Abnormal bidirectional plasticity-like effects in Parkinson’s disease

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Levodopa-induced dyskinesia is a major complication of long-term dopamine replacement therapy for Parkinson’s disease that becomes increasingly problematic in advanced Parkinson’s disease. Although the cause of levodopa-induced dyskinesias is still unclear, recent work in animal models of the corticostriatal system has suggested that levodopa-induced dyskinesias might result from abnormal control of synaptic plasticity. In the present study, we aimed to explore control of plasticity in patients with Parkinson’s disease with and without levodopa-induced dyskinesias by taking advantage of a newly developed protocol that tests depotentiation of pre-existing long-term potentiation-like synaptic facilitation. Long-term potentiation-like plasticity and its reversibility were studied in the motor cortex of 10 healthy subjects, 10 patients with Parkinson’s disease and levodopa-induced dyskinesias, who took half of the regular dose of levodopa and 10 patients with Parkinson’s disease without levodopa-induced dyskinesias, who took either half or the full dose of levodopa. Patients with Parkinson’s disease without levodopa-induced dyskinesias had normal long-term potentiation- and depotentiation-like effects when they took their full dose of levodopa, but there was no long-term potentiation-like effect when they were on half dose of levodopa. In contrast, patients with levodopa-induced dyskinesias could be successfully potentiated when they were on half their usual dose of levodopa; however, they were unresponsive to the depotentiation protocol. The results suggest that depotentiation is abnormal in the motor cortex of patients with Parkinson’s disease with levodopa-induced dyskinesias and that their long-term potentiation-like plasticity is more readily affected by administration of levodopa than their clinical symptoms.

Keywords: long-term potentiation; depotentiation; Parkinson’s disease; levodopa; dyskinesia

Abbreviations: DARRP = dopamine- and cyclic AMP-regulated phosphoprotein; LID = levodopa-induced dyskinesia; TBS = theta burst stimulation

Introduction

Dopaminergic drugs, including levodopa (L-3,4-dihydroxyphenylalanine) and dopamine agonists, are highly effective symptomatic agents in the treatment of Parkinson’s disease. However, as the disease progresses, long-term dopamine-replacement therapy is almost invariably accompanied by development of motor complications, including dyskinesia. Such levodopa-induced dyskinesias (LIDs), are more likely to occur in patients who have younger age at Parkinson’s disease onset, or who have a larger accumulated dose of levodopa (Cenci, 2007; Fabbrini et al., 2007; Jenner, 2008). Although LIDs seldom cause a large negative effect on
the quality of life in patients with Parkinson’s disease (Marras et al., 2004; Pechevis et al., 2005), they can become a significant limitation in treatment.

The exact underlying mechanism of LID is still obscure. Chronic dopaminergic denervation together with prolonged dopamine replacement therapy leads to a series of pre- and postsynaptic changes in the striatum such as hypersensitivity of D1 receptors and redistribution of D2 receptors (Aubert et al., 2005; Hurley and Jenner, 2006). In the 6-hydroxydopamine rat model of Parkinson’s disease, this is accompanied by reduced or absent long-term potentiation at corticostriatal synapses that can be restored by chronic levodopa treatment. Work in the same model has found that rats who develop LIDs as a consequence of levodopa treatment additionally fail to show depotentiation of long-term potentiation, whereas this is present in treated non-dyskinetic animals (Picconi et al., 2003). Absent depotentiation might mean that corticostriatal synapses that had been recruited to a potentiated state by a previous burst of firing would continue to show an enhanced response to incoming cortical inputs, irrespective of their salience for the animal’s ongoing behaviour, thus playing a direct role in the development of dyskinesias.

At a molecular level, dyskinetic mice have been found to have a persistent and irreversible increase in phosphorylation of DARPP-32 (dopamine- and cyclic AMP-regulated phosphoprotein, molecular weight = 32,000) at Thr34 (Santini et al., 2007). DARPP-32 may mediate dyskinesia through modulating two mitogen-activated protein kinases, ERK1 and ERK2, which have been implicated in synaptic plasticity (Sweatt, 2004; Thomas and Huganir, 2004; Santini et al., 2008). At the receptor level, the GluR1 component of the N-methyl-D-aspartate receptor in dyskinetic mice shows increased phosphorylation of the Ser845 residue. Dephosphorylation of this site normally induces long-term depression of synaptic strength (Lee et al., 2000), so that maintained phosphorylation might lead to the opposite effect, again with consequences for control of synaptic plasticity.

