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Title

Paradoxical Facilitation After Depotentiation Protocol Can Precede Dyskinesia Onset in Early Parkinson's Disease.

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Abstract

Loss of dopamine, a key modulator of synaptic signalling, and subsequent pulsatile non-physiological levodopa replacement is believed to underlie altered neuroplasticity in Parkinson's Disease (PD). Animal models suggest that maladaptive plasticity (e.g.: deficient depotentiation at corticostriatal synapses) is key in the development of Levodopa Induced Dyskinesia (LID), a common complication following levodopa replacement in PD. Human studies using Transcranial Magnetic Stimulation protocols have shown similar depotentiation deficit in patients with LID. We hypothesized that subtle depotentiation deficits should precede LID if these deficits are mechanistically linked to LID onset. Moreover, patients on pulsatile levodopa-based therapy may show these changes earlier than those treated with levodopa sparing strategies.

We recruited 22 early non-dyskinetic PD patients (<5 years since diagnosis) and 12 age-matched Healthy Controls, and grouped patients into those on Levodopa-Based (n=11) and Levodopa-Sparing therapies (n=11). Patients were selected to obtain groups matched for age and disease severity. We used a Theta Burst Stimulation protocol to investigate potentiation and depotentiation in a single session.

We report significant depotentiation deficits in the Levodopa-Based group, compared to both Levodopa-Sparing and Healthy Control groups. Potentiation and Depotentiation responses were similar between Levodopa-Sparing and Healthy Controls. Although differences persist after accounting for potential confounds (e.g.: levodopa equivalent dose), these results may yet be caused by differences in disease severity and cumulative levodopa-equivalent dose as discussed in the text.

In conclusion, we show for the first time that paradoxical facilitation in response to depotentiation protocols can occur in PD even prior to LID onset.

Keywords: synaptic plasticity, potentiation, depotentiation, levodopa-induced dyskinesia, Parkinson's disease.

Introduction

Loss of dopaminergic neurons, and consequent loss of a key modulator of synaptic signalling, results in abnormal cortical excitability in early Parkinson's Disease (Bareš et al. 2007; Ridding et al. 1995), that can be largely restored with Levodopa replacement. However, chronic pulsatile non-physiological levodopa replacement is believed to underlie subsequent altered neuroplasticity in Parkinson's Disease (PD).

Abnormal involuntary movements that develop in patients with Parkinson's disease on dopamine replacement therapies, grouped under the umbrella term Levodopa Induced Dyskinesia (LID), are believed to be the consequence of maladaptive neural plasticity in response to non-physiological pulsatile dopamine replacement. Established risk factors for LID are Parkinson's disease severity and duration. Other risk factors include high initial and cumulative doses of L-DOPA, and young age at Parkinson's disease onset (reviewed in Manson and Schrag 2006). However, time to onset of dyskinesia varies considerably among patients and currently the individualized risk cannot be predicted accurately. Studies in rat models of Parkinson's disease have shown that normal plasticity of corticostriatal synapses, measured as the ability to undergo long-term potentiation or depression (LTP, LTD) is reduced or abolished by dopaminergic denervation following chemical lesions of the nigrostriatal tract. LTP is restored by chronic levodopa therapy, but in some animals synaptic depotentiation is not restored, and these rats then go on to develop LID (Picconi et al. 2003).

Advances in neurostimulation have now allowed a similar process of synaptic potentiation and depotentiation to be studied in humans. Using Transcranial Magnetic Stimulation techniques, investigators have been able to demonstrate abnormalities in synaptic plasticity in cortical motor areas of patients diagnosed with Parkinson's disease (for a review see Bologna et al., 2016). In an experiment similar to those from animal models, Huang *et al.* (2011) employed an elegant Theta Burst Stimulation (TBS) based protocol to demonstrate that potentiation and depotentiation were restored with levodopa in non-dyskinetic Parkinson's disease patients. However, in dyskinetic patients just half of the levodopa dose was sufficient to restore potentiation, but subsequent depotentiation could not be induced. Instead, the depotentiation paradigm resulted in a characteristic paradoxical increase in cortical excitability in dyskinetic patients.

