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Pain-motor integration in the primary motor cortex in Parkinson's disease

A. Suppa^{a,b}, C. Leone^a, F. Di Stasio^b, L. Marsili^a, A. Di Santo^{a,c}, A. Biasiotta^a, S. La Cesa^a, A. Truini^a, G. Cruccu^a, A. Berardelli^{a,b,*}^a Department of Neurology and Psychiatry, Sapienza University of Rome, Italy^b IRCCS Neuromed Institute, Pozzilli IS, Italy^c Neurology Unit, Campus Bio-Medico University, Rome, Italy

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ABSTRACT

Background: In Parkinson's disease (PD), the influence of chronic pain on motor features has never been investigated. We have recently designed a technique that combines nociceptive system activation by laser stimuli and primary motor cortex (M1) activation through transcranial magnetic stimulation (TMS), in a laser-paired associative stimulation design (Laser-PAS). In controls, Laser-PAS induces long-term changes in motor evoked potentials reflecting M1 long-term potentiation-like plasticity, arising from pain-motor integration.

Objective: We here examined the possible influence of chronic pain on motor responses to Laser-PAS in patients with PD, with and without chronic pain.

Methods: We compared motor responses to Laser-PAS in healthy subjects and in patients with PD, with and without chronic pain.

Results: Unlike controls, we found reduced responses to Laser-PAS in patients with PD, with and without pain. Patients off and on dopaminergic therapy had similar responses to Laser-PAS. When comparing responses to Laser-PAS in patients with and without pain, the two patients' subgroups had similar abnormalities. When we compared patients with pain in the body region investigated with Laser-PAS, with those with pain in other body regions, we found prominent changes in patients with homotopic pain. Finally, when comparing Laser-PAS with the original PAS protocol, which combines electric peripheral nerve stimuli and TMS, in patients without pain and those with homotopic pain, we found similar responses to both techniques in patients without pain, whereas Laser-PAS induced greater abnormalities than PAS in patients with pain.

Conclusions: In PD, chronic pain degrades response to Laser-PAS through abnormal pain-motor integration.

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Abbreviations: ACC, anterior cingulate cortex; ADM, abductor digiti minimi; ANOVA, analysis of variance; La DN4, douleur neuropathique en 4 questions; HAM-D, Hamilton Depression Rating Scale; Laser-PAS, laser-paired associative stimulation; LEDDs, L-Dopa equivalent daily doses; LEP, laser evoked potential; LTP, long-term potentiation; LTD, long-term depression; M1, primary motor cortex; MEP, motor evoked potential; MMSE, Mini Mental State Evaluation; NRS, numerical rating scale; PAS, paired associative stimulation; PD, Parkinson's disease; RMT, resting motor threshold; S2, secondary somatosensory area; STDP, spike timing-dependent plasticity; TMS, transcranial magnetic stimulation; MDS-UPDRS, Movement Disorders Society-Unified Parkinson's Disease Rating Scale.

* Corresponding author. Department of Neurology and Psychiatry, Neuromed Institute, Sapienza University of Rome, Viale dell'Università, 30, 00185 Rome, Italy.

E-mail address: alfredo.berardelli@uniroma1.it (A. Berardelli).

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Chronic pain is a non-motor symptom observed in 40–85% of patients with Parkinson's disease (PD) [1–4]. In PD, chronic pain is currently classified in nociceptive (i.e. musculoskeletal, visceral and cutaneous pain) and neurophatic pain (i.e. radicular and central pain) [1,5,6]. Similarly to other non-motor symptoms in PD, pain is currently considered to reflect extranigral pathology [7,8], but the pathophysiology of chronic pain in PD remains largely unknown [1,2,5,6,9].

Laser stimulation is widely considered a reliable tool for investigating pain pathways in humans [10]. Laser stimulation selectively activates A δ and C nociceptors and evokes [11] scalp potentials (LEPs) comprising an early lateralized component (N1), generated by secondary sensory area (S2) area and a late negative-

positive complex (N2-P2) generated by anterior cingulate cortex (ACC) [11–13]. Previous studies in patients with PD demonstrated LEP abnormalities in patients with nociceptive and neuropathic pain [14,15], suggesting abnormal cortical processing of nociceptive inputs as a key pathophysiological mechanism underlying chronic pain in PD [2,4,9].

Recently we have designed a new technique, namely Laser-paired associative stimulation (Laser-PAS₅₀) [16]. This protocol combines laser pulses delivered to the hand with transcranial magnetic stimulation (TMS) of M1 in a PAS design. In healthy subjects, Laser-PAS₅₀ elicits long-term increase of motor evoked potential (MEP) amplitude through mechanisms of long-term potentiation (LTP)-like plasticity in M1 possibly related to changes in N-Methyl-D-aspartate transmission [16].

No studies have previously investigated M1 plasticity as assessed with the Laser-PAS₅₀ technique in PD with and without chronic pain. A better understanding of pain-related M1 plasticity might clarify the pathophysiological basis of chronic pain in PD and open new perspectives in the treatment of this non-motor symptom.

In this study, we applied Laser-PAS₅₀ in a cohort of patients with PD. Then to clarify whether in PD chronic pain influences the responses to Laser-PAS₅₀, we compared Laser-PAS₅₀ in patients with and without pain. To understand whether pain has a segmental or generalized effect, we compared responses to Laser-PAS₅₀ in patients with pain in the right upper limb, the same body region examined by Laser-PAS₅₀, and patients with pain in other body regions. Finally, to verify whether abnormal responses to Laser-PAS₅₀ in patients with chronic pain depends on intrinsic M1 plasticity abnormalities regardless of the specific PAS protocol used, we compared responses to Laser-PAS₅₀ and to the original PAS protocol, which combines electric peripheral nerve stimuli and transcranial magnetic stimulation (TMS) at interstimulus interval of 25ms (PAS₂₅) [17], in the same cohort of patients, with and without pain.