However, animal models of Parkinson’s disease may not be identical to human patients even if they can successfully replicate some of the major features of the disease. Animal models often rely on highly reduced (e.g. slice) preparations in which neurons are not firing at their normal rates, and membrane potentials may lie outside their usual range. In addition, levels of neurotransmitters crucially involved in synaptic plasticity including dopamine, acetylcholine and nitric oxide may all differ from physiological levels seen in the intact behaving animal. Thus, it is of some importance to test how relevant these models may be to our understanding of dyskinesias in human patients with Parkinson’s disease.

Morgante et al. (2006) used transcranial magnetic stimulation to probe synaptic plasticity in the human cerebral motor cortex with a paired associative method and found that it was absent in patients with Parkinson’s disease when they were studied OFF their normal therapy. Plasticity returned to normal when they were given levodopa. More recently, Prescott et al. (2009) investigated plasticity in the substantia nigra pars reticulata of patients undergoing neurosurgery for treatment with deep brain stimulation. They also reported that plasticity was absent when patients were OFF therapy, but that this recovered when the patients were given a single dose of levodopa. Thus, these two probes of synaptic plasticity in cortex and substantia nigra pars reticulata appeared to have the same sensitivity to levodopa as the corticostriatal plasticity reported in animals, suggesting that there may be a general role for dopamine in regulating synaptic plasticity in many brain areas in addition to striatum.

Morgante et al. (2006) also compared the response with levodopa in dyskinetic patients with Parkinson’s disease. Unlike the non-dyskinetic patients, plasticity was not restored by treatment with levodopa. This differs from the rodent 6-hydroxydopamine model in which long-term potentiation was restored equally well by levodopa therapy in all animals. There are many possible reasons for this including differences in behaviour of cortical versus striatal circuits, differences in the protocols used to produce long-term potentiation-like effects, and differences in behaviour of acute lesions of the dopamine system in animals compared with slow degeneration in humans.

In the present study, we tried to readdress some of these problems using a new method to induce long-term potentiation-like effects in the cortex, theta burst stimulation (TBS) (Huang et al., 2005, 2009, 2010; Jhih-Hung Fang, 2010). This also allowed us to examine depotentiation for the first time in human patients with and without LIDs using a recently described technique (Huang et al., 2010).

Subjects and methods

Subjects

Twelve patients with Parkinson’s disease with LID (five males, age: 64.0 ± 5.7 years) and 12 patients with Parkinson’s disease without LID (six males, age: 62.2 ± 6.5 years) were recruited from the movement disorder clinics at the Chang Gung Memorial Hospital, Taipei, Taiwan. Parkinson’s disease was diagnosed based on UK Parkinson’s Disease Society Brain Bank clinical diagnostic criteria. Clinical details of these patients are given in Table 1. Patients with LID and without LID were matched for disease duration (t = 1.280, P = 0.214) and motor disability (ON: t = 1.064, P = 0.299; OFF: t = 1.167, P = 0.256). Only patients with peak-dose dyskinesia were recruited in the LID group; they were taking a larger daily equivalent dose of levodopa than the patients without LID (t = 3.314, P = 0.003). Twelve healthy control subjects (four males, age: 61.3 ± 7.3 years) were recruited from a departmental register of volunteers. We aimed to have a statistical power of ≥ 0.8 when comparing the depotentiation protocol between control, LID and non-LID groups. By assuming a medium effect size (0.3) (Cohen, 1988), three groups (control, LID, non-LID) and two measures (before and after TBS), a total sample size of 30 would meet the minimal requirement. Here we recruited 12 subjects in each group, which gave a power of 0.88. All participants gave their informed consent prior to participation. The experiments were performed with the approval of the Institutional Review Board of the Chang Gung Memorial Hospital, Taiwan.