While prior studies have reported increasing deficits in LTP/LTD and depotentiation with

advancing Parkinson's disease, becoming most prominent in patients with LID (Huang et al. 2011; Kishore et al. 2012b), the effects of type of drug treatment have not been explored systematically. Patients receiving dopamine agonists rather than levodopa as initial monotherapy have shown a reduced risk for developing LID (Constantinescu et al. 2007; Parkinson Study Group 2000; Holloway 2000; Holloway 2004; Rascol et al. 2000; Rascol et al. 2006; Schrag and Quinn 2000; Watts et al. 2010).

Our hypothesis was that patients on pulsatile levodopa-based therapy may show depotentiation deficits earlier than patients treated with levodopa sparing treatment strategies. We studied neural plasticity in the less affected hemisphere of Parkinson's disease patients in the best 'On' state, using an optimized version of the TBS potentiation-depotentiation paradigm previously used by Huang *et al.* (2011). Parkinson's disease patients were free from LID or significant motor fluctuations, within 5 years of diagnosis, and on dopamine replacement therapies. We also studied the dominant hemisphere in a group of age-matched healthy controls with the same protocol.

Materials and methods

Subjects

All participants gave their informed consent prior to participation. The study was performed with the approval of the Oxfordshire Research Ethics Committee and carried out according to the Declaration of Helsinki.

We recruited 34 participants within an age range of 58-74 years; 22 participants with Parkinson's disease (8 females; mean age: 66.0 ± 4.9, range 59 – 74 years), and 12 age-matched healthy control subjects (5 females; mean age: 65.7 ± 4.3, range 58 – 74 years). Parkinson's disease patients were recruited into two groups based on their therapy: 11 patients on levodopa treatment (with or without co-treatment with dopamine agonists, but levodopa was the mainstay of therapy) (Levodopa-Based group; 3 females; mean age: 68.0 ± 4.8; range 59-74), and 11 patients were on levodopa sparing treatment strategies (e.g.: ropinirole, pramipexole) (Levodopa-Sparing group; 5 females; mean age: 64.1 ± 4.4; range 59-72). We matched for disease severity (On-state UPDRS III scores) between groups of Parkinson's disease patients on Levodopa-Based and Levodopa-Sparing treatment.

All patients with Parkinson's disease were recruited based on the following criteria: they were free from LID; no more than 5 years after diagnosis of Parkinson's disease, and on dopamine replacement therapies at the time of the study. Patients on Amantadine were excluded as NMDA antagonism limits TBS effects (Blanpied et al. 2005; Teo et al. 2007), which would consequently limit our ability to interpret the results. Amantadine used as an adjuvant for Levodopa may also mask the onset of Dyskinesia, further limiting interpretation of results (Warren and Burn 2004). Age-matched control participants did not have a history of neurological or psychiatric disorders, and were not on any central nervous system (CNS) active medication. Details of patients with Parkinson's disease are shown in Table 1.

TMS procedure

We tested synaptic plasticity in the less affected hemisphere of Parkinson's disease patients in the ON medication state, whereas control participants were tested on their dominant hemisphere. Several authors have shown that repetitive transcranial magnetic stimulation (rTMS) paradigms may not have their intended effect in the Off medication state (Morgante et al. 2006; Suppa et al. 2011), particularly with advancing disease (see discussion later in text). Moreover, we hypothesized that any between group differences in depotentiation would be most evident in the On Medication state. Testing the less affected hemisphere in the On state also enabled us to minimise rigidity and rest tremor, which can affect NIBS measures. Finally, testing the less affected hemisphere may enable us to 'look back' at changes earlier in the course of Parkinson's disease (Djaldetti et al. 2006).

Single pulse TMS

Single pulse transcranial magnetic stimulation (TMS) was delivered over the primary motor cortex (M1) through a normal figure-of-eight coil (70 mm external diameter) connected to a monophasic Magstim 200^2 stimulator (The Magstim Company Ltd, Whitland, UK). The coil was held over the motor hot-spot, with the handle pointing backwards and laterally at 45˚ relative to the mid-sagittal plane (Brasil-Neto et al. 1992). We defined the motor hot-spot as the optimal scalp position for eliciting motor evoked potentials (MEP) in the first dorsal interosseous muscle (FDI) of the hand contralateral to the stimulated hemisphere. We directly marked the motor hot-spot on the participants' scalp with a soft-tipped pen in order to keep an accurate coil position along the experimental session. For single M1 stimulation we looked for the TMS intensity that elicited MEPs of ~1 mV amplitude

with subjects at rest. Data was discarded when a stable baseline MEPs of ~1 mV amplitude could not be recorded reliably.