Material and methods

Subjects

Twenty patients with PD (14 men and 6 women, mean age \pm SD: 62.5 \pm 9.5) and 20 age-matched healthy subjects (10 men and 10 women, mean age \pm SD: 59.7 \pm 15.8) participated in the study. All participants were right-handed. The diagnosis of idiopathic PD was made according to the Queen Square Brain Bank criteria and the EFNS/MDS-ES recommendations [18,19]. Patients were recruited from the Movement Disorder Clinic at the Department of Neurology and Psychiatry, Sapienza University of Rome. Patients had a predominantly akinetic-rigid syndrome without dementia. Patients were studied while they were under their usual dopaminergic treatment (on) and after drugs had been withdrawn for at least 12 h (off). None of the patients received other neuropsychiatric medications. Patients were clinically evaluated before starting each experimental session. Motor signs were scored using the motor section of the Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) [20,21] and the Hoehn & Yahr (H&Y) scale. Cognitive function was evaluated using the Mini Mental State Evaluation (MMSE) [22] and the Frontal Assessment Battery (FAB) [23]. Depression was assessed with the Hamilton Depression Rating Scale (HAM-D) [24]. According to the criteria suggested by Wasner et al. [5], we first evaluated the presence of chronic pain. None of the healthy controls reported any type of pain. Patients were asked to rate the intensity of pain on an 11-point numerical rating scale (NRS) from 0 (no pain) to 10 (strongest imaginable pain) [10] before the first experimental session and

relative to the previous 6 months. Those rating pain \geq 4 and reporting it from at least 6 months were considered "chronic-pain" patients. Patients with chronic pain also underwent the screening tool for the detection of neuropathic pain (La douleur neuropathique en 4 questions - DN4) [25]. No patients were being administered with any treatment for pain at the time of the study. Demographic and clinical features of parkinsonian patients, with and without pain are summarized in Table 1. Subjects gave their written informed consent. The study was approved by the institutional review Board of Sapienza, University of Rome, Italy and conformed to the Declaration of Helsinki.

Laser stimulation technique and LEP recordings

Laser stimuli were delivered with a neodymium:yttrium-aluminium-perovskite laser stimulator (Nd:YAP, wave length 1.34 μ m, pulse duration 2–20 ms, maximum energy 7 J El.En - Florence, Italy) under fiber-optic guidance. The laser perceptive threshold was determined by increasing and decreasing the stimulus energy in steps of 0.25 J in series of three stimuli, and defined as the lowest intensity at which the subjects perceived at least the 50% of the stimuli [26]. To evoke clear and stable LEPs, laser stimuli were set to induce a clear painful pinprick sensation (intensity 119.4–150 mJ/mm²; duration 5 ms; spot diameter 5 mm) and directed to the ulnar region of the right hand dorsum. The target of the laser beam was shifted by at least 1 cm in a random direction, to allow for passive skin cooling and avoid nociceptor fatigue or sensitization and the interstimulus interval was varied pseudo-randomly (10–15 s) [26]. Subjects, wearing protective goggles, rested comfortably on a medical examination table. LEPs were recorded through surface electrodes from the scalp: T3 referenced to Fz for recording the early lateralized N1 component, and Cz referenced to the nose, for recording the late vertex N2-P2 complex. Electro-oculographic recordings monitored possible eye movements or blinks. We averaged 15 artefact-free trials off line using dedicated equipment (Synergy 10.1; EL.EN, Florence, Italy). The recording bandpass was between 0.3 and 30 Hz and the sampling frequency 50 kHz. We measured the peak latency and amplitude of the lateralized N1 and the vertex N2-P2 complex. These methods adhered to the recommendations given by the International Federation of Clinical Neurophysiology [11]. LEP recordings were taken at least 30 min before TMS, while the patients were on therapy (1 h after taking their usually antiparkinsonian therapy).

TMS and MEP recordings

TMS was delivered through a repetitive magnetic stimulator (Magstim Super Rapid-The Magstim Company Ltd, Whitland, United Kingdom) connected to a figure-of-eight coil (external wing 9 cm in diameter) placed tangentially to the scalp on the left hemisphere, with the handle pointing back and away from the midline at 45° inducing postero-anterior and antero-posterior biphasic currents in the brain. The coil was placed over the optimum scalp position (hot spot) to elicit MEPs in the abductor digiti minimi (ADM) muscle of the right hand. To ensure that the stimulating coil remained in a constant position throughout the experiments, the hot spot was marked on the scalp with a soft-tipped pen. Motor threshold was determined at rest (RMT) as the lowest intensity able to evoke a MEP of more than 50 μ V in at least 5 of 10 consecutive trials in the ADM muscle. RMT was determined in steps of 1% maximum stimulator output intensity. Electromyographic activity was recorded through a pair of surface electrodes placed over the right ADM muscle, using a belly-tendon montage. Electromyographic raw signals were recorded, sampled at 5 kHz with a CED 1401 A/D laboratory interface (Cambridge Electronic Design,

Table 1

Demographic and clinical features of 20 patients with Parkinson's disease (PD), with and without chronic pain, participating into the experiments. Note that 15 out of 20 patients (75%) reported chronic pain and none of the patients reported neuropathic pain. MDS-UPDRS: Movement Disorders Society-Unified Parkinson's Disease Rating Scale; H&Y: Hoehn & Yahr scale. MMSE: Mini Mental State Evaluation; FAB: Frontal Assessment Battery; HAM-D: Hamilton Depression Rating Scale; LEDDs: L-Dopa equivalent daily doses (mg) calculated for each patient according to the criteria of Tomlinson et al. [88]; RUL: right upper limb; DN4: Neuropathic Pain in 4 questions; NRS: numerical rating scale for pain; AV: average. SD: standard deviation. The 0 scores from the questionnaire for the detection of neuropathic pain (DN4) are not shown.

Patient	Age (years)	Gender	Disease Duration	UPDRS		H&Y	MMSE	FAB	HAM-D	LEDDs	ChronicPain	RUL Pain	DN4	NRS
				On	Off									
1	71	M	1	22	32	2	28	16	2	300	–	–	–	–
2	74	M	14	9	30	2	30	17	2	850	+	–	1	4
3	69	M	5	26	40	2	24	14	5	650	–	–	–	–
4	80	M	7	11	20	1	30	16	0	550	+	+	0	6
5	48	F	7	20	30	2	30	18	3	405	+	–	0	7
6	61	F	8	21	32	2	28	17	3	460	–	–	–	–
7	63	F	7	18	33	2	30	18	2	400	+	+	0	8
8	59	M	1	4	12	1	30	18	7	310	+	+	0	4
9	69	M	5	12	20	2	30	18	0	310	–	–	–	–
10	60	M	2	10	12	1	30	18	0	260	–	–	–	–
11	69	M	10	17	40	2	28	17	2	942	+	+	0	5
12	63	M	7	8	22	1	30	18	3	400	+	+	0	6
13	62	M	3	14	25	2	30	18	7	310	+	–	0	4
14	60	F	5	12	20	2	30	18	7	405	+	–	0	5
15	58	M	1	11	16	2	30	18	7	160	+	–	0	5
16	40	F	3	35	38	1	30	18	20	310	+	+	3	5
17	75	M	2	20	23	1	29	8	13	400	+	+	3	7
18	61	M	1	22	26	1	30	18	4	400	+	+	0	5
19	53	M	1	14	15	1	30	17	7	340	+	+	0	4
20	55	F	2	16	20	1	30	18	0	300	+	+	0	5
Av	62.5		4.6	16.1	25.3	1.6	29.4	16.9	4.7	423.1			0.5	5.3
SD	9.5		3.6	7.2	8.8	0.5	1.5	2.3	4.9	193.0			1.1	1.2