Recording and stimulation

Subjects were seated in a comfortable chair. EMGs were recorded using Ag–AgCl electrodes from the first dorsal interosseus muscle in the more affected hand in patients and the dominant hand in healthy subjects. EMG activity was recorded with a gain of 1000 × and
Table 1 Clinical features of dyskinetic and non-dyskinetic patients with Parkinson’s disease

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<th>UPDRS-III (OFF)</th>
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<td>L, P, B</td>
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*A number was randomly assigned to patients in LID and ND groups for anonymity purposes. Rx = prescription drug; A = amantadine; B = biperiden; C = pergolide; E = entacapone; F = female; L = levodopa; LID = levodopa-induced dyskinesia; LED = equivalent dose of levodopa; M = male; NA = not applicable; ND = non-dyskinesia; P = pramipexole; R = ropinirole; UPDRS-III = Unified Parkinson’s Disease Rating Scale part III (motor examination); UPDRS-IV = complication of therapy (Items 32 and 33).

Raw EMG signal on the screen was provided as visual feedback to the subject to help maintain a constant muscle contraction of the correct force.

Depotentiation paradigm

The protocols used in the present study are based on those previously reported (Huang et al., 2010) using theta burst form of repetitive transcranial magnetic stimulation (TBS) over the primary motor cortex (Huang et al., 2005, 2007, 2008). The patterns of TBS all consist of bursts containing three pulses at 50 Hz at an intensity of 80% active motor threshold repeated at 200 ms intervals (i.e. at 5 Hz). In a pilot study (not published), we noticed that the potentiation effect of intermittent TBS was not consistent in patients with Parkinson’s disease. We therefore decided to replace intermittent TBS with continuous TBS followed by 1-min contraction (cTBSc0) for producing long-term potentiation-like effect in the present study. In cTBSc0, subjects were asked to perform a voluntary contraction (10% maximum) of the target first dorsal interosseus muscle for 1 min immediately after a 20-s train of uninterrupted continuous TBS. This procedure reverses the usual inhibitory effect of continuous TBS and produces significant potentiation of motor evoked potentials for 20 min or so (Huang et al., 2008). A shorter form of continuous TBS (cTBS150) that consisted of a 10-s train of uninterrupted TBS (150 pulses in total) was given at 1 min after cTBSc0 to test the reversibility of potentiation induced in the
motor cortex by cTBSc0. The intensity of stimulation for motor evoked potential assessment was set to that required to produce a motor evoked potential of ~1 mV in the baseline condition.

To quantify the contraction levels, subjects were asked to squeeze a 4-cm block between their thumb and index finger. Visual feedback about the intensity of muscle contraction was provided to the subjects on an oscilloscope. Subjects were firstly asked to squeeze using the maximum force at the beginning of the experiments, and were instructed to maintain a constant muscle contraction at ~20 and 10% of maximum amplitude for active motor threshold measurement and the 1-min contraction for cTBSc0, respectively. The contraction level was monitored and visual feedback was provided to the subjects continuously.

Experimental design

Subjects came for two sessions in a random order. (i) The cTBSc0 session: baseline motor evoked potential recording was performed using 30 pulses delivered every 4.5–5.5 s. cTBSc0 was then applied to the subject. Motor evoked potential size was assessed using single pulses of transcranial magnetic stimulation delivered in trains of 12 pulses given every 4.5–5.5 s every 2 min for 22 min after the end of cTBSc0. (ii) The depotentiation session: the procedure was similar to that of the cTBSc0 session, except that cTBS150 was given over the motor hot spot after the first 1 min assessment of motor evoked potential following cTBSc0. In addition, the effect of cTBS150 given alone on the size of motor evoked potentials was tested in 8 of the 12 patients with LID taking half their regular levodopa dosage and 8 of the 12 patients without LID taking full dose of levodopa. Motor evoked potential size was assessed using single pulses of transcranial magnetic stimulation delivered in trains of 12 pulses given every 4.5–5.5 s before and at 0, 5, 10, 15 and 20 min after the end of cTBS150.

