



Abnormal cortical facilitation and L-dopa-induced dyskinesia in Parkinson's disease

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ABSTRACT

Background: Animal models of Parkinson's Disease (PD) demonstrated increased facilitatory cortico-striatal activity, reflecting overactive glutamatergic neurotransmission and contributing to the pathophysiology of L-dopa induced dyskinesias (LIDs).

Objective: To assess different facilitatory intracortical circuits in the primary motor cortex (M1) in patients with PD and LIDs by means of a combination of transcranial magnetic stimulation (TMS) protocols. **Methods:** We tested the Input/Output (I/O) curve, intracortical facilitation (ICF) and short-interval intracortical facilitation (SICF) at baseline (T0), 'OFF' and 'ON' state, in 20 PD patients with LIDs. The same parameters were examined after 2 weeks of chronic intake of 50 mg (T1) and 100 mg/day (T2) of safinamide. Finally, we tested SICF in a further group of patients without LIDs.

Results: At T0, patients with LIDs showed increased I/O curve steepness, which was partly ameliorated by L-dopa. These patients also had normal ICF, and abnormally increased SICF, which did not change with L-dopa. Safinamide improved the I/O curve both at T1 and T2, it reduced SICF at T1 and normalized this measure at T2. In patients with PD and LIDs, SICF correlated with the severity of dyskinesia. In patients without LIDs, SICF was less prominently abnormal and responsive to L-dopa.

Conclusions: Patients with PD and LIDs have abnormal cortical facilitation, possibly suggesting overactive glutamatergic neurotransmission in specific circuits within M1. Although not responsive to L-dopa, this dysfunction is restored by the anti-glutamatergic properties of safinamide 100 mg. The results suggest that the abnormal cortical facilitation in M1 contributes to the pathophysiology of LIDs.

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Introduction

Animal models of Parkinson's disease (PD) demonstrated increased facilitation and abnormal plasticity in the cortico-striatal system reflecting changes in non-dopaminergic circuits, such as overactive glutamatergic transmission [1,2]. These changes are believed to play a role in the pathophysiology of motor complications and L-dopa-induced dyskinesias (LIDs) [3–9]. In patients with LIDs, several studies investigated primary motor cortex (M1) excitability and plasticity [10–13] by means of transcranial magnetic stimulation (TMS) techniques. These studies revealed

abnormal plasticity and reduced inhibition within M1 which did not improve under L-dopa, supporting the hypothesis that changes in non-dopaminergic pathways contribute to the pathophysiology of LIDs. However, whether and through which mechanisms dopaminergic and non-dopaminergic changes contribute to LIDs, remain a matter of debate in patients with PD [4]. For this purpose, a possible strategy would be to perform a pharmac-TMS study in PD by using a compound that acts on both dopaminergic and glutamatergic transmission and to examine in detail the excitability of facilitatory intracortical circuits, possibly reflecting glutamatergic activity in M1, in patients with LIDs, under or not under L-dopa. This approach would shed light on the relative weight of dopaminergic and glutamatergic changes in M1 and their putative role in the pathophysiology of LIDs.

Safinamide was recently approved by the FDA/EMA for the treatment of mid-to-late stage fluctuating patients with PD as add-

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on therapy to a stable dose of L-dopa [14,15]. *In vitro* studies have shown that, besides its ability to reversibly and selectively inhibit monoamine oxidase-type B (MAO-B), safinamide also has a non-dopaminergic mechanism of action, i.e. inhibition of glutamate release through blockage of voltage-gated sodium channels (VGSCs) [16–19]. Moreover, the dopaminergic mechanism of action (MAO-B inhibition) of safinamide operates similarly at both lower and higher doses, whereas the anti-glutamatergic effect of the drug is believed to occur only at higher doses.

Here we applied different TMS protocols designed to test facilitatory intracortical circuits in the human M1, possibly reflecting glutamatergic activity [20], including the Input/Output (I/O) curve, intracortical facilitation (ICF) and short-interval intracortical facilitation (SICF). We compared all the TMS measures in patients with LIDs, both 'OFF' and 'ON' L-dopa. We also assessed neurophysiological changes when patients were treated or not with safinamide at doses of 50 or 100 mg/day. In addition, to verify whether safinamide modulates GABA-A-ergic activity, we tested short-interval intracortical inhibition (SICI) [21,22]. Finally, to clarify whether possible changes in facilitatory intracortical circuits are specific for PD patients with LIDs, we evaluated an additional group of patients with PD who never manifested LIDs.

Material and methods

Participants

Twenty patients with PD manifesting LIDs when 'ON' L-dopa therapy (6 females; mean age \pm standard deviation [SD]: 67.4 \pm 10.7 years) and 20 age-matched healthy subjects (HS; 9 females; mean age \pm SD: 64.7 \pm 4.7 years) participated. We also enrolled 11 patients with PD who never manifested LIDs when 'ON' L-dopa (2 females; mean age \pm SD: 68.8 \pm 7.1 years). All the patients manifested motor fluctuations, including wearing off at \leq 3.5 h after the intake of L-dopa. The diagnosis of PD was based on the current clinical criteria [23,24]. Patients were recruited from the Department of Human Neurosciences, Sapienza University of Rome. The clinical assessment of motor signs included the Hoehn and Yahr (H&Y) scale and the motor section of the Unified Parkinson's Disease Rating Scale (UPDRS-III [25]). Cognitive functions were evaluated using the Mini-Mental State Evaluation (MMSE [26]) and Frontal Assessment Battery (FAB [27]). Depression was assessed using the Beck Depression Inventory-II (BDI-II [28]) (see Tables 1 and 2). The intensity of LIDs was scored using the impairment section of the Unified Dyskinesia Rating Scale (UDysRS-III [29]). No patient had relevant additional neurological or psychiatric comorbidities or contraindications to the use of TMS [30]. The study was approved by the local institutional review board (CE 4411) and conducted according to the Declaration of Helsinki. All the participants gave their informed consent to the study.