Cambridge, UK), amplified ($\times 1000$), and filtered (bandwidth 5–1 kHz) with the Digitimer D360 (Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) and stored on a laboratory computer for on-line visual display and further off-line analysis (Signal software, Cambridge Electronic Design, Cambridge, UK). The baseline electromyographic activity level before, during and after TMS was controlled by visual-feedback through an oscilloscope screen and auditory feedback through a loudspeaker. To exclude possible confounding effects from involuntary muscular contraction, trials with background electromyographic activity were rejected. MEP amplitude was measured peak-to-peak (mV) and then averaged.

Experimental design

The study consisted of two separate experimental sessions (Experiments 1–2; Fig. 1). All controls and patients participated in Experiment 1, whereas a subgroup of 10 patients participated in Experiments 2. All experimental sessions took place at comparable daytime and at least 1 week elapsed between each session. Participants were tested fully relaxed and with their eyes open.

Experiment 1: Effect of Laser-PAS₅₀

In this experiment, designed to investigate the response to Laser-PAS₅₀ protocol in healthy controls and patients with PD, with and without pain, when off and on therapy, we used a “test-conditioning” TMS approach. The “test” stimulation consisted of 20 single TMS pulses able to elicit MEPs of 1 mV from the right ADM at baseline (T₀). We then recorded and measured a clear-cut peak LEP N1 component according to the international recommendations [11]. We applied “conditioning” Laser-PAS₅₀ consisting of 60 repetitive TMS pulses at 0.1 Hz, each TMS pulse following a single laser stimulus delivered at an interstimulus interval of LEP N1 latency+50 ms (total duration of intervention: 10 min) [16]. Laser stimuli were delivered at the same intensity used during LEP recording. To investigate the time course of after-effects induced by

Laser-PAS₅₀, we compared baseline MEPs (T₀) with those recorded immediately after Laser-PAS₅₀ ended (T₁) and at 10 (T₂), 20 (T₃), 30 (T₄), 40 (T₅), 50 (T₆) and 60 (T₇) after it ended. All patients were studied at T₁–T₇. Patients were randomly assigned to participate in two separate sessions, when off and on therapy.

Experiment 2: Effect of Laser-PAS₅₀ and PAS₂₅

This experiment was designed to compare responses to Laser-PAS₅₀ and to PAS₂₅ protocol in patients with PD, with and without pain, while they were on therapy. A subgroup of 10 patients, 5 with pain in the right upper limb, and 5 patients without pain participated in two separate experimental sessions (Laser-PAS₅₀ and PAS₂₅), randomly. We delivered Laser-PAS₅₀ as described in Experiment 1, and recorded 20 MEPs before (T₀) and after conditioning (T₁–T₇). PAS₂₅ was delivered using peripheral ulnar nerve electric stimulation (cathode proximal) and TMS over the cortical hot-spot for ADM at an interstimulus interval of 25 ms. The intensity of TMS was set to evoke an MEP of 1 mV in the ADM muscle, while the intensity of the ulnar nerve stimulus (0.2 ms duration) was set at 3 times perceptible threshold (by calculating the minimum intensity at which 5 electric stimuli, delivered over the ulnar nerve at the wrist, are all clearly perceived). Two hundred pairs of stimuli were given at 0.25 Hz. MEPs were recorded from the ADM muscle at baseline (T₀) and at T₁–T₇ after PAS₂₅, and then averaged.

Statistical analysis

We used the unpaired Student's *t*-test to compare the RMT, the intensity to evoke baseline MEPs and for Laser-PAS₅₀, heat sensation, the intensity of laser pulse during Laser-PAS₅₀, the latency and amplitude of MEP and of main LEP components (N1 and N2/P2), in controls and in patients, when off and on therapy. To assess the effect of dopaminergic therapy on MEP and LEP measurements we used the paired Student's *t*-test.

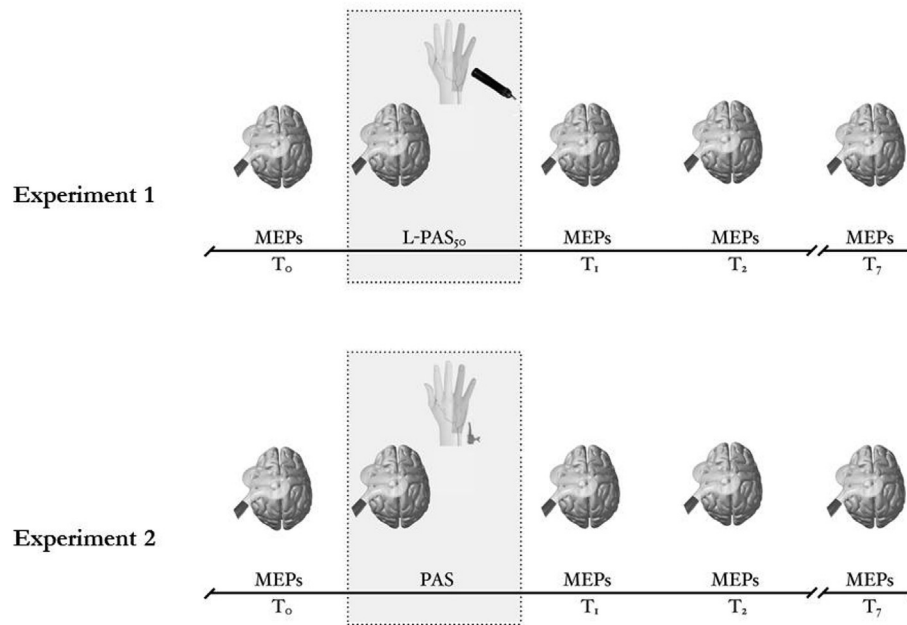


Fig. 1. Experimental paradigm. Experiment 1 began with laser-evoked potentials (LEPs) recordings. We tested the excitability of the primary motor cortex (M1) recording 1 mV motor evoked potentials (MEPs). We then applied 10 min of Laser-PAS₅₀ and examined long-term changes in MEP amplitude, for 60 min after Laser-PAS₅₀ ended, in healthy subjects (HS) and patients with Parkinson's disease (PD), with and without pain, off and on therapy. In the experiment 2, we compared MEPs before (T₀) and for 60 min following Laser-PAS₅₀ and PAS₂₅ in a subgroup of patients with PD with chronic pain in the right upper limb and in a subgroup of PD patients without pain. MEPs were recorded from the right abductor digiti minimi muscle (ADM).