In patients, the experiments were performed ~1.5 h after medication was taken. To avoid any LIDs that may have affected the results, patients with LID were asked to take their normal levodopa dosage and keep any other medicines unchanged for the dose before the experiment. Because the patients with LID were taking a higher daily equivalent dose of levodopa than the non-LID group, this meant that the half dose they took in this part of the experiment was the same as the full dose taken by patients without LID (LID: 144.6 ± 46.7, non-LID: 178 ± 65.6 mg; t = −1.437, P = 0.165). If dyskinesia was seen during the experiment, the experiment was cancelled and the patient was asked to come back to repeat the experiment on another day. For comparison, patients without LID came in a pseudo-randomized order. None of the patients experienced any discomfort or significant disability with the reduced single dose of levodopa. This is probably because the patients still took their regular dose of other drugs, such as a dopamine agonist, catechol-O-methyl transferase (COMT) inhibitor, anti-cholinergic drug and amantadine (Table 1), and did not move much during the experiments. Moreover, they were allowed to take the removed dose immediately after the experiment. Most of the patients with LID presented no dyskinesia with reduced dose. Two patients with LID (Patients 2 and 5) showed minimal dyskinesia in one of their experiments. The patients managed to come back in order to repeat the same experiment on another day. On that occasion they had no dyskinesias even though the reduced dose remained unchanged.

The amplitudes of baseline motor evoked potentials were not different between healthy controls (cTBSc0: 10.8 ± 0.09 mV; depotentiation: 1.10 ± 0.07 mV), patients with LID (cTBSc0: 1.13 ± 0.05 mV; depotentiation: 1.18 ± 0.14 mV) and patients without LID taking half (cTBSc0: 1.19 ± 0.13 mV; depotentiation: 1.18 ± 0.11 mV) or full levodopa dose (cTBSc0: 1.07 ± 0.07 mV; depotentiation: 1.13 ± 0.09 mV). This was confirmed by a two-way ANOVA showing no effect of group (LID, non-LID, control) [F(2,33) = 0.234, P = 0.793] and pattern [F(1,33) = 0.552, P = 0.463] and no group × pattern interaction [F(2,33) = 0.064, P = 0.983].

The time course of the after effects of the TBS protocols was initially analysed with a three-way ANOVA with the between-subject effect group (LID, non-LID taking full dose, control) and the within-subject effects pattern (cTBSc0, depotentiation) and time showed significant effects of pattern [F(1,33) = 20.477, P < 0.0001, partial η² = 0.38] and time [F(12,396) = 5.675, P < 0.0001], a significant pattern × time interaction [F(1,33) = 2.099, P = 0.016, partial η² = 0.06] and a marginal group × pattern interaction [F(2,33) = 2.947, P = 0.066, partial η² = 0.15]. A similar ANOVA comparing patients with LID, patients without LID taking half dose levodopa and controls showed significant pattern [F(1,30) = 6.135, P = 0.019, partial η² = 0.17] and time [F(12,360) = 4.061, P < 0.0001] effects, a significant group × pattern interaction [F(2,30) = 3.351, P = 0.049, partial η² = 0.18] and a marginal group × time interaction [F(24,360) = 1.512, P = 0.060, partial η² = 0.09]. We therefore evaluated the individual effects separately below.

Depotentiation in healthy subjects

Depotentiation in Parkinson’s disease with dyskinesia Brain 2011: 134; 2312–2320 | 2315

Data analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS). A three-way repeated measures ANOVA was performed to compare the results of different sessions of the three subject groups. One-way repeated measures ANOVA or two-way repeated measures ANOVA were used to examine the time course of changes in motor evoked potential amplitude and to test the effect of cTBS150 given alone, the effects of cTBSc0 and the influence of the cTBS150 intervention. Pearson correlation was used to correlate the amount of depotentiation and the clinical data. To calculate the amount of depotentiation, motor evoked potential amplitudes were normalized to the pre-TBS baseline amplitude of each session. The averaged per cent change of normalized motor evoked potential amplitudes between cTBSc0 and depotentiation sessions [(MEP_cTBS0 − MEPdepotentiation)/MEP_cTBS0] at 2–22 min after cTBSc0 was calculated as the amount of depotentiation. A P < 0.05 was considered statistically significant.
abolished the facilitation. This was confirmed in the two-way ANOVA of the time points after application of cTBS150, which showed a significant main effect of pattern (cTBS0 and depotentiation) \(F(1,11) = 17.073, P = 0.002, \text{ partial } \eta^2 = 0.61\). Paired t-tests demonstrated that there was no difference between the initial amount of facilitation in the first minute after cTBS0 (\(t = 0.691, P = 0.504\)). An additional one-way ANOVA confirmed that motor evoked potential returned to the baseline level after cTBS150 \(F(11,121) = 1.432, P = 0.167\).