TMS and recordings

TMS was delivered by using MAGSTIM 200 (Magstim Company Limited) and motor-evoked potentials (MEPs) were recorded from the right first dorsal interosseous (FDI) muscle. The optimal scalp position ('hotspot') to elicit MEPs, the resting (rMT) and active motor threshold (AMT), and the intensity for eliciting MEPs of \approx 1 mV (MT_{1mV}) were determined according to the international guidelines [31]. The I/O curve was assessed by collecting 12 MEPs at seven intensities (100–160% rMT). ICF and SICI were tested at interstimulus intervals (ISI) of 10 and 3 ms, respectively, and by using a conditioning stimulus at 80% AMT [21,22]. In addition, in a subgroup of 8 PD patients with LIDs (4 females; mean age \pm SD: 68.5 \pm 11.0 years), we also tested SICI at ISI 1.5 ms. SICF was

examined by delivering paired-pulses at ISI 1.5, 2, 2.5, 3, 3.5, 4 and 4.5 ms according to standard methods [32,33]. The intensities of the first and second stimulus were set at MT_{1mV} and 90% rMT, respectively. Twelve MEPs were recorded for ICF, SICI and SICF at each ISI, and randomized with single-pulse MEPs at MT_{1mV} . Peak-to-peak MEP amplitudes were measured and averaged per condition. Each trial was visually inspected and those displaying EMG activity \geq 0.1 mV in a 200-ms time-window preceding TMS were rejected.

Experimental design

PD patients with LIDs: each patient underwent three separate experiments: 1) in the first session (T0), patients were studied in 'OFF' state (i.e. at least 1 h after the occurrence of the wearing off) and then in the 'ON' state (i.e. 1 h after the intake of their usual dose of L-dopa); 2) in the second session (T1), patients were tested after 2 weeks of chronic intake of safinamide given at 50 mg/day as add-on to their usual L-dopa regimen; similarly to T0, patients were examined in 'OFF' and 'ON' state; 3) the last session (T2) was identical to T1, with the only difference that safinamide was given at 100 mg/day. The three sessions were systematically conducted at the same time of day for each patient. The same methodology was adopted both in patients experimentally examined in the morning and in the afternoon. The details of our clinical and neurophysiological assessments are shown in Fig. 1.

PD patients without LIDs: each patient participating in our additional experiment underwent the same clinical evaluation conducted in patients with LIDs at T0. The neurophysiological investigation consisted in the assessment of SICF in 'OFF' and 'ON' state.

Statistical analysis

Possible differences in age and gender between HS and patients were evaluated using the Mann-Whitney U and the Fisher-exact test, respectively. Unpaired Student's *t*-test was used to compare rMT, AMT, SICI and ICF in patients and HS, whereas paired *t*-test was used when comparing patients in 'OFF' and 'ON' state. To verify whether SICF produced the typical curve with specific peaks of facilitation, in HS rmANOVA with the factor 'ISI' (8 levels: TS, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 ms) was tested on raw MEPs data. RmANOVA with factors 'group' (2 levels: PD and HS) and 'intensity' (7 levels: 100, 110, 120, 130, 140, 150 and 160% rMT) was used to evaluate differences in the I/O curve between patients and HS, whereas factors 'group' and 'ISI' (7 levels: 1.5, 2, 2.5, 3, 3.5, 4 and 4.5 ms) were adopted to test differences in SICF. To evaluate the effects of L-dopa on the I/O curve rmANOVA with factors 'state' (2 levels: OFF, ON) and 'intensity' was used, while factors 'state' and 'ISI' were used to assess changes in SICF. When comparing SICF in PD patients without LIDs and HS, rmANOVA with factors 'group' (2 levels: PD without LIDs and HS) and 'ISI' (2 levels: 1.5 and 3 ms) was tested. RmANOVA with factors 'state' and 'ISI' was used to evaluate the effects of L-dopa on SICF in patients without LIDs. Also, rmANOVA with factors 'group' (2 levels: PD with LIDs and PD without LIDs), 'ISI' (2 levels: 1.5 and 3 ms) and 'state' was tested to compare SICF in patients with and without LIDs. Safinamide-related changes in UPDRS-III, BDI-II and UDysRS-III were evaluated by Friedman test with the factor 'session' (3 levels: T0, T1, T2). Wilcoxon test was used for the post-hoc analyses. The effect of safinamide on neurophysiological measures was examined by using the following tests: separate rmANOVAs with factors 'session' (3 levels: T0, T1, T2) and 'state' (2 levels: OFF, ON) were used to evaluate changes in rMT, AMT, SICI and ICF. RmANOVA with factors 'session', 'state' and 'intensity' (7 levels: 100, 110, 120, 130, 140, 150 and 160% rMT) was

Table 1
Clinical-demographic characteristics of patients with PD and LIDs.

Subject	Age (Y)	Gender	Disease duration (Y)	UPDRS-III		H&Y	MMSE	FAB	BDI-II	UDysRS-III	LEDDs
				OFF	ON						
1	65	F	10	27	9	2	30	17	15	7	865
2	65	M	9	43	27	3	30	17	20	1	500
3	67	M	6	29	19	2	27	13	12	1	915
4	66	M	22	48	38	4	29	13	19	3	670
5	49	M	15	45	26	3	25	17	6	4	1530
6	68	M	14	47	24	4	30	18	5	8	950
7	79	M	19	52	37	5	28	16	12	7	750
8	49	M	5	19	9	2	30	14	20	1	400
9	67	F	11	40	27	3	28	15	14	5	750
10	76	M	6	38	27	3	25	12	4	1	1055
11	57	M	9	25	11	2	30	16	0	5	425
12	79	M	10	64	56	5	29	12	20	19	1000
13	81	M	6	30	24	2	28	16	22	1	400
14	48	F	10	32	13	2	26	12	26	8	550
15	77	F	18	48	35	2	26	14	34	19	625
16	63	F	8	39	26	2	30	18	9	1	400
17	66	M	5	26	13	2	30	18	6	1	800
18	82	F	15	50	35	4	25	12	35	12	800
19	74	M	12	52	41	3	26	14	10	11	800
20	73	M	10	37	31	2	29	14	12	2	400
mean	67.4	—	11.0	39.6	26.4	2.9	28.0	14.9	15.0	5.8	729.3
SD	10.7	—	4.8	11.5	12.1	1.0	1.9	2.2	9.5	5.6	287.0

UPDRS-III, Unified Parkinson's Disease Rating Scale, part III; H&Y, Hoehn and Yahr scale; MMSE, Mini Mental State Examination; FAB, Frontal Assessment Battery; BDI-II, Beck Depression Inventory II; UDysRS-III, Unified Dyskinesia Rating Scale, part III; LEDDs, L-dopa Equivalent Daily Doses; Y, years; SD, Standard Deviation.