In Experiment 1, to test the effect of Laser-PAS₅₀ on MEP amplitude in controls and patients, off and on therapy, we used between-group analysis of variance (ANOVA) with “Group” (healthy subjects vs patients off therapy, healthy subjects vs patients on therapy) and “Time” (T₀ vs T₁-T₇). To clarify the effect of dopaminergic therapy on responses to Laser-PAS₅₀ in patients, we also used the two-ways repeated-measures ANOVA with factor “dopaminergic therapy” (off vs on) and factor “Time”. In a subsequent analysis, to assess the effect of chronic pain on plasticity induced by Laser-PAS₅₀ in patients, off and on therapy, we used two separate between-group ANOVAs with factor “Pain” (patients with vs patients without pain, off and on therapy) and “Time”. To assess the effect of chronic pain in homotopic and heterotopic body regions in PD, we first compared responses to Laser-PAS₅₀ in controls and patients with homotopic or heterotopic pain, off and on therapy, by using separate between-group ANOVAs with factor “Group” (healthy subjects vs patients with pain in the right upper limb or patients with pain in other body regions, off and on therapy) and factor “Time”. Then, we also used two separate between-group ANOVAs with factor “Pain Localization” (patients with pain in the right upper limb vs patients with pain in other body regions, off and on therapy) and “Time” to better assess the specific effect of chronic pain in homotopic and heterotopic body regions in patients, off and on therapy.

In the Experiment 2, to compare the effect of Laser-PAS₅₀ and PAS₂₅ on MEP amplitude in patients with chronic pain in the right upper limb, and in patients without chronic pain, on therapy, we used a mixed-design ANOVA with “Group” (patients with pain in the right upper limb vs patients without pain), “Protocol” (Laser-PAS₅₀ vs PAS₂₅) and “Time” (T₀ vs T₁-T₇) as main factors of analysis.

We used the Spearman's R correlation test to assess correlation between patients' clinical features (UPDRS, MMSE, FAB, HAM-D, NRS, disease duration and total L-Dopa equivalent daily doses - LEDDs) and changes in MEP amplitude induced by Laser-PAS₅₀ respect to baseline (T₀), at all time intervals. P values less than 0.05

were considered to be statistically significant. The Greenhouse-Geisser correction was used when necessary to correct the non-sphericity.

All data are reported as average \pm SD.

Results

None of the subjects experienced any adverse effects during or after the Laser-PAS₅₀. All subjects perceived laser stimuli as painful in all experimental sessions and had LEP values within the normative ranges established in our laboratory [11,26].

In our cohort of patients, 15 out of 20 patients (10 men and 5 women, mean age \pm SD: 61.3 \pm 10.4) reported at least one type of chronic pain (patient number 2, 4–5, 7–8, 11–20), all of them underwent the DN4 questionnaire and obtained a score <4, thus excluding the presence of neuropathic pain (Table 1).

The Student's *t*-test showed comparable RMT, TMS intensity, perceptive threshold, laser pulse intensity, latency and amplitude of MEPs and LEPs, in controls and in patients, when off and on therapy (all *p* values > 0.05) (Tables 2 and 3).

Experiment 1

ANOVA showed that, after Laser-PAS₅₀, MEPs increased in amplitude at T₁-T₇ in healthy controls, whereas in patients it remained unchanged. The amount of MEP changes induced by Laser-PAS₅₀ differed in healthy controls and in patients off therapy. ANOVA showed a significant interaction between factors “Group” and “Time” ($F_{7,266} = 6.16$; $p < 0.001$), and a significant effect of factor “Group” ($F_{1,38} = 6.13$; $p = 0.02$) and “Time” ($F_{7,266} = 8.67$; $p < 0.001$). In controls, ANOVA showed a significant effect of the factor “Time” ($F_{7,133} = 8.34$; $p < 0.001$); after Laser-PAS₅₀, MEP amplitude increased significantly at T₂-T₇ ($p < 0.01$ for all comparisons). By contrast, in patients off therapy, the factor “Time” was not significant ($F_{7,133} = 1.02$; $p = 0.42$). Although MEP amplitude

was similar at T0 in controls and patients off therapy, after Laser-PAS₅₀, MEP amplitude differed significantly at T2-T7 in the two groups ($p < 0.05$ for all comparisons) (Fig. 2).

Comparing healthy controls and patients on therapy, ANOVA showed significant interaction between factors “Group” and “Time” ($F_{7,266} = 4.80$; $p < 0.001$), and a significant effect of factor “Group” ($F_{1,38} = 7.10$; $p = 0.01$) and “Time” ($F_{7,266} = 9.48$; $p < 0.001$). In controls, ANOVA confirmed a significant effect of the factor “Time” ($F_{7,133} = 8.34$; $p < 0.001$); after Laser-PAS₅₀, MEP amplitude significantly increased at T2-T7 ($p < 0.01$ for all comparison). By contrast, in patients on therapy, the factor “Time” was not significant ($F_{7,133} = 1.50$; $p = 0.17$). Although MEPs were comparable at T0 in healthy subjects and patients on therapy, after Laser-PAS₅₀, MEPs differ at T2-T7 ($p < 0.05$ for all comparison) (Fig. 2).

When we assessed the effect of dopaminergic therapy in patients, ANOVA showed no significant effect of the factor “dopaminergic therapy” ($F_{1,19} = 0.62$; $p = 0.44$) nor the effect of the factor “Time” ($F_{7,133} = 1.26$; $p = 0.22$) (Fig. 2).

When testing the effect of chronic pain on responses to Laser-PAS₅₀ in patients, off and on therapy, ANOVA showed a non significant effect of the factor “Pain” ($F_{1,18} = 0.26$; $p = 0.62$) and “Time” ($F_{7,126} = 0.63$; $p = 0.73$) when off and on state of therapy ($F_{1,18} = 0.22$; $p = 0.65$ and $F_{7,126} = 1.24$; $p = 0.29$, respectively) (Fig. 3).

