibly prodromal feature of Parkinson's disease

The third aim of the present paper was to investigate whether and to what extent cortical disinhibition may reflect the clinical state of the patients and the evolution of Parkinson's disease. SICI was virtually indistinguishable in levodopa-naïve ($n = 57$), non-dyskinetic ($n = 50$) and dyskinetic ($n = 59$) patients, and did not correlate with disease severity (as measured by either MDS-UPDRS-III or disease duration). It is important to acknowledge that even though the MDS-UPDRS-III score was lower in levodopa-naïve patients, it did not differ between non-dyskinetic and dyskinetic patients, possibly because of underestimation of the true OFF state and/or some selection bias towards a more benign evolution in the dyskinetic compared to non-dyskinetic group (e.g. due to exclusion criterion of neuropsychiatric comorbidities). The absence of SICI differences between patients at different disease stages is in line with three relatively small studies showing (i) no significant differences in SICI between *de novo* untreated and chronically-treated (but OFF medication) patients with Parkinson's disease (Kaçar *et al.*, 2013); (ii) absence of longitudinal SICI changes in early Parkinson's disease (Kojovic *et al.*, 2015); and (iii) absence of SICI differences between non-dyskinetic and dyskinetic patients OFF medication (Barbin *et al.*, 2013). These findings suggest that neither disease severity and evolution, nor chronic exposure to levodopa, nor levodopa-induced motor complications seem to determine the anomalies of cortical inhibitory circuits underlying SICI in Parkinson's disease. SICI *per se* is thus unlikely to directly contribute to the pathophysiology of parkinsonian motor features: it is altered early and appears to remain consistently altered throughout the evolution of the disease. However, longitudinal observations will be required to firmly establish whether and how SICI changes (or not) with the progression of Parkinson's disease.

We observed only minor group differences in SICI between the more affected and less affected side. Notably, in the less affected side SICI was also significantly reduced compared to controls, even in very early *de novo* patients in whom the less affected side was minimally symptomatic (lateralized MDS-UPDRS-III = 0 or 1, $n = 23$). This observation appears to contrast with the seemingly normal SICI previously reported in the minimally affected side of highly-asymmetric patients with Parkinson's disease (Kojovic *et al.*, 2012, 2015). Again, the discrepancy is likely due to the higher intensity of conditioning stimuli used by Kojovic *et al.* (90% RMT), which implies a substantial contribution of SICF in SICI as discussed above. SICF, rather than 'genuine' SICI, may thus be normal in pre-symptomatic Parkinson's disease and correlate with the severity of motor features after their onset (Ni *et al.*, 2013) (Fig. 6). A somewhat parallel pre-symptomatic alteration has been described at the network level with ^{18}F-fluorodeoxyglucose (FDG) PET, showing an increase of the Parkinson's disease motor-related pattern not only in the symptomatic side, but also in the minimally symptomatic side of patients with highly-asymmetric early Parkinson's disease (Tang *et al.*, 2010). Overall, these findings suggest that the alteration of 'genuine' SICI might develop in the prodromal stage of Parkinson's disease, before the onset of motor features (Fig. 6).

Prodromal cortical disinhibition may play a pathophysiological or compensatory role in the adaptation to the initial dopamine depletion (Blesa *et al.*, 2017). Furthermore, it is also tempting to speculate a pathogenic relevance for the

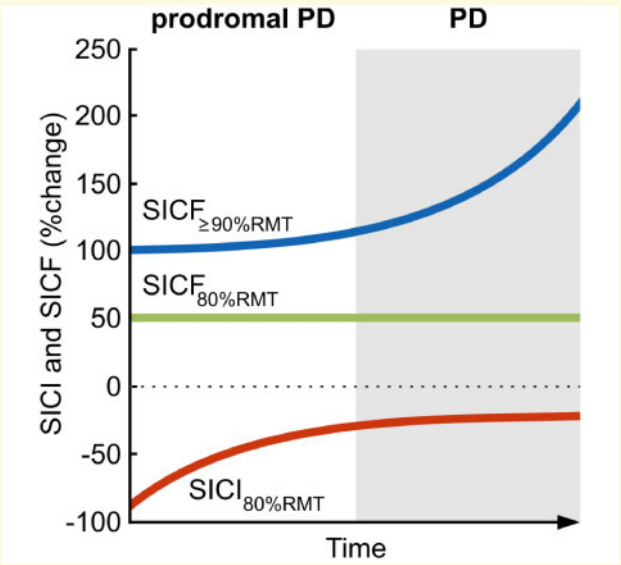

Figure 6 Cortical disinhibition in Parkinson's disease. Schematic representation of the proposed alteration of the equilibrium between excitation and inhibition in the motor cortex throughout the evolution of Parkinson's disease (PD). After the appearance of motor features (grey), Parkinson's disease is characterized by both a stable loss of inhibition, as measured by SICI, and a progressive hyper-excitation, as measured by SICF at high-intensity conditioning (from literature). Conversely, prodromal Parkinson's disease might be characterized by a more specific alteration of inhibition, leading to cortical disinhibition before the onset of motor features.

progression of Parkinson's disease. First, cortical disinhibition may reflect a hitherto unrecognized widespread deficit of GABA function, which could promote neurotoxicity via calcium-mediated mechanisms (Hurley *et al.*, 2013; Błaszczyk, 2016). Second, a loss of cortical inhibition may contribute to corticostriatal hyperactivity, which causes glutamate-dependent dendritic spine loss at striatal projection neurons in animal models of dopamine depletion (Neely *et al.*, 2007; Garcia *et al.*, 2010). In turn, corticostriatal hyperactivity represents a possible stress factor for the degeneration of nigrostriatal terminals in the early evolution of the disease (Foffani and Obeso, 2018). Overall, even though the exact role of cortical disinhibition remains speculative and calls for further investigation, the alterations of cortical inhibitory circuits in Parkinson's disease appear to start much earlier than previously suspected.

Conclusions

The results of the present study suggest that (i) SICI is decreased in Parkinson's disease; (ii) this reduction likely reflects alteration of cortical inhibitory circuits; and (iii) the resulting cortical disinhibition is a very early, possibly prodromal feature of Parkinson's disease.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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