Table 2
Clinical-demographic characteristics of patients with PD who never manifested LIDs.

Subject	Age (Y)	Gender	Disease duration (Y)	UPDRS-III		H&Y	MMSE	FAB	BDI-II	UDysRS-III	LEDDs
				OFF	ON						
1	68	M	6	32	20	2	28	13	12	0	900
2	75	M	7	37	28	3	26	13	5	0	1100
3	80	M	6	31	25	2	26	16	20	0	550
4	67	M	5	28	16	2	30	17	11	0	800
5	75	M	10	46	37	2	25	13	10	0	300
6	53	F	12	52	35	3	30	17	3	0	605
7	65	M	6	28	19	2	30	16	3	0	605
8	67	M	10	40	29	2	29	16	7	0	700
9	73	M	7	46	32	3	30	17	11	0	700
10	65	M	7	35	20	2	25	13	11	0	470
11	69	F	7	37	25	2	28	15	9	0	655
mean	68.8	—	7.5	37.5	26.0	2.3	27.9	15.1	9.3	0	671.4
SD	7.1	—	2.2	7.9	6.9	0.5	2.1	1.8	4.8	—	213.6

UPDRS-III, Unified Parkinson's Disease Rating Scale, part III; H&Y, Hoehn and Yahr scale; MMSE, Mini Mental State Examination; FAB, Frontal Assessment Battery; BDI-II, Beck Depression Inventory II; UDysRS-III, Unified Dyskinesia Rating Scale, part III; LEDDs, L-dopa Equivalent Daily Doses; Y, years; SD, Standard Deviation.

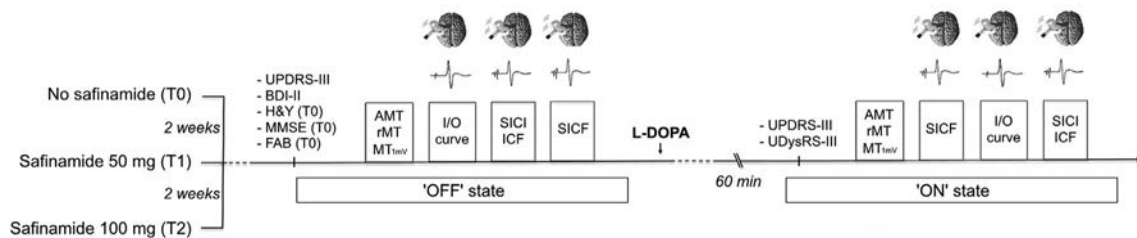


Fig. 1. Clinical evaluation (all sessions): UPDRS-III ('OFF' and 'ON' state), UDysRS-III ('ON' state), BDI-II ('OFF' state). T0 also included H&Y, MMSE and FAB ('OFF' state). Neurophysiological assessment (all sessions): I/O curve, SICI-ICF and SICF delivered in a randomized order.

adopted to compare the I/O curve. RMANOVA with factors 'session', 'state' and 'ISI' (7 levels: 1.5, 2, 2.5, 3, 3.5, 4 and 4.5 ms) was used to assess changes in SICF. Greenhouse-Geisser correction was applied in case of violation of sphericity. The level of significance was initially set at $p < 0.05$, with Bonferroni's correction subsequently

being applied to multiple comparisons. Neurophysiological correlations were assessed by using Pearson's correlation coefficient, while clinical-neurophysiological correlations were assessed by using Spearman's rank-correlation test. For these analyses, SICF was considered as the average of the values obtained at 1.5 and 3 ms

(SICF_{AV}). All the values are expressed as mean \pm 1 standard error (SE). Statistical analyses were performed using SPSS Statistics and Statistica® software.

Results

There were no differences in age and gender distribution between HS, patients with LIDs and patients without LIDs (p always >0.05). The two groups of patients (with and without LIDs) also had comparable clinical characteristics (H&Y, disease duration, UPDRS OFF/ON, LEDDs, MMSE, FAB and BDI-II: p always >0.05).

Neurophysiological measures in HS and patients, without safinamide

RMT ($p = 0.15$) and AMT ($p = 0.12$) were similar in HS and patients ('OFF' state), and they did not change with L-dopa (rMT: $p = 0.23$; AMT: $p = 0.4$)(Table 3).

I/O curve

The I/O curve differed between HS and PD in 'OFF' state, as shown by the significant effect of the factor 'group' ($F_{1,38} = 18.04$, $p < 0.001$) and the interaction 'group' x 'intensity' ($F_{6,228} = 10.72$, $p < 0.001$). The post-hoc analysis revealed increased MEP amplitudes in patients at all the intensities tested, but 100% rMT ($p = 0.71$), which points to a steeper I/O curve in PD. L-dopa reduced the steepness of the I/O curve, as demonstrated by the 'state' x 'intensity' interaction ($F_{6,114} = 2.26$, $p = 0.04$)(Fig. 2A).

SICI and ICF

SICI ($p = 0.09$) and ICF ($p = 0.61$) were comparable in HS and patients ('OFF' state), and they did not change in 'ON' state (SICI: $p = 0.3$; ICF: $p = 0.09$)(Fig. 2B). Also, there were no differences between patients 'ON' state and HS (SICI: $p = 0.07$; ICF: $p = 0.17$).