Comparing controls with patients with pain in the right upper limb ($n = 10$, patient number 4, 7, 8, 11, 12 and 16–20), ANOVA showed a significant interaction between factors “Group” and “Time”, when off and on therapy ($F_{7,196} = 3.01$; $p < 0.01$ and $F_{7,196} = 4.07$; $p < 0.01$, respectively) and a significant effect of factor “Group” ($F_{1,28} = 4.22$; $p < 0.01$ and $F_{1,28} = 6.38$; $p < 0.01$, respectively) and “Time” ($F_{7,196} = 5.07$; $p < 0.01$ and $F_{7,196} = 3.58$; $p < 0.01$, respectively). Post hoc analysis showed that MEPs increased in amplitude at T3-T7 (all p values < 0.05) in controls but not in patients with pain in the right upper limb, when off and on therapy. Differently, when we tested responses to Laser-PAS₅₀ in controls and patients with pain in other body regions ($n = 5$, patient number 2, 5, 13, 14, 15), off and on therapy, ANOVA showed a significant effect of the factor “Time” ($F_{7,16} = 4.80$; $p < 0.01$ and $F_{7,16} = 4.53$; $p < 0.01$, respectively), whereas factor “Group” ($F_{1,23} = 0.46$; $p = 0.5$

Table 3

Transcranial magnetic stimulation (TMS) data in the experiment testing primary motor cortex (M1) plasticity in 20 healthy subjects (HS) and in 20 patients with Parkinson's disease (PD), off and on therapy. RMT = Resting motor threshold; Int. for MEPs = Intensity for 1 mv MEP.

	RMT (%)	Int. for MEPs (%)
Controls	41.1 ± 4.9	54.7 ± 9.4
PD off	41.3 ± 15.2	54.3 ± 20.2
PD on	43.9 ± 15	57.2 ± 17.4

and $F_{1,23} = 0.67$; $p = 0.42$, respectively) and the interaction between “Group” and “Time” ($F_{7,161} = 0.49$; $p = 0.84$ and $F_{7,161} = 0.52$; $p = 0.82$, respectively) were both non significant.

When comparing responses to Laser-PAS₅₀ in patients, off and on therapy, with pain in the right upper limb with patients with pain in other body regions, ANOVA showed a significant interaction between factors “Pain Localization” and “Time” ($F_{7,91} = 3.33$; $p < 0.01$) and a significant effect of the factor “Pain Localization” ($F_{1,13} = 4.68$; $p = 0.04$) and “Time” ($F_{7,91} = 6.46$; $p < 0.01$) in patients off therapy. Post hoc analysis showed that MEPs decreased in amplitude at T3-T5 (all p values < 0.05) in patients with pain in the right upper limb compared with patients with pain in other body regions. Similarly, in patients on therapy ANOVA showed a significant interaction between factors “Pain Localization” and “Time” ($F_{7,91} = 2.39$; $p = 0.03$) and a significant effect of the factor “Pain Localization” ($F_{1,13} = 7.64$; $p = 0.02$) and “Time” ($F_{7,91} = 2.20$; $p = 0.04$). Post hoc analysis showed that MEPs decreased in amplitude at T4-T7 (all p values < 0.05) in patients with pain in the right upper limb compared with patients with pain in other body regions (Fig. 4).

Experiment 2

In the Experiment 2, ANOVA showed a significant effect of the factor “Protocol” ($F_{1,8} = 8.48$; $p = 0.02$) and “Time” ($F_{7,56} = 3.61$; $p < 0.01$). In patients with pain in the right upper limb, post hoc analysis showed an interaction between the factors “Protocol” and “Time” ($F_{7,28} = 4.07$; $p < 0.01$) and a significant effect of the factor “Protocol” ($F_{1,4} = 139.98$; $p < 0.01$) and “Time” ($F_{7,28} = 4.40$;

Table 2

Analytical results for Laser-evoked potential (LEP) recordings in the 20 patients with Parkinson's disease (PD). Stim. Int: Stimulation Intensity; Lat: Latency; Amp: Amplitude.

Patients	Perceptive Threshold (mj/mm ²)	Stim Int. (mj/mm ²)	N1 lat. (ms)	N1 amp. (μV)	N2 lat. (ms)	N2 amp. (μV)	P2 lat. (ms)	P2 amp. (μV)
1	51	102	188	8.6	210	9.2	314	13.2
2	51	127	200	5.0	240	4.0	350	8.0
3	70	153	198	3.0	236	7.0	438	2.0
4	76	153	220	6.5	284	4.5	420	16.0
5	38	102	152	6.0	194	16.0	330	14.0
6	64	127	182	3.0	216	4.0	298	20.0
7	39	115	220	6.0	248	10.0	350	18.0
8	51	127	188	10.0	239	7.2	360	13.0
9	50	127	216	6.0	244	6.0	318	1.5
10	50	114	218	13.0	252	7.0	366	6.0
11	38	127	220	5.0	240	10.0	395	7.5
12	51	127	138	9.0	180	8.0	300	12.0
13	50	127	198	6.5	238	13.0	358	7.0
14	51	127	188	6.0	230	3.0	294	9.0
15	40	140	244	5.0	246	8.4	390	7.0
16	38	102	186	5.0	244	1.0	330	17.0
17	38	127	164	1.0	223	6.0	356	14.0
18	25	178	230	4.3	274	4.3	384	6.0
19	76	153	178	13.2	226	9.6	318	22.6
20	51	127	193	6.5	230	7.0	350	9.0
Av	49.9	129.1	195.05	6.4	234.7	7.26	350.95	11.1
SD	13.3	19.1	26.4	3.1	23.7	3.5	41.0	5.8