Theta-burst stimulation

We applied theta burst stimulation over the primary motor cortex using a standard figure-of eight coil (70mm external diameter) connected to a Super Rapid high-frequency biphasic magnetic stimulator (The Magstim Company Ltd, Whitland, UK). Previous studies have shown that this specific TMS protocol leads to modulation on brain activity (Huang et al. 2010; Huang et al. 2007; Huang et al. 2005; Huang et al. 2008).

The pattern of TBS consists of bursts of three pulses delivered at a frequency of 50 Hz (e.g.: every 20ms), repeated every 200 ms intervals (i.e. 5 Hz). Stimulation intensity was set at 80% of the active motor threshold (aMT). In order to calculate the aMT we first measured participants' maximum voluntary contraction (MVC) on the FDI muscle contralateral to the stimulated hemisphere. We then delivered single pulse of TMS over participants' motor hot spot while they kept a FDI muscle contraction of 20 % of their MVC. We defined aMT as the lowest intensity necessary to evoke a MEP of ~200μV amplitude in the recorded muscle in 50% trials from a series of ten consecutive stimuli.

To elicit long-term potentiation-like effects (LTP), we used a protocol of continuous theta burst stimulation (cTBS_Potentiation) previously studied by Huang et al. (Huang et al. 2008). The cTBS_Potentiation protocol consisted of a total of 300 pulses delivered over the hot-spot at 80% of the aMT for ~20s, followed immediately by FDI muscle contraction at 10% of the MVC for 1 min. This TBS protocol has been shown to reverse the usual inhibitory effect of cTBS, resulting in significant MEP increase (potentiation) for 20 min after the stimulation (see (Huang et al. 2008).

In order to test the reversibility of previously induced potentiation, we used a second block of cTBS (cTBS_Depotentiation). The cTBS_Depotentiation protocol consisted of a ~10 s train of uninterrupted TBS (150 pulses in total) delivered at 80% of the aMT. This protocol has been shown to reverse LTP-like effects previously elicited, leading to a reduction in MEP amplitude (Huang et al. 2008).

Experimental design

Subjects were comfortably seated in an armchair and were asked to maintain their arm relaxed throughout the entire protocol, except during the assessment of the aMT (~20% MVC) and for 1 min immediately after cTBS_Potentiation (~10% MVC). EMG of the FDI muscle contralateral to the stimulated hemisphere was monitored online throughout the experimental session. Trials where muscle activation was observed were discarded. In Parkinson's disease patients, studying the less affected side in the on-state minimized effects of rigidity. Patients with significant rest tremor in the On state in the less affected arm were excluded, as the effect of muscle contraction on results would not be controlled for.

We calculated MVC of the FDI muscle by asking subjects to squeeze as much as they could a cylinder (~4 cm diameter) positioned between the thumb and index finger of the hand contralateral to the hemisphere to be stimulated. We defined MVC as the difference between the maximum and minimum peaks from the recorded EMG signal. Visual and verbal feedback was continuously provided to allow participants to maintain an accurate muscle contraction during measurement of aMT (~20% MVC), and after the cTBS_Potentiation protocol (~10% MVC).

Subjects participated in a single session where TBS was applied over M1 in order to examine brain plasticity and its reversibility (to a review see (Huang 2012). We first recorded baseline motor evoked potentials (Baseline measurement) on 24 trials of single TMS pulses. We then assessed effects of cTBS_Potentiation by delivering 12 stimuli of single pulse TMS (Potentiation measurement) with subjects at rest. The experimental session concluded with a block of 12 single TMS pulses 2 minutes after cTBS_Depotentiation (Depotentiation measurement) in order to assess reversibility of synaptic plasticity (see Fig. 1).

Data acquisition

We recorded EMG traces from the FDI muscle contralateral to the stimulated hemisphere, using 9 mm diameter Ag-AgCl surface cup electrodes. The active electrode was placed over the muscle belly and the reference electrode over the metacarpophalangeal joint of the index finger. Responses were amplified with a Digitimer D360 amplifier (Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) through filters set at 20 Hz and 2 kHz with a sampling rate of 5 kHz. EMG signals were then collected using SIGNAL software (Cambridge Electronic Devices, Cambridge, UK) through a Power 1401 data acquisition interface (Cambridge Electronic Design Ltd.). Magstim stimulators were triggered using

Signal software and CED data acquisition interface.