SICF

In HS, SICF demonstrated the typical curve with peaks of facilitation, as suggested by the significant factor 'ISI' in the rmANOVA ($F_{7,133} = 12.92$, $p < 0.001$). Significant MEPs facilitation occurred at ISI 1.5 ($p = 0.007$) and 3 ms ($p = 0.03$). SICF differed between PD ('OFF' state) and HS, as shown by the significant factor 'group' ($F_{1,38} = 19.58$, $p < 0.001$) and the significant 'group' x 'ISI' interaction ($F_{6,228} = 4.51$, $p < 0.001$). In patients, SICF increased markedly at 1.5 ms ($p < 0.001$) and to a lesser extent at 3 ms ($p = 0.048$) with respect to HS. No significant differences were detected in the other ISIs between patients and HS (p always >0.05). Comparing patients 'OFF' and 'ON', the rmANOVA did not demonstrate any effect of the

factor 'state' ($F_{1,19} = 1.84$, $p = 0.19$), nor a 'state' x 'ISI' interaction ($F_{6,114} = 1.34$, $p = 0.24$)(Fig. 2C).

Comparing the two peaks of SICF facilitation (1.5 and 3 ms) in HS and patients without LIDs ('OFF' state), the rmANOVA revealed a significant effect of the factor 'group' ($F_{1,29} = 5.16$, $p = 0.03$) and a non-significant 'group' x 'ISI' interaction ($F_{1,29} = 0.93$, $p = 0.34$). SICF decreased in patients 'ON' state (factor 'state': $F_{1,10} = 15.15$, $p = 0.003$), and it was comparable to HS (factor 'group': $F_{1,29} = 0.44$, $p = 0.51$; 'group' x 'ISI' interaction: $F_{1,29} = 0.08$, $p = 0.77$). Comparing SICF in patients with and without LIDs, the rmANOVA demonstrated a significant effect of the factor 'group' ($F_{1,29} = 4.06$, $p = 0.048$) and non-significant 'group' x 'ISI' x 'state' ($F_{1,29} = 1.58$, $p = 0.22$), 'group' x 'ISI' ($F_{1,29} = 2.53$, $p = 0.12$) or 'group' x 'state' interaction ($F_{1,29} = 0.01$, $p = 0.92$), indicating that SICF differed in the two patients' subgroups (Fig. 3).

Clinical and neurophysiological measures in patients with LIDs, with safinamide

Safinamide improved UPDRS-III scores in 'OFF' and 'ON' state at both T1 and T2 (T0 vs T1: 'OFF' state, $p < 0.001$; 'ON' state, $p = 0.01$; T0 vs T2: 'OFF' state, $p < 0.001$; 'ON' state, $p = 0.006$). Conversely, UDysRS-III did not change in patients at T1 and T2 compared with the baseline ($p = 0.28$). Finally, BDI-II scores decreased in patients taking safinamide ($p = 0.018$) with a comparable effect at T1 and T2 (T0 vs T1: $p = 0.04$; T0 vs T2: $p = 0.05$; T1 vs T2: $p = 0.14$)(Fig. 4, upper panels).

RMT and AMT did not change among sessions ($F_{2,38} = 0.49$, $p = 0.62$) or clinical states ($F_{1,19} = 2.96$, $p = 0.1$).

I/O curve

RmANOVA demonstrated a 'session' x 'state' x 'intensity' interaction ($F_{12,228} = 2.66$, $p = 0.002$). Separate rmANOVAs for the 'OFF' and 'ON' state demonstrated a 'session' x 'intensity' interaction ('OFF' state: $F_{12,228} = 3.29$, $p < 0.001$; 'ON' state: $F_{12,228} = 2.25$, $p = 0.01$) and a significant effect of the factor 'session' ('OFF' state: $F_{2,38} = 6.23$, $p = 0.004$; 'ON' state: $F_{2,38} = 3.80$, $p = 0.03$). Post-hoc analyses indicated decreased MEP amplitudes under safinamide 50 and 100 mg in 'OFF' state at 130% (T0 vs T1: $p = 0.01$; T0 vs T2: $p = 0.02$), 140% (T0 vs T1: $p < 0.001$; T0 vs T2: $p < 0.001$), 150% (T0 vs T1: $p < 0.001$; T0 vs T2: $p < 0.001$), and 160% rMT (T0 vs T1: $p = 0.003$; T0 vs T2: $p = 0.04$). In 'ON' state MEPs decreased under safinamide 100 mg at 150% ($p = 0.003$) and 160% rMT ($p < 0.001$). Notably, no difference was detected between T1 and T2 at any intensity both for the 'OFF' and the 'ON' state (p always >0.05)(Fig. 4A, lower panels). Finally, rmANOVAs with 'group' (2 levels: HS and PD) and 'intensity' as factors, demonstrated a 'group' x 'intensity' interaction at T1 ('OFF': $F_{6,228} = 5.12$, $p < 0.001$; 'ON': $F_{6,228} = 7.02$, $p < 0.001$) and T2 ('OFF': $F_{6,228} = 3.81$, $p = 0.001$; 'ON': $F_{6,228} = 3.44$, $p = 0.003$), suggesting that the I/O curve steepness was reduced, but not restored, by safinamide.

Table 3
TMS thresholds.

	rMT (%)			AMT (%)		
	HS	PD		HS	PD	
		OFF	ON		OFF	ON
No safinamide (T0)	48.4 \pm 13.1	43.3 \pm 7.6	42.3 \pm 7.4	36.7 \pm 7.9	33.3 \pm 8.8	32.3 \pm 7.9
Safinamide 50 mg (T1)	–	43.5 \pm 7.9	42.4 \pm 7.4	–	33.3 \pm 8.4	32.0 \pm 8.0
Safinamide 100 mg (T2)	–	44.6 \pm 9.2	44.0 \pm 8.0	–	33.6 \pm 8.6	33.4 \pm 8.0

rMT, resting motor threshold; AMT, active motor threshold; PD, patients with PD and LIDs; HS, healthy subjects. Values are reported as mean \pm 1 Standard Deviation.