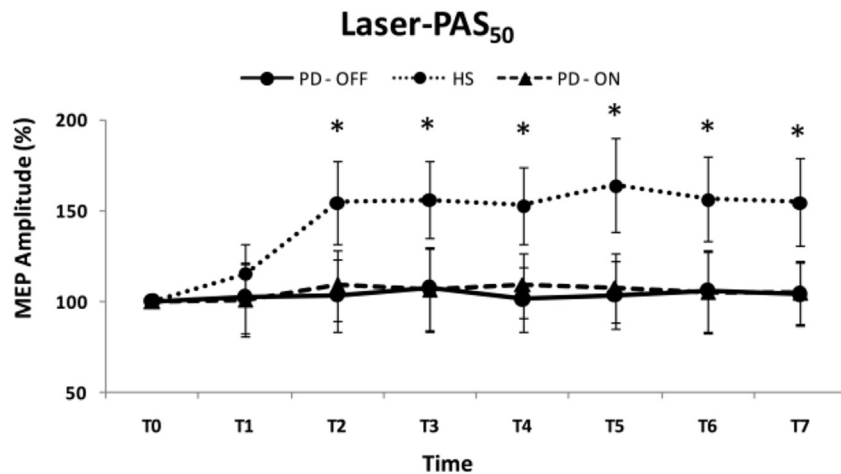


Fig. 2. Effects of Laser-PAS₅₀ on motor evoked potential (MEP) amplitude in healthy subjects (HS) and in patients with Parkinson's disease (PD), off and on therapy. Each point corresponds to the mean MEP amplitude recorded at baseline and for 60 min after Laser-PAS₅₀. Vertical bars represent SD. Asterisks indicate significant P values. Note that Laser-PAS₅₀ increased MEP amplitude in healthy subjects but not in patients with PD, off and on therapy.

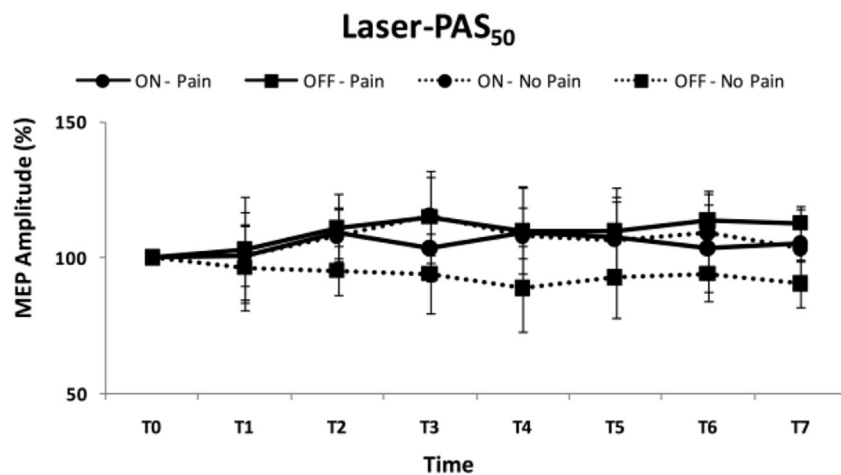


Fig. 3. Effect of Laser-PAS₅₀ on motor evoked potential (MEP) amplitude in patients with Parkinson's disease (PD), with and without pain, off and on therapy. Each point corresponds to the mean MEP amplitude recorded at baseline and for 60 min after Laser-PAS₅₀. Vertical bars represent SD. Note that Laser-PAS₅₀ induced no changes on MEP amplitude in PD patient, with and without pain, off and on therapy.

$p < 0.01$). Although in patients with pain in the right upper limb, MEPs were comparable before Laser-PAS₅₀ and PAS₂₅ (T0) ($p = 1$), compared to PAS₂₅, Laser-PAS₅₀ elicited lower amplitude MEPs at T2-T7 (all p values < 0.05). Conversely, in patients without pain, post hoc analysis showed a non significant effect of the factors "Protocol" ($F_{1,4} = 0.81$; $p = 0.42$) and "Time" ($F_{7,28} = 1.58$; $p = 0.18$) demonstrating that MEPs were comparable before and after Laser-PAS₅₀ and PAS₂₅ (all $p > 0.05$) (Fig. 5).

The Spearman's R correlation test found no correlation between patients' clinical features at all the time intervals. In contrast, Spearman's correlation test showed a significant negative correlation between the severity of pain reported in the right upper limb, as assessed by the NRS, and the percentage of MEP amplitude increase at T5 after Laser-PAS₅₀ in patients off ($R = -0.78$ and $p = 0.01$) and on therapy ($R = -0.70$ and $p = 0.02$) (Fig. 6A and B).

Discussion

In this study, we demonstrated abnormal MEP responses to Laser-PAS₅₀ in PD. Dopaminergic therapy left responses to Laser-PAS₅₀ globally unchanged. The response to Laser-PAS₅₀ was

comparable in patients with and without chronic pain. In patients with pain in the limb directly investigated with Laser-PAS₅₀, we found prominent abnormal responses than in patients with pain in other body regions. We provide here the first evidence of a reduced responses to Laser-PAS₅₀ in patients with and without chronic pain, off and on therapy, reflecting an altered pain-motor integration.

When testing the effects of Laser-PAS₅₀ in PD, we have excluded a number of methodological factors possibly leading to misinterpretation of our data. Given that we found comparable RMT values, intensity to evoke MEPs of 1 mV amplitude and intensity for TMS during Laser-PAS₅₀, in healthy controls and patients, off and on therapy, we excluded possible confoundings due to altered integrity of the cortico-spinal tract as well as difference in M1 baseline excitability. We also used comparable laser intensity during Laser-PAS₅₀, in controls and patients, off and on therapy. During each experimental session, we checked the patients' muscle relaxation and none of the patients showed voluntary or involuntary muscular activity before, during or after Laser-PAS₅₀ excluding homeostatic or non-homeostatic interaction between muscular activity and TMS [27–31]. In this study, when we applied repeated sessions of Laser-PAS₅₀ in the same patient, we used between-session intervals of at