Statistical analysis:

We performed two separate one-way independent ANOVA for MEP values registered from the FDI contralateral to the stimulated hemisphere after cTBS_Potentiation, and cTBS_Depotentiation respectively. MEPs values were normalized relative to Baseline. We used Group (Levodopa-Based, Levodopa-Sparing, and Healthy Control) as between subject factor. Post-hoc comparisons were performed when a significant result was found.

We calculated Effective Depotentiation as the reduction in MEP amplitudes resulting from cTBS_Depotentiation ([1-(Depotentiation/Potentiation)]*100). Crucially, this summary statistic determines the amount of depotentiation relative to the amount of potentiation previously achieved. We performed a univariate ANOVA for Effective Depotentiation, with Group (Levodopa-Based, Levodopa-Sparing, and Healthy Control) as between subject factor.

To evaluate whether groups were age-matched, we performed a univariate ANOVA for Age, with Group (Levodopa-Based, Levodopa-Sparing, and Healthy Controls) as between subject factor.

To test for potential confound factors between groups of PD patients, we performed separate univariate ANOVAs for UPDRS III score, Disease Duration, and Equivalent dose of levodopa (LED; calculated according to (Tomlinson et al. 2010; Zangaglia et al. 2010), with Group (Levodopa-Based, Levodopa-Sparing) as between subject factor. When significant between-group differences were found, we performed an analysis of covariance (ANCOVA) for Effective Depotentiation, with Group (Levodopa-Based, Levodopa-Sparing) as between subject factor.

A p-value <= 0.05 was considered significant. The statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS; version 22.0).

Results

Effect of cTBS_Potentiation and cTBS_Depotentiation on MEP amplitudes.

We did not find a significant effect for Group factor as a result of a one-way independent ANOVA performed for the normalized MEP values registered after cTBS_Potentiation ($F_{2, 31} = 0.574$, $p = 0.57$). Conversely, we found a significant effect for Group when we performed a one-way independent ANOVA for the normalized MEP values registered after cTBS_Depotentiation ($F_{2, 31} = 4.959$, $p = 0.014$). Post-hoc comparisons revealed that amplitude of MEPs elicited following cTBS_Depotentiation (normalized with Baseline) were significantly larger for the Levodopa-Based group, when compared with both Levodopa-Sparing ($p = 0.012$) and Healthy Controls ($p=0.009$) (see Fig. 2).

Similarly, a univariate ANOVA on the Effective Depotentiation summary statistic revealed significant between group difference ($F_{2, 31} = 3.2$, $p = 0.05$). Post-hoc comparisons revealed that Effective Depotentiation was significantly less (i.e. lack of depotentiation) for Levodopa-Based group, when compared with Levodopa-Sparing group ($p = 0.04$), and with Healthy Controls ($p = 0.03$) (see Fig. 3).

Potential confound variables.

Univariate ANOVA for Age, revealed no effect for Group ($F_{2, 31} = 2.1$, $p = 0.14$). Similarly, we did not find significant effect of Group (Levodopa-Based, Levodopa-Sparing) factor for UPDRS III score ($F_{1, 20} = 0$, $p = 0.98$) or Disease Duration ($F_{1, 20} = 1.4$, $p = 0.25$).

Univariate ANOVA for LED revealed that Levodopa-Based group had (unsurprisingly) significantly larger LED values than Levodopa-Sparing group ($F_{1, 20} = 9.4$, $p = 0.006$). The Ancova for Effective Depotentiation, with Group (Levodopa-Based, Levodopa-Sparing) as between subject factor, and LED as covariate, revealed that the significant effect for Group ($F_{1, 19} = 4.7$, $p = 0.04$) persisted after accounting for the difference in LED between Levodopa-Based and Levodopa-Sparing groups.