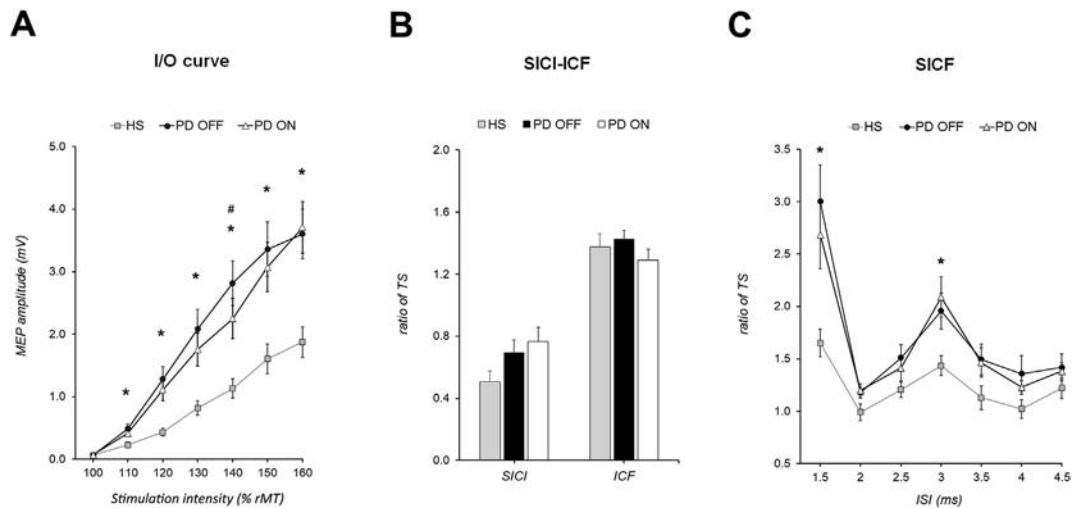


Fig. 2. I/O curve, SICI-ICF and SICI-ICF in HS and patients with LIDs, without safinamide, 'OFF' and 'ON' state. Patients showed steeper I/O curve and higher SICI than HS. Asterisks: differences between HS and PD OFF. Hash: differences between patients OFF and ON. Bars denote SE.

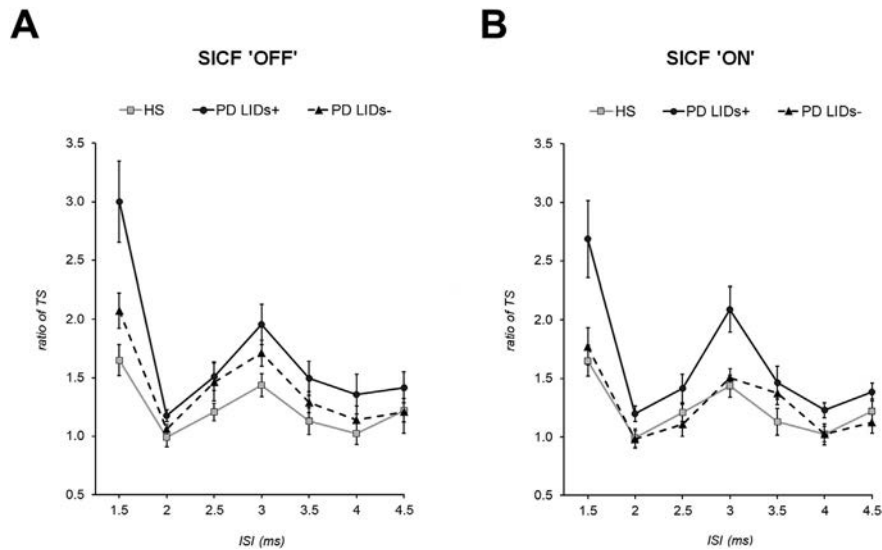


Fig. 3. SICI-ICF in HS, patients with LIDs (PD LIDs+) and patients without LIDs (PD LIDs-). SICI-ICF in LIDs-was higher than HS but lower than LIDs+, and improved with l-dopa. Bars denote SE.

SICI and ICF

Safinamide did not change SICI or ICF, as revealed by the non-significant effect of the factors 'session' (SICI: $F_{2,38} = 3.44$, $p = 0.07$; ICF: $F_{2,38} = 0.65$, $p = 0.57$) and 'state' (SICI: $F_{1,19} = 0.04$, $p = 0.84$; ICF: $F_{1,19} = 0.71$, $p = 0.41$), and the lack of a 'session' x 'state' interaction (SICI: $F_{2,38} = 1.32$, $p = 0.28$; ICF: $F_{2,38} = 2.51$, $p = 0.17$) (Fig. 4B, lower panels).

SICF

RMANOVA demonstrated a 'session' x 'ISI' interaction ($F_{12,228} = 4.72$, $p < 0.001$) and a significant effect of the factor 'session' ($F_{2,38} = 21.65$, $p < 0.001$). No effect of the factor 'state' emerged ($F_{1,19} = 0.19$, $p = 0.66$), nor a 'session' x 'state' ($F_{2,38} = 1.24$, $p = 0.3$), 'state' x 'ISI' ($F_{6,114} = 1.60$, $p = 0.15$) or 'session' x 'state' x 'ISI' interaction ($F_{12,228} = 0.68$, $p = 0.77$). Post-hoc analyses revealed that SICF decreased under safinamide 50 mg at ISI 1.5 ms

($p = 0.001$) and under safinamide 100 mg at ISI 1.5 ($p < 0.001$) and 3 ms ($p = 0.03$). Notably, SICF at ISI 1.5 ms was dose-dependently modulated, being the effect of safinamide more pronounced at 100 than at 50 mg (T1 vs T2: $p = 0.01$). No significant change of SICF was observed at the remaining ISIs (Fig. 4C, lower panels). These results indicate that safinamide modulates SICF, with a prominent effect at 1.5 ms and 100 mg/day.