### Depotentiation in patients with levodopa-induced dyskinesia

Motor evoked potentials were facilitated by cTBS0 in patients with LID who had received half of their normal dose of levodopa \(F(12, 132) = 2.261, P = 0.012\) (Fig. 2). The facilitation was similar to that in healthy subjects (group effect: \(F(1,22) = 0.244, P = 0.626\); group \times time interaction: \(F(12, 264) = 0.609, P = 0.834\)). In contrast to healthy controls and patients without LID, cTBS150 given at 1 min after cTBS0 did not modify the facilitation produced by cTBS0. This was confirmed in the two-way ANOVA of the time points after cTBS150 that showed no significant main effect of pattern (cTBS0 and depotentiation) \(F(1,11) = 0.901, P = 0.363\) nor a pattern \times time interaction \(F(10,110) = 0.773, P = 0.654\). One-way ANOVAs of baseline and the time points after cTBS150 further supported the finding that facilitation produced by cTBS0 was not abolished by cTBS150 \(F(11,121) = 1.981, P = 0.036\).

We further correlated the amount of depotentiation and the clinical data, including the clinical scale of dyskinesia, daily levodopa equivalent dose, levodopa equivalent dose before test and disease duration (Table 1). None of the clinical data significantly correlated with the amount of depotentiation, although the clinical scale and daily levodopa equivalent dose showed weak non-significant negative correlation to the amount of depotentiation (\(r = -0.365\) and \(-0.454\), respectively).

### Depotentiation in patients without levodopa-induced dyskinesia

When patients with Parkinson’s disease without LID received half of their normal dose of levodopa, neither cTBS0 alone nor the depotentiation protocol produced significant effect on motor evoked potentials (one way ANOVA: \(F(12,96) = 0.534, P = 0.887; F(12,96) = 1.069, P = 0.395\), respectively) (Fig. 3A). In contrast, cTBS0 successfully facilitated motor evoked potentials \(F(12,132) = 1.859, P = 0.045\) in the same patients when they took their full dose of levodopa (Fig. 3B). Two-way ANOVA of the time points after application of cTBS150 showed a significant main effect of pattern (cTBS0 and depotentiation) \(F(1,11) = 11.635, P = 0.006, \text{ partial } \eta^2 = 0.51\), while paired t-tests demonstrated that there was no difference between the initial amount of facilitation in the first minute after cTBS0 (\(t = 0.140, P = 0.892\)). This suggested that the initial facilitation was abolished by addition of cTBS150 (Fig. 3B). A further comparison of the cTBS0 induced facilitation in patients without LID with full dose of levodopa and healthy subjects showed no effect of group (control and non-LID) \(F(1,22) = 0.178, P = 0.677\) nor a time \times group interaction \(F(12,264) = 1.024, P = 0.427\).

### Effect of cTBS150 alone

A two-way ANOVA showed no effect of group (LID and non-LID) \(F(1,14) = 0.615, P = 0.446\), time \(F(5, 70) = 0.527, P = 0.755\) and no group \times time interaction \(F(5,70) = 1.031, P = 0.406\), suggesting cTBS150 produced no effect on the size of motor evoked potentials in either patients with LID taking half dose or patients without LID taking full dose of levodopa.
the baseline EMG of patients with LID was stable during the 0.694]. Furthermore, a two-way ANOVA confirmed that $P=\left[\begin{array}{c}
\end{array}\right]$ in cTBSc0 and depotentiation sessions [group effect: $F(1,22) = 0.488, P = 0.492$; group $\times$ time interaction: $F(12,264) = 0.603, P = 0.839$; group $\times$ time $\times$ pattern interaction: $F(12,264) = 0.758, P = 0.694$]. Furthermore, a two-way ANOVA confirmed that the baseline EMG of patients with LID was stable during the experiments by showing no effect of time [$F(1,11) = 0.040, P = 0.846$] or pattern [$F(12,132) = 0.907, P = 0.542$] and no pattern $\times$ time interaction [$F(12,132) = 0.912, P = 0.537$].