Discussion

Our results show a depotentiation deficit in non-dyskinetic PD patients on levodopa based therapy, which resulted in a paradoxical facilitation pattern, previously reported only in patients with established Levodopa Induced Dyskinesia. This depotentiation deficit accounts for the differences seen in the Levodopa-Based group compared to patients in the Levodopa-Sparing group, and Healthy

Controls. The group of patients on levodopa sparing therapies mostly show preserved depotentiation, similar to Healthy Controls. The depotentiation deficits in the Levodopa-Based treatment group are significant despite matching for potential confounds (Age, UPDRS III, Disease Duration), and remain significant after correcting for confounds that could not be matched (LED). Our results, in patients with no clinical evidence of LID, may lend support to the idea that abnormal synaptic plasticity may play a mechanistic role in the development of LID in PD (Picconi et al. 2003).

A number of studies have utilized Non-invasive Brain Stimulation (NIBS) techniques to evaluate bidirectional synaptic plasticity in cortical excitability in Parkinson's disease. Most studies report the loss of NIBS-induced motor cortex plasticity in the off-drug state in Parkinson's disease patients (Morgante et al. 2006; Suppa et al. 2011). This is in line with experimental models of Parkinson's disease, that have consistently shown that dopamine plays a key role in the modulation of the altered mechanisms of synaptic plasticity detected in the basal ganglia (see review (Koch 2013). The ability of chronic levodopa therapy to restore NIBS-induced potentiation was less certain in studies of non-dyskinetic Parkinson's disease patients. Studies have reported that chronic dopamine-replacement therapy restored (Morgante et al. 2006), or failed to restore (Suppa et al. 2011) NIBS induced LTP-like motor cortex potentiation in Parkinson's disease. In an elegant series of experiments, Kishore et al. (Kishore et al. 2012b) used TBS protocols to demonstrate that there is an increasing deficit in cortical plasticity with advancing Parkinson's disease in the on-drug state, and suggested wide variance in disease duration may have influenced the results of prior studies.

Few studies have explored the effects of NIBS-induced plasticity in Parkinson's disease patients with LID, with obvious technical challenges in delivering NIBS and obtaining reliable outcome measures in patients with involuntary movements. Morgante et al. (2006) reported that LTP-like plasticity is deficient in Parkinson's disease off medication, and is restored by levodopa in non-dyskinetic but not in dyskinetic patients. The Paired Associative stimulation NIBS paradigm employed in the study required median nerve electrical stimulation to be paired with TMS pulses delivered to Abductor Pollicis Brevis engram in M1 for 30 minutes. The authors have acknowledged that the results in dyskinetic patients may have been influenced by involuntary movements. Huang et al. (2011) overcame this technical challenge by studying depotentiation—a phenomenon closely related to LTD, but perhaps more relevant to LID than LTD (Dunnett 2003; Picconi et al. 2008; Prescott et al. 2014)—

in dyskinetic patients after taking half their regular dose of levodopa, as severity of LID is dose dependent. The authors demonstrated that half the regular dose was sufficient to restore LTP-like potentiation in Parkinson's disease patients with LID, but not sufficient for Parkinson's disease without LID. Our results are in contrast with one aspect of their findings, since PD patients without LID on full treatment were reported to have normal potentiation and depotentiation-like responses. However, it should be noted that disease duration in this study was 7.9±2.9 years. Considering the fact that 40-60% of patients with Parkinson's Disease develop LID within 4-6 years of treatment, the patients in this cohort are evidently less prone to LID. Patients on Levodopa-Based therapies in our study were 2.3±2.0 years on the disease, just prior to the typical disease duration window to develop LID. The key result from the study by Huang et al. however, was that depotentiation was abolished in patients with LID, in keeping with results from animal studies of LID (Picconi et al. 2003).

Kishore et al. (2012b) further clarified these results by studying the LTP and LTD-like (not depotentiation) response to TBS in separate sessions, in patients at various stages of Parkinson's disease (i.e.: stable drug response phase, motor fluctuation, and motor fluctuation with LID). They concluded that LTD-like response to the continuous TBS paradigm is affected earlier in the course of treatment, with a reduced LTD-like response in Parkinson's disease patients without motor fluctuations, absent LTD-like response in Parkinson's disease patients with motor fluctuations, and a paradoxical LTP-like response in patients with LID. We found that non-dyskinetic PD patients on levodopa based therapy showed a paradoxical LTP-like response to cTBS_Depotentiation (a depotentiation deficit) similar to that reported by Huang et al. (2011) in patients with established LID. Our results are also in agreement with Kishore et al. (2012b) and futhermore show that depotentiation deficits occur in patients on levodopa based therapies before developing LID.