To verify whether the effect of safinamide on SICF is contaminated by concurrent changes in GABA-A-ergic neurotransmission, in a subgroup of 8 patients we compared MEPs elicited by SICF and SICI both induced by paired-pulses at ISI 1.5 and 3 ms. RMANOVA with factors 'session', 'state', 'ISI' and 'protocol' (2 levels: 'SICI', 'SICF') demonstrated a 'session' x 'protocol' interaction ($F_{2,14} = 13.70$, $p = 0.001$), indicating that safinamide modulated SICF ($p = 0.001$), but not SICI ($p = 0.09$). The analysis showed no interaction 'session' x 'protocol' x 'state' x 'ISI' ($F_{2,14} = 0.21$, $p = 0.82$), 'session' x 'protocol' x 'state' ($F_{2,14} = 0.95$, $p = 0.42$), or 'session' x 'protocol' x 'ISI' ($F_{2,14} = 3.51$, $p = 0.06$).

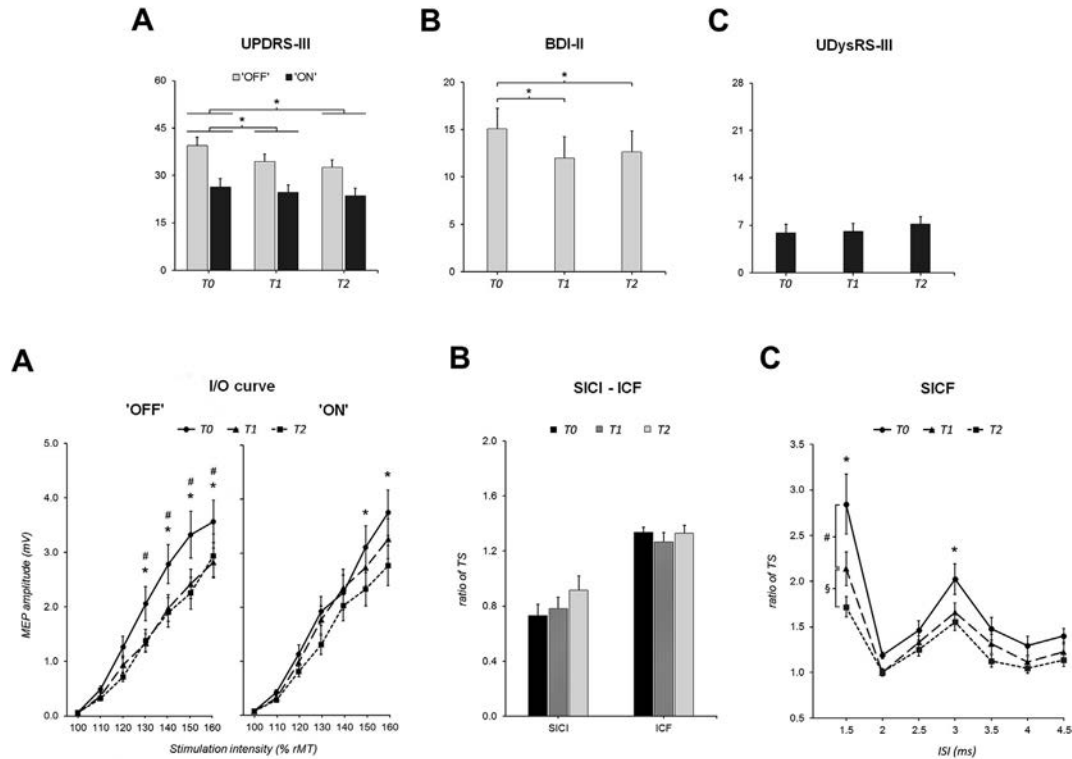


Fig. 4. Clinical (upper panels) and neurophysiological (lower panels) effect of safinamide in patients with LIDs. Upper panels: asterisks denote differences between sessions. Lower panels: asterisks show differences between T0 and T2; hashes indicate differences between T0 and T1; double-s denotes differences between T1 and T2; since SICI, ICF and SICF were comparable in patients 'OFF' and 'ON' state under safinamide, the average of 'OFF' and 'ON' values is shown for these measures. Bars denote SE.

RmANOVAs with factors 'group' and 'ISI' revealed that SICF differed between HS and patients ('OFF' state) at T1 (factor 'group': $F_{1,38} = 4.94$, $p = 0.03$), but not at T2 ($F_{1,38} = 1.27$, $p = 0.27$). Similar results were observed when patients were 'ON' state (T1: $F_{1,38} = 9.44$, $p = 0.01$; T2: $F_{1,38} = 0.52$, $p = 0.47$).

Clinical and neurophysiological correlations

Since safinamide reduced both the steepness of the I/O curve and SICF, we tested the possible correlation between these measures. In order to estimate safinamide-induced changes of I/O curve, we calculated the ratio between the slope of the curve at T1 and T2 with respect to T0 (I/O T1/T0; I/O T2/T0). Similarly, we calculated the ratio between SICF_{AV} at T1 and T2 with respect to T0 (SICF_{AV} T1/T0; SICF_{AV} T2/T0). The analysis demonstrated no correlation between I/O T1/T0 and SICF_{AV} T1/T0 ('OFF': $r = 0.16$, $p = 0.49$; 'ON': $r = 0.22$, $p = 0.36$) or I/O T2/T0 and SICF_{AV} T2/T0 ('OFF': $r = 0.40$, $p = 0.08$; 'ON': $r = 0.38$, $p = 0.10$).

SICF_{AV} at T0 in the 'ON' state positively correlated with the baseline UDysRS-III scores ($r = 0.60$; $p = 0.005$), i.e. the higher the MEP facilitation, the more severe the LIDs. The effect of safinamide on LIDs was calculated by measuring changes in UDysRS-III between T2 and T0 (UDysRS-III T2-T0). The analysis showed that the effect of safinamide on SICF and LIDs was positively correlated, i.e. the higher the reduction in SICF, the lower the induction of LIDs. The correlation was significant when SICF was measured in the 'OFF' state ($r = 0.56$, $p = 0.01$). A strong trend was also present in the 'ON' state ($r = 0.43$, $p = 0.07$) (Fig. 5).