**Discussion**

Like Suppa and colleagues (2010), we found in an initial pilot study that it was not possible to induce a reliable long-term potentiation-like effect in patients with Parkinson’s disease using the standard intermittent TBS protocol. However, a modified cTBSc0 protocol was successful; it led to clear motor cortex facilitation in non-dyskinetic patients after taking their normal dose of levodopa, but not after taking half a dose. In contrast, facilitation was observed in dyskinetic patients after only half their normal dose of levodopa, suggesting that their long-term potentiation-like plasticity was more sensitive to levodopa than their clinical symptoms that required a full dose to achieve maximum benefit. In addition, we report for the first time the response to a depotentiation protocol that was applied shortly after induction of long-term potentiation-like effects. As reported by Picconi et al. (2003) at corticostratial synapses in the rodent 6-hydroxydopamine model of Parkinson’s disease, we found that depotentiation occurred normally in non-dyskinetic patients but was absent in dyskinetic patients. The difference was not due to any discrepancies in the level of muscle contraction during the 1-min contraction nor to differing responses to cTBSc0 between the groups. Thus we suggest that aberrant plasticity is most likely due to the disease itself rather than any extraneous factors. The results are compatible with the idea that reversal of plasticity is more difficult to achieve not only in the animal model of Parkinson’s disease with LID but also in human patients with Parkinson’s disease with LID.

**Induction of long-term potentiation-like plasticity in Parkinson’s disease**

Our results confirm previous work that dopamine is critically important for expression of long-term potentiation-like plasticity in both animal models and in patients with Parkinson’s disease. Long-term potentiation at the corticostratial synapse is absent in 6-hydroxydopamine-lesioned rats but can be restored by chronic levodopa treatment (Picconi et al., 2003). Similarly, dopamine is necessary for expression of long-term potentiation-like effects within the substantia nigra pars reticulata of patients with Parkinson’s disease undergoing surgery for deep brain stimulation (Prescott et al., 2009). In the motor cortex, Morgante and colleagues (2006) demonstrated that paired associative stimulation could induce long-term potentiation-like changes only when patients with Parkinson’s disease were on medication, while Eggers and colleagues (2010) found that an inhibitory protocol, continuous TBS for 40s, failed to induce any long-term depression-like effects in patients with Parkinson’s disease OFF medication for $\sim$15h. Even in healthy subjects, plasticity-like effects in the motor cortex are capable of being modulated by activation of dopamine receptors (Monte-Silva et al., 2009). We conclude that dopamine can have widespread effects on synaptic plasticity induced by many different protocols in both the basal ganglia and
cortex. Whether the latter is due to a direct effect of levodopa on cortex (Molina-Luna et al., 2009; Xu and Yao, 2010) or to concurrent changes in output of the basal ganglia is unknown at the present time. The long-term potentiation-like effects of the present protocol (cTBS150) had no effect in patients without LID when they were receiving only half their normal levodopa dosage, and appeared only after their full normal dose. This sensitivity to levels of dopamine might also explain why the usual intermittent TBS failed to induce long-term potentiation-like effects in patients with Parkinson’s disease both in our pilot study, and that reported by Suppa and colleagues (2010). cTBS150 involves a short period of voluntary contraction. A protocol incorporating physical activity may be more powerful than repetitive transcranial magnetic stimulation given alone to overcome the deficit in the dopamine system.