Our study design was informed by the strengths and weaknesses of these preceding studies. We chose to study patients earlier in the course of disease (<5 years since disease onset), prior to the development of significant motor fluctuation and on stable treatments. We decided to study the less affected hemisphere in the On state to avoid potential confounds like rigidity, and also to explore if changes such as those reported by Kishore et al. (2012a) could be seen in the less affected hemisphere (which is 'earlier' in the course of disease). Matching groups as closely as possible for UPDRS III scores, age and disease duration has enabled us to compare the effects of treatment strategies on

LTP/Depotentiation in Parkinson's disease for the first time. Inter and intra subject variability in response to rTMS protocols (López-Alonso et al. 2014) is an issue with study designs where LTP and LTD like responses are studied on different days or in different subjects (Kishore et al. 2012a). Thus, we decided to use an established TBS Depotentiation protocol, which allowed us to assay LTP and Depotentiation-like response within a single session, and within a relatively short time window (Huang et al. 2011). This short data-sampling window ensures that 'wearing off' of levodopa effects in Parkinson's disease patients does not contaminate results. Moreover, the TBS rTMS paradigm used in our study is N-methyl-D-aspartate receptor (NMDAR)-dependent (Huang et al. 2007; Teo et al. 2007), and consequently a useful assay to evaluate the pathophysiology of LID, where abnormal glutamate signalling plays a critical role (Ahmed et al. 2011; Ghiglieri et al. 2012).

The trend of initiating Parkinson's disease therapy with dopamine receptor agonists, rather than with levodopa, was largely driven by evidence suggesting that it could delay the onset of dyskinesia (Holloway 2004; Olanow and Obeso 2000; Rascol et al. 2000). However, the reasons underlying the delayed time to onset of LID in patients initiated on treatment with dopamine agonists remains under debate. Long-acting dopamine agonists provide more continuous, rather than pulsatile, dopaminergic stimulation that may avoid dyskinesia induction. Although more commonly associated with levodopa, dyskinesias can also occur with dopamine agonist monotherapy (Parkinson Study Group 2000; Rascol et al. 2000). Most currently used dopamine agonists are selective for D2-like receptors, with only pergolide and apomorphine potentially interacting with D1 receptor populations. Differences in the balance between D1/D2 type receptor stimulation have also been proposed as a potential mechanism for the development of LID (Calabresi et al. 2010; Feyder et al. 2011; Missale et al. 1998). It is also worth remembering that all Parkinson's disease patients eventually require therapy with levodopa, and the combination of levodopa and dopamine agonist is associated with a rise in the frequency of dyskinesia (Rascol et al. 2000). The results of our study should not be interpreted as supporting the use of dopamine agonists as initial therapy.

A key limitation of our study was that we could not match for levodopa-equivalence between groups, as the priority was to match for disease severity and duration (the key risk factors). Thus, we have attempted to evaluate the effects of LED differences between groups with an ANCOVA, and we did not find an effect on our main results. However, it is still possible that differences seen here are due

to LED or cumulative LED over duration of treatment, rather than the effect of treatment type. **Moreover, as the groups are not matched for levodopa-equivalence, it is possible that disease severity is greater in the Levodopa group as matching for disease severity was based on On-state UPDRS III.** Therefore, levodopa-equivalence and disease severity may also explain these results. Nevertheless, the fundamental finding, that paradoxical depotentiation can occur early in PD, and prior to the onset of LID, still holds.

In conclusion, we report depotentiation deficits severe enough to cause a paradoxical facilitation pattern in non-dyskinetic early PD patients. These deficits were only found for patients on levodopa-based therapy, whereas patients treated with dopamine-agonists showed responses similar to healthy controls. An effect of disease severity or cumulative levodopa dose cannot be fully excluded. A similar pattern of paradoxical depotentiation was previously reported to follow the onset of LID (Huang et al. 2011). This supports the notion that paradoxical facilitation in response to a depotentiation protocol is the mechanism, and not just the consequence, of LID. If confirmed, NIBS measures of depotentiation may be a valuable biomarker for LID in Parkinson's disease. However, continued follow-up of our cohort is necessary to confirm this notion.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent:

Informed consent was obtained from all individual participants included in the study.