Discussion

We demonstrate that PD patients with LIDs are characterized by an increased steepness of the I/O curve in comparison to HS, which

is only partly improved by L-dopa. We also show that PD patients with LIDs have an abnormally enhanced SICF, which is not responsive to L-dopa. The enhanced SICF correlates with the intensity of LIDs, and the degree of SICF abnormality is higher in patients with LIDs than in those without LIDs. Safinamide reduces the steepness of the I/O curve at both dosages. The drug also improves SICF when given at the dose of 50 mg/day and restores this measure at 100 mg/day.

The systematic evaluation of patients at fixed timing allowed us to exclude several possible confounding factors. Based on pharmacokinetic evidence showing that steady-state concentrations of safinamide are reached within a week [34], we started the experiments 14 days after the first administration of the drug to ensure a complete and stable MAO-B inhibition. Moreover, at T1 and T2, patients were assessed 2–3 h after acute administration of safinamide, a timing compatible with the most effective pharmacological action of the VGSC block [34]. Since all the patients manifested motor fluctuations, including wearing off at ≤ 3.5 h after the intake of L-dopa, any significant long-term response to L-dopa was unlikely [35]. Experiments in the 'OFF' state were nevertheless conducted ≥ 1 h after the clinical occurrence of wearing off. Lastly, we did not modify any of the patients' antiparkinsonian therapy, except safinamide, during the experiment to avoid undesired clinical-neurophysiological changes caused by different levels of dopaminergic stimulation.

Abnormal cortical facilitation in M1 in PD, without safinamide

In keeping with previous observations in PD [21,36–38], patients manifesting LIDs (when 'ON' L-dopa), demonstrated an increased steepness of the I/O curve in the 'OFF' state. This alteration improved, although it was not restored under L-dopa. Given

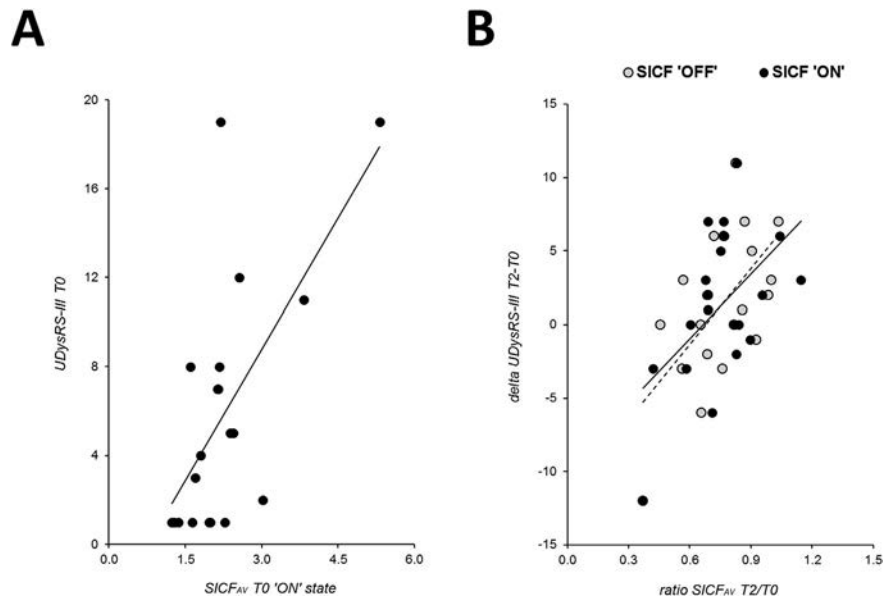


Fig. 5. Panel A: correlation between SICF and UDysRS-III at T0 ('ON' state), i.e. the higher the SICF the greater the intensity of LIDs. Panel B: correlation between changes in SICF and in UDysRS-III at T2 ('OFF' and 'ON' state), i.e. the higher the reduction of SICF the lower the worsening of LIDs. SICF_{AV}: average of SICF at 1.5 and 3 ms.

that the I/O curve is a measure of global corticospinal excitability, our data suggest increased excitability in the corticospinal system in patients with LIDs, that is only partly improved by L-dopa. We also found normal ICF in these patients 'OFF' state, which did not change with L-dopa. This finding is line with previous data in PD patients with and without LIDs [21,38,39], further suggesting that cortical circuits responsible for ICF are normal in PD, regardless of the clinical state of the patients. Given that previous pharmacological studies have suggested that ICF may reflect intracortical facilitation mediated by glutamatergic NMDA transmission [40,41], our observation points to the integrity of these circuits in PD.

The main finding of the study is that SICF was abnormally increased (prominently at ISI 1.5 ms) in patients who had LIDs, in both 'OFF' and 'ON' state. The comparable values of SICF in 'OFF' and 'ON' state suggest that this abnormality is not influenced by dopaminergic effects. SICF is a measure of cortical facilitation which depends on the specific timing of inputs on corticospinal neurons [33,41–43]. However, the specific circuits mediating SICF have not been fully elucidated, and various neurotransmitter systems may contribute to this facilitatory measure [41]. SICF implies a specific timing of peaks of MEPs facilitation corresponding to the intervals between I-waves, as demonstrated by direct epidural recordings in humans [42]. The facilitatory effect is likely produced by the synchronization of neural elements within M1, even though different cortical circuits may mediate the various peaks of facilitation [44,45]. Hence, the abnormally enhanced SICF in patients manifesting LIDs suggests a pathological neuronal synchronization in M1. Important insights into the pathophysiological role of abnormally enhanced SICF in PD come from our control experiment in patients who never manifested LIDs. Although patients with and without LIDs had comparable clinico-demographic features, patients without LIDs had a lower degree of SICF abnormalities. Moreover, differently from patients manifesting LIDs, SICF improved with L-dopa [46]. In patients with LIDs, the abnormal SICF increase and the lack of L-dopa responsiveness of this measure likely reflect pathophysiological mechanisms contributing to LIDs.