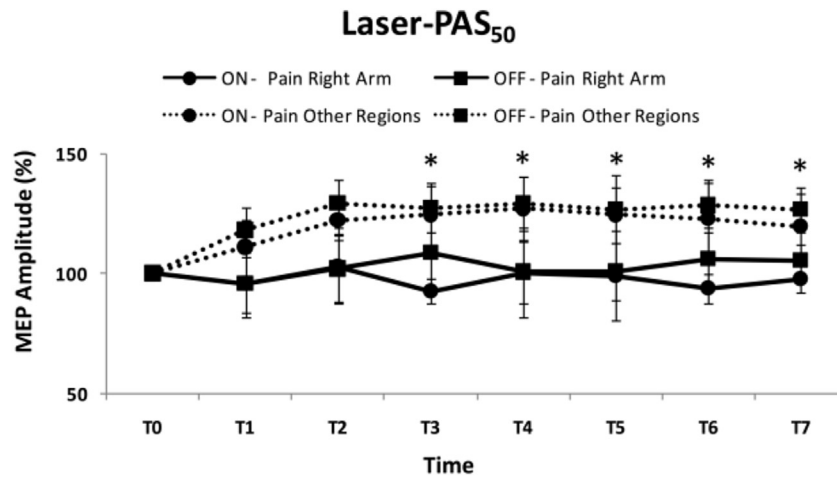


Fig. 4. Effect of Laser-PAS₅₀ on motor evoked potential (MEP) amplitude in patients with Parkinson's disease (PD), with pain in the right upper limb or in other body regions. Each point corresponds to the mean MEP amplitude recorded at baseline and for 60 min after Laser-PAS₅₀. Vertical bars represent SD. Asterisks indicate significant P values (significant values at T3-T5 in patients off, and at T4-T7 in patients on therapy). Note that Laser-PAS₅₀ induced no changes in MEP amplitude in all patients' subgroups. However, Laser-PAS₅₀ induced lower responses in patients with pain in the right upper limb than those observed in patients with pain in other body regions, off and on therapy.

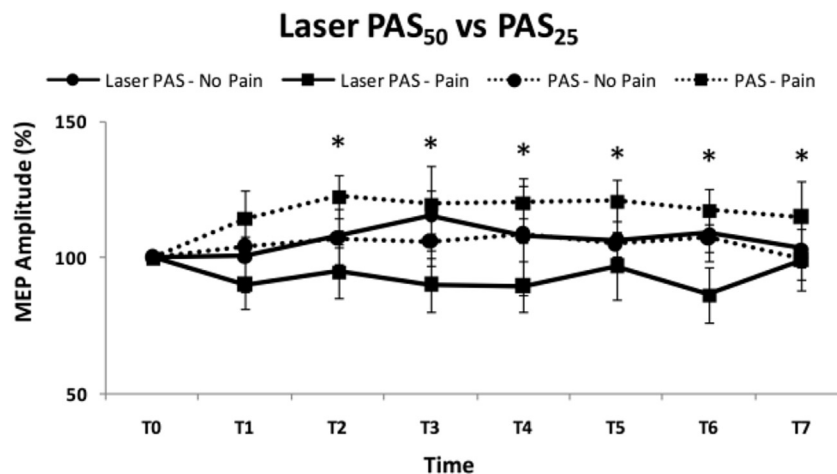


Fig. 5. Effect of Laser-PAS₅₀ and PAS₂₅ on motor evoked potential (MEP) amplitude in patients with Parkinson's disease (PD), with pain in the right upper limb and in patients without pain, on therapy. Each point corresponds to the mean MEP amplitude recorded at baseline and for 60 min after Laser-PAS₅₀ and PAS₂₅. Vertical bars represent SD. Asterisks indicate significant P values. Note that Laser-PAS₅₀ and PAS₂₅ induced comparable changes in PD patients without pain, whereas in PD patients with pain in the right upper limb they did not. Compared to PAS₂₅, Laser-PAS₅₀ induced lower responses in patients with pain in the right upper limb than those observed in patients without pain, on therapy.

least one week making highly unlikely the possible interference between the two experimental sessions [28,31,32].

Laser-PAS₅₀ elicits reduced responses in Parkinson's disease

The reduced responses to Laser-PAS₅₀ we observed in patients off therapy, may reflect a number of pathophysiological mechanisms. We can reasonably exclude a significant contribution of small-nerve fibre damage, previously demonstrated in a skin biopsy study in patients with PD [33] since none of the participants manifested or referred any dysautonomic symptom and none had pure neuropathic pain. Moreover, we found comparable laser perceptive thresholds, latency and amplitude of N1- and N2/P2-LEP components in healthy controls and in patients, with and without pain. Similar laser perceptive thresholds and latency/amplitude of the N1 and N2/P2-LEP components in healthy controls and in patients, with and without pain, also exclude damage of peripheral and central nociceptive afferent pathways. These data therefore indicate that our patients with pain did not suffer from neuropathic

pain [34]. The observation of comparable LEP amplitudes in controls and in patients with PD argues against two previous laser studies, that using a CO₂ laser stimulator reported abnormal LEPs in PD. Schestatsky et al. [14] found an increased LEP amplitude in patients with PD with neuropathic (central) pain; conversely, Tinazzi et al. [15] stimulating the shoulder, reported a decreased LEP amplitude in patients with nociceptive (musculoskeletal) pain. In our study, however, we used a different laser stimulator, investigated the hand and none of our patients suffered from neuropathic (central) pain, as assessed with DN4. Hence, the hypothesis that possible changes in N2/P2 LEP component influenced our findings is very unlikely. A more likely hypothesis implies that the reduced responses to Laser-PAS₅₀ we observed in patients, off therapy, reflects changes in M1 plasticity mechanisms. In a previous study in healthy subjects [16], we found that, Laser-PAS₅₀ induces MEP amplitude changes that might reflect spike-timing-dependent plasticity (STDP) in M1 [16,17]. STDP in M1 is supposed to reflect the coincidence detection of excitatory postsynaptic potentials in the apical dendrites (layer 2/3) and back-