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Table 1. Personal details and clinical features of Parkinson's disease patients involved in this study. We calculated Levodopa equivalent dose (LED) according to Tomlinson *et al.* (2010), and followed Zangaglia's *et al.* (2010) suggestion for calculation of LED for patients on melevodopa+carbidopa.

Figure 1. Schematic representation of the experimental design.

Figure 2. Averaged Motor Evoked Potential (MEP) amplitude, relative to baseline, for each experimental group. MEP values at Depotentiation are significantly larger in Levodopa-Based group, when compared to both Levodopa-Sparing, and Healthy Control groups (paradoxical facilitation response). Values are shown as mean ± SEM.

Figure 3. Effective Depotentiation: The summary variable Effective Depotentiation measures depotentiation relative to the preceding level of potentiation achieved. It was calculated using the formula Effective Depotentiation = [1-(MEP amplitude Depotentiation/MEP Amplitude Potentiation)]*100. Effective Depotentiation was significantly smaller for the Levodopa-Based group, when compared with both Levodopa-Sparing and Healthy Controls. Triangles represent the mean (horizontal dotted line) value for each group ± SEM. Individual values for each experimental group are also shown as a superimposed scatterplot. Area shaded dark gray represents mean ± 1SD value for the Healthy Control group, and area shaded light gray represents mean ± 2SD value for the Healthy Control group. This illustrates that 5/11 patients from the Levodopa-Based group (but only 1/11 in Levodopa-Sparing group) show very low Effective Depotentiation (lower than the 2SD value of Healthy Controls).

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Patient	UPDRS III	Disease Duration (months)	Gender	Age	Levodopa equivalent dose	Prescribed drugs
LEVODOPA						
1	11	18	M	74	450	L+C
2	18	54	F	63	745	L+B, Rot, Ra
3	23	50	F	69	214	L+B, P
4	26	11	M	70	300	L+B
5	40	4	M	69	300	L+B
6	39	8	M	67	300	L+B
7	9	0	M	74	375	L+B
8	14	57	F	73	605	L+C, Rot

9	15	13	M	64	335	L+B, P
10	16	51	M	66	605	ML+C, P, Ra
11	14	27	M	59	840	L+B, ML+C,P, Ra
MEAN ± SD	**20.5 ± 10.6**	**26.6 ± 22.1**		**68.0 ±4.8**	**460.8 ± 206.9**	
LEVODOPA SPARING						
1	38	37	M	59	310	P_XL, Ra
2	25	48	M	69	310	P, Se
3	25	22	M	60	280	Ra, Rop
4	30	12	M	62	120	Rop_XL
5	15	7	F	66	150	Rop
6	29	22	M	61	210	P
7	20	10	M	62	120	Rop
8	10	19	F	63	420	Ra, Rop

9	11	2	F	70	205	P, Ra
10	12	7	F	72	310	P_XL, Ra
11	9	3	F	61	315	P_XL
MEAN ± SD	**20.4 ± 9.7**	**22.1 ± 14.5**		**64.1 ± 4.4**	**250.0 ± 96.0**	

Abbreviations: L + B = levodopa + benserazide; L + C = levodopa + carbidopa; ML + C = melevodopa + carbidopa; P = pramipexole; P_XL = pramipexole extended release; Ra = rasagiline; Rop = ropinirole; Rop_XL = ropinirol extended release; Rot = rotigotine; Se = selegiline.

Figure

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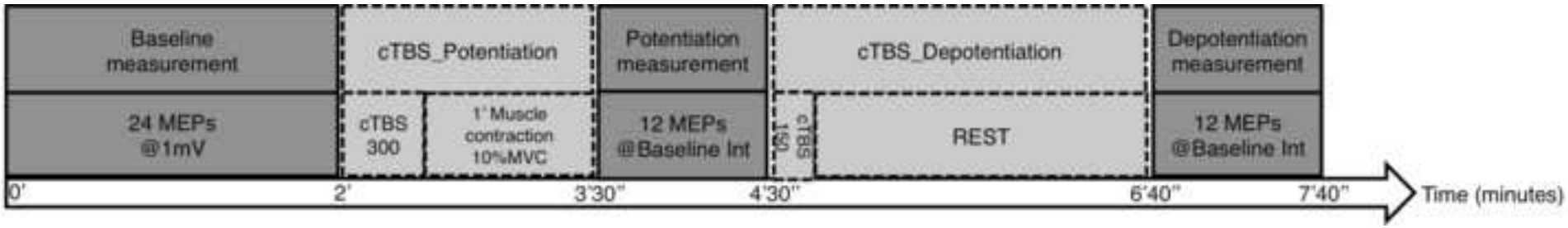