Effects of safinamide

Safinamide improved UPDRS-III in both 'OFF' and 'ON' state. This effect was likely due to the MAO-B reversible inhibition exerted by safinamide [47], leading to an increase in dopaminergic transmission. The combined dopaminergic and anti-glutamatergic properties of the drug might explain the improvement in mood, as tested by BDI-II [48]. In agreement with clinical studies [14,15], the intensity of LIDs was not significantly modified by safinamide, on average.

Neurophysiologically, safinamide reduced the abnormal M1 excitability observed in patients with LIDs, as indicated by the decreased steepness of the I/O curve. This beneficial effect of safinamide was not dose-dependent, as the comparable curves at T1 and T2 demonstrate. Since the steepness of the I/O curve was sensitive to L-dopa, we speculate that the similar changes observed at T1 and T2 may reflect increased baseline dopaminergic stimulation due to MAO-B inhibition, which is known to be similar at both low and high dosages of safinamide [14,47]. In line with this hypothesis, safinamide comparably improved UPDRS-III scores at T1 and T2. The observation that the I/O curve was even more flattened in patients 'ON' state at T2 may indicate that the pharmacological properties of safinamide 100 mg promote the beneficial effect of L-dopa on corticospinal excitability.

Another relevant finding of this study is that the abnormally enhanced SICF improved under safinamide at both 50 and 100 mg/day. Given the similar effect of safinamide on SICF and on the I/O curve (reduction of excessive MEP facilitation), it might be argued that the drug modulates these two TMS measures by acting on common neurophysiological mechanisms. However, we found no correlation between safinamide-induced changes in SICF and in the I/O curve. Also, differently from the I/O curve, the effect of safinamide on SICF was comparable in 'OFF' and 'ON' state, indicating that safinamide and L-dopa do not interact in circuits responsible for SICF. Hence, the altered SICF in patients with LIDs might reflect non-dopaminergic pathophysiological mechanisms. More importantly, unlike the changes in the I/O curves, SICF was clearly modulated in a dose-dependent manner, with the

strongest effect being observed at 100 mg/day. Based on *in vitro* and animal studies demonstrating that safinamide express specific anti-glutamatergic effects, particularly at high doses [16,17,19,49], we speculate that SICF reflects, at least in part, the activity of glutamatergic circuits within M1. These circuits may differ from those underlying ICF, which reflect NMDA-glutamatergic transmission not modulated by safinamide. The similar SICF in HS and patients at T2 indicates that safinamide restores the putative glutamatergic dysfunction in patients with LIDs. Accordingly, we conjecture that safinamide targets the glutamatergic activity in the intracortical circuits specifically altered in PD. The observation that safinamide activity was prominent at ISI 1.5 ms (the shortest interval used for SICF corresponding to the highest frequency of paired stimulation) may suggest that this drug exerts its greatest effects in a frequency-dependent manner. All these findings agree with those reported *in vitro* and in kindling models of seizures in animals [50], demonstrating that the anti-glutamatergic mechanism of action of safinamide is related to its ability to inhibit VGSCs in a state- and frequency-dependent manner [16,50]. Alternatively, we hypothesize that this drug acts prominently on neuronal elements responsible for the first peak of facilitation of SICF [44].

A further observation is that safinamide modulated SICF but not SICI. Human TMS studies have demonstrated that SICF can be reduced by drugs acting on GABA-A-ergic transmission [43]. Accordingly, it might be argued that safinamide reduced SICF by increasing GABA-A-ergic activity rather than decreasing glutamatergic neurotransmission. However, this hypothesis can be excluded by several reasons: first, animal studies have demonstrated that safinamide, in addition to its anti-glutamatergic effect, may inhibit, and not facilitate, cortical GABA release [49]. Second, our results in the human M1 showed that safinamide failed to modulate SICI, a measure of cortical GABA-A-ergic neurotransmission [20,22]. Third, our control experiment comparing SICF and SICI at ISI 1.5 and 3 ms demonstrated a specific effect of safinamide on SICF. Hence, our results point to a prominent anti-glutamatergic effect of the drug.

Relationship between abnormal cortical facilitation, LIDs and safinamide

Without safinamide, the degree of SICF in the 'ON' state positively correlated with the intensity of LIDs. Converging evidence from parkinsonian animal models points to an important role of overactive glutamatergic transmission in the cortico-striatal pathway in the pathophysiology of LIDs [3–7]. Also, a recent neuroimaging study in humans demonstrated that LIDs are accompanied by glutamate receptor overactivity in the precentral gyrus [51]. Our neurophysiological data are in line with this evidence and suggest a possible pathophysiological relationship between the abnormal cortical facilitation in M1 (SICF) and LIDs in patients with PD, likely reflecting dysfunctional glutamatergic neurotransmission.

Under safinamide 100 mg/day, despite the neurophysiological restoration of SICF, we found no clinical improvement of LIDs in our cohort of PD patients. This would suggest that the restoration of SICF is not sufficient to drive the clinical overt improvement of LIDs and that additional pathophysiological mechanisms may contribute to dyskinesia. The correlation we found between changes in SICF and in LIDs in patients treated with safinamide 100 mg/day (the higher the suppression of abnormal SICF, the lower the worsening of LIDs) would raise the hypothesis that the anti-glutamatergic effect of the drug may prevent the onset or worsening of LIDs, in agreement with animal data [18]. Future studies in humans would clarify this hypothesis.

Conclusions

Our study demonstrates that abnormal cortical facilitation is present in M1 in mid-to-late stage PD, being prominent in patients manifesting LIDs. However, this dysfunction is limited to specific facilitatory circuits within M1. Dopaminergic stimulation improves the overall M1 excitability in PD (i.e. I/O curve) though not the abnormal facilitation within M1, as tested by SICF. This specific circuit is modulated by safinamide and even restored when the drug is used at 100 mg/day, suggesting a possible link between abnormal SICF and overactive glutamatergic neurotransmission in M1 in patients manifesting LIDs. Overall, our neurophysiological data support the classical model of LIDs pathophysiology in PD, in which excessive glutamate release represents a key endogenous mediator of LIDs [4–7,9].

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Conflict of interest/financial disclosures

A.G., A.S., V.D.O., F.D.S., F.A., G.F. and A.B. have nothing to report.

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