The patients with LID were taking a higher daily equivalent dose of levodopa than the non-LID group. We were unable to study them while they were taking this dose because most of them had visible dyskinesias. We therefore studied them when taking half their usual dose, which was close to the full dose taken by the patients without LID. At this dose, patients with LID showed normal long-term potentiation-like plasticity but failed to demonstrate depotentiation. The lack of depotentiation is unlikely to resolve at a higher dose of levodopa since high levels of dopamine in animal experiments interfere with depotentiation (see below). We conclude that although their long-term potentiation-like plasticity was more sensitive to levels of levodopa than their clinical symptoms, depotentiation was absent. In contrast, patients without LID who took their full dose of levodopa had normal long-term potentiation-like plasticity and normal depotentiation. When we halved their dose, plasticity disappeared and depotentiation was untestable. In this case, it appears that there is a more even match between the effect of levodopa on long-term potentiation-like plasticity, depotentiation and clinical symptoms.

Increased levels of D1 receptor messenger RNA were found after dopaminergic treatment in 6-hydroxydopamine-lesioned rats with LID (Gerfen et al., 1990) and a study in non-human primates further confirmed that increased D1 downstream signal transduction cascades were due to hypersensitivity of D1 receptors following long-term dopamine depletion and chronic levodopa treatment (Aubert et al., 2005). Moreover, overactivity of other receptors, e.g. AMPA and N-methyl-o-aspartic acid receptors that are known to be crucial for synaptic plasticity, has been demonstrated in animal models of LID (Chase and Oh, 2000; Ouattara et al., 2010). It may be that the result of these changes is to decrease the requirement for levodopa to produce long-term potentiation-like effects in patients with Parkinson’s disease with dyskinesia. However, we cannot exclude the possibility that the threshold for dopaminergic effects on plasticity induction are lower in patients with LID from the very beginning of the disease. This could make patients experience LDIs more easily than those with a higher threshold. In this case, the threshold level of dopamine required for plasticity induction could be a useful parameter to predict future development of LID at the early stage of Parkinson’s disease. A longitudinal study on this point is required to address this question.

Our result, however, contrasts with a previous report of Morgante et al. (2006) who found that it was not possible to produce long-term potentiation-like plasticity in patients with LID, even after giving a full dose of medication. The most likely reason for the difference between the two studies is the protocol used to induce the long-term potentiation-like effects. In the current study, we used a method (cTBS150) that activates only motor cortical circuitry whereas Morgante and colleagues (2006) used paired associative stimulation, a method that involves combined sensory and motor stimuli. Thus, any change in transmission of sensory input to motor cortex as well as in the interaction between these two could potentially affect the response. Another possibility is that LDIs were more severe in the current study than in the previous study (Items 32 and 33 of Unified Parkinson’s disease Rating Scale IV: 2.6 ± 1.6 versus 1–2, respectively), so that the hypersensitivity of dopamine receptors could be more profound in our patients resulting in a clearer response to low doses of levodopa on plasticity. Subclinical dyskinesia could be another important issue, since physical activities, e.g. muscle contraction, before, during and after plasticity induction is known to modulate motor plasticity (Gentner et al., 2008; Huang et al., 2008; Iezzi et al., 2008). In the present experiments, intermittent subtle dyskinesia was sometimes noticed in few of our patients with LID taking half dose of levodopa. We had to ask those patients to return on a different day to make sure the experiments were performed in the absence of any dyskinesia. It is possible that patients with LID given a full dose of medication had some subclinical dyskinesia even during the experiment, which could interfere with paired associative stimulation-induced plasticity-like effects.

**Synaptic depotentiation in Parkinson’s disease**

The present experiments employed a new method for testing probable synaptic depotentiation following induction of long-term potentiation-like effects (Huang et al., 2010). It relies on applying a short train of continuous TBS that when given alone has no lasting effects on cortical excitability. Following our previous arguments (Huang et al., 2010), we consider that the phenomenon tested here was depotentiation rather than homeostatic metaplasticity for three reasons: (i) in metaplasticity paradigms, the second protocol for testing metaplastcity is usually a protocol that produces or potentially produces a plasticity effect on its own, while cTBS150 used here is far from producing any significant after-effect when given alone; (ii) inhibitory continuous TBS did not prime cTBS150 to produce facilitation or at least to block the ongoing depression from continuous TBS; and (iii) cTBS150 can only reverse the potentiation effect within a certain time window. This is compatible with the known time-dependent properties of depotentiation.