Figure

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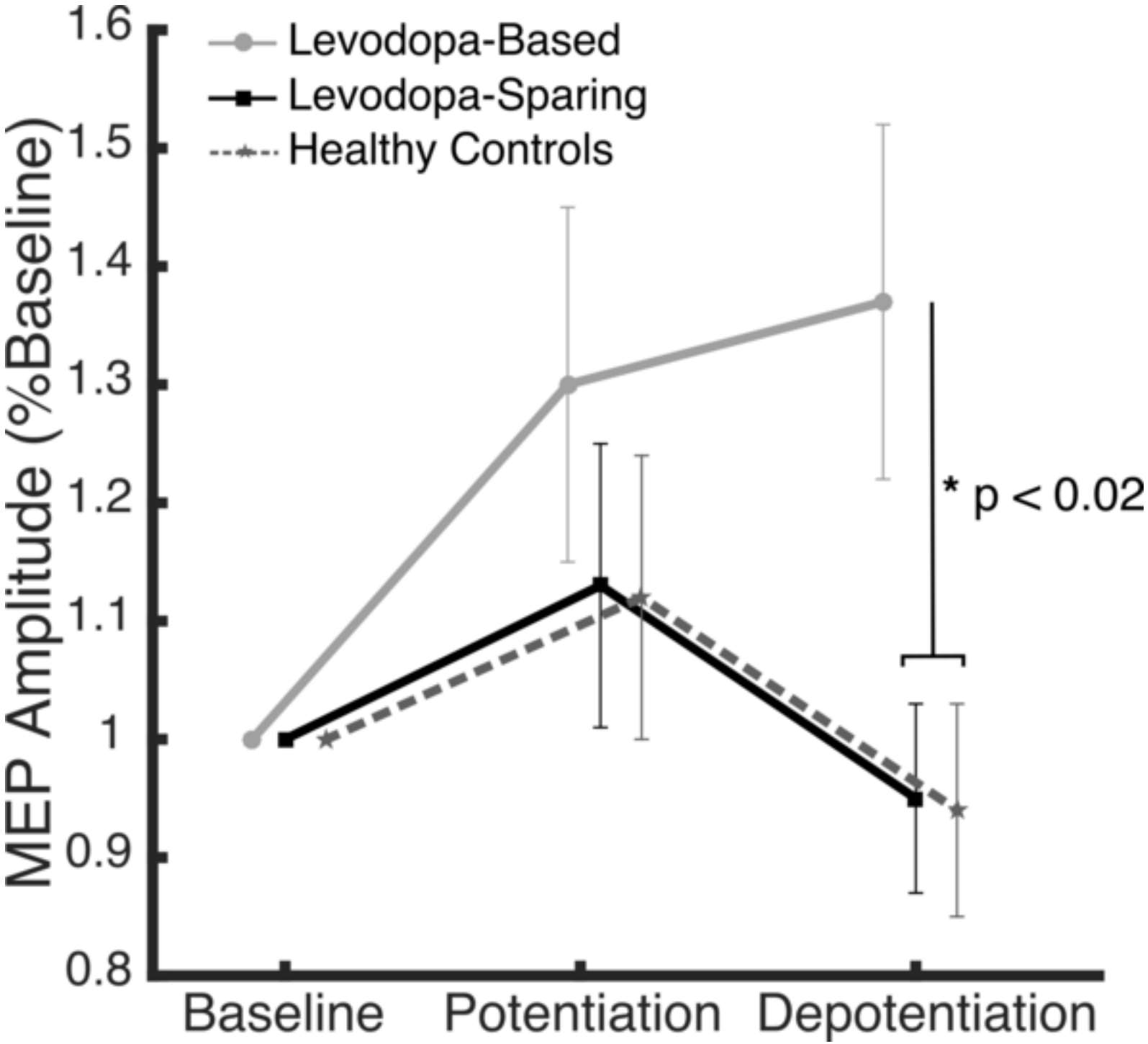

Figure

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