We found that the long-term potentiation-like effect induced in non-dyskinetic patients could be reversed by this method as normal, but that it was absent in patients with LDIs. These data parallel those seen at corticostriatal synapses in the rodent 6-hydroxydopamine model of Parkinson’s disease (Picconi et al., 2003), and may therefore indicate that a lack of control of synaptic plasticity in patients (or animals) could be one factor responsible for appearance of LID.
Depotentiation in Parkinson’s disease with dyskinesia

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The underlying mechanism of loss of depotentiation in Parkinson’s disease with LID remains unclear. Antagonists of the N-methyl-D-aspartic acid receptor have been reported to suppress dyskinesia in patients with Parkinson’s disease and in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-treated animals (Blanchet et al., 1998; Chase et al., 2000; Del Dotto et al., 2001; Nash et al., 2004; Morissette et al., 2006). In dyskinetic rats that had lost depotentiation, Picconi and colleagues (2003) found increased levels of Thr34-phosphorylated DARPP-32, a downstream signalling molecule in the D1 dopamine receptor pathway. Together these results are compatible with hypersensitivity of D1 receptors and overactivity of N-methyl-D-aspartic acid receptors in dyskinetic animals.

Dopamine receptors, including D1 receptors, are capable of regulating depotentiation in animal studies (Kulla and Manahan-Vaughan, 2000). Excessive dopamine augments long-term potentiation but prevents depotentiation in several pathways in the brain (Otmakhova and Lisman, 1998; Centonze et al., 2006). Chronic treatment of parkinsonian rats with high-dose levodopa results in higher rates of motor complication associated with a lack of depotentiation than low-dose levodopa (Gerfen et al., 1990; Aubert et al., 2005; Picconi et al., 2008). These observations all suggest that the higher the level of dopamine the greater the loss of depotentiation. The daily levodopa-equivalent dose was significantly higher in our patients with LID as compared with patients without LID, although both groups had similar disease duration and severity. This further supports the idea that the same mechanism may be responsible for the reduced depotentiation in our patients with LID.

Depotentiation is commonly implicated as a mechanism of ‘forgetting’. In the motor system, motor memories are vulnerable to interference by subsequent practice of a different task within a short time of the initial learning (Walker et al., 2003; Robertson et al., 2004; Krakauer and Shadmeer, 2006). Similarly, improper or excessive learning could be wiped out through the same mechanism. The loss of depotentiation in the present patients may therefore lead to unbalanced and unnecessary motor learning that contributes eventually to abnormal motor programming and aberrant muscle control causing involuntary excessive movement that appears to be dyskinesias. The data also suggest that such effects may occur not only in the basal ganglia, but also in the motor cortex of dyskinetic patients with Parkinson’s disease.

The amount of change in depotentiation, however, does not significantly correlate the severity of dyskinesia in patients with LID. This could be due to the inter- and intrasubject variability in response to plasticity induction, the limited patient numbers in the present study, the uneven patient distribution in the different scales of dyskinesia and insufficient accuracy of the rating scale itself (Fabbriani et al., 2007). Nevertheless, the aberrant pattern of depotentiation could still be a useful marker for early detection of developing dyskinesia and might allow adjustment of the amount of medication, and evaluation of the therapeutic benefit of new interventions to treat LID (Koch et al., 2005, 2009).

In conclusion, we have demonstrated that dopamine is required to demonstrate a response to a standard motor plasticity protocol in patients with Parkinson’s disease without LID and that patients with LID fail to respond normally to a protocol that reverses plasticity. In addition, the present group of patients with LID were hypersensitive to levodopa such that their response to the motor plasticity protocol was restored by half their normal dose of levodopa. These results are very similar to descriptions of abnormal bidirectional plasticity in the animal model of Parkinson’s disease. Moreover, the newly developed protocols and the finding of the decreased sensitivity to levodopa for plasticity induction in patients with LID could be useful for early detecting or predicting LID and for evaluating the therapeutic benefit of new interventions to treat LID.

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