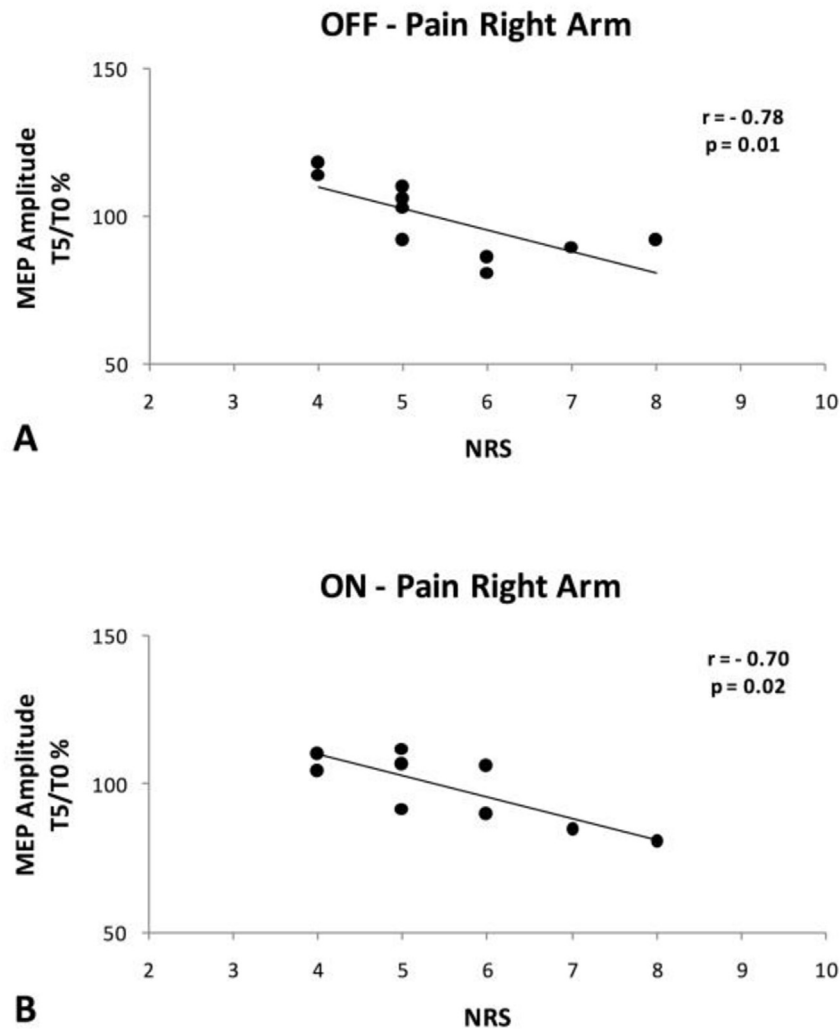


Fig. 6. Correlation between the amount of pain as assessed by the numerical rating scale for pain (NRS), and the MEP amplitude recorded at T5 (40 min after the end of Laser-PAS₅₀), in patients with Parkinson's disease (PD), with pain in the right upper limb, off (A) and on therapy (B). Note the negative correlation between pain severity as tested by NRS and the amount of MEP amplitude changes following Laser-PAS₅₀ in patients with PD off (A) and on therapy (B).

propagating action potentials from layer 5 neurons [16,35–38]. Since TMS activates layer 5 cortical-spinal neurons [39,40] and cortical projections originating from remote cortical pain-related areas reach M1 in cortical layers 2/3 [41–43], it is likely that Laser-PAS₅₀ induces STDP in cortical layer 2/3 of M1. Hence, the hypothesis we favor is that in PD patients off therapy, the reduced response to Laser-PAS₅₀ reflects abnormal STDP in M1.

Dopaminergic therapy does not restore responses to Laser-PAS₅₀

We found that L-Dopa left responses to Laser-PAS₅₀ unchanged in patients with and without chronic pain, suggesting that dopaminergic therapy does not restore abnormal STDP in M1 in PD, in line with previous observations with other types of plasticity-inducing protocols [16,44–48]. Our findings indirectly support the hypothesis that non-dopaminergic pathways contribute to the pathophysiology of pain in PD [2,4]. Extranigral pathology in PD, including neurodegeneration in the noradrenergic, serotonergic, and cholinergic systems, contributes to pain modulation [7,8,49,50]. The hypothesis that a non-dopaminergic pathway contributes to the pathophysiology of chronic pain in PD is also in

line with the observation that dopaminergic therapy only partly improve pain [2,4].

The response to Laser-PAS₅₀ differs according to pain localization in Parkinson's disease

When comparing patients with chronic pain (regardless of the specific body region affected by pain) and patients without pain, we found comparable responses to Laser-PAS₅₀ in the two groups. This observation would suggest that in PD, chronic pain does not influence *per se* the response to Laser-PAS₅₀. When we compared however, patients with pain in the right upper limb and patients with pain in other body regions (but not in the right upper limb), we found different responses to Laser-PAS₅₀ in the two patients' subgroup: patients with pain in the right upper limb had prominent alterations in responses to Laser-PAS₅₀, whereas patients with pain in other body regions were characterized by less severe neurophysiological abnormalities. It should be noted, that in our analysis, we found different responses to Laser-PAS₅₀ when comparing controls and patients with homotopic but not heterotopic pain probably due to the low number of subjects analysed in the third group (controls: $n = 20$; patients with homotopic pain:

$n = 10$; patients with heterotopic pain: $n = 5$). Interestingly, we found a negative correlation between the severity of pain in the right upper limb, as tested by NRS scores, and the amount of response to Laser-PAS₅₀. Given that patients with pain in the right upper limb had similar clinical features including MDS-UPDRS motor scores compared to those with pain in other body regions, we can exclude that the prominent abnormalities in MEP changes observed after Laser-PAS₅₀, in patients with pain in the right upper limb, merely arose from more severe motor symptoms. Overall our findings demonstrate that in patients with chronic pain, the severity of M1 STDP abnormalities is prominent when Laser-PAS₅₀ examines the body region specifically affected by pain further suggesting abnormal pain-motor integration in PD.

Pain-motor and sensori-motor integration in Parkinson's disease

The reduced responses to Laser-PAS₅₀ might arise from abnormal STDP in M1 that does not strictly depend on the specific PAS protocol used. Previous studies with the original PAS₂₅ protocol have demonstrated abnormal responses to PAS₂₅ in PD [45,48,51–59]. In more detail, the response to PAS₂₅ has been consistently found to be reduced in PD following PAS₂₅ over the most affected M1, as in our study. According to the hypothesis that the reduced responses to Laser-PAS₅₀ arise from abnormal M1 STDP not strictly depending on the specific PAS protocol used, patients with PD, with and without pain, should have manifested comparable responses to Laser-PAS₅₀ and PAS₂₅, since both PAS protocols are known to elicit STDP in M1. In this study, however, this was not the case. In our second set of experiments, we found comparable responses to Laser-PAS₅₀ and PAS₂₅ only in patients without pain. By contrast, in patients with homotopic pain, Laser-PAS₅₀ elicited greater abnormalities than PAS₂₅. Previous studies have shown that chronic musculoskeletal pain may lead to cortical reorganization processes in M1 muscle representation [60–63]. Accordingly, it might be argued that in PD, pain-induced cortical reorganization contributed to the differential response to Laser-PAS₅₀ and PAS₂₅ we observed in patients with homotopic pain. In this study, we did not examine and compare cortical maps of ADM muscles in patients with and without pain and thus we cannot fully exclude that a pain-related cortical map reorganization might have contributed at least in part to our findings. However, this hypothesis seems unlikely since in PD patients with homotopic pain, a pain-induced cortical reorganization would have similarly affected responses to both Laser-PAS₅₀ and PAS₂₅ protocols. We therefore suggest that the reduced responses to Laser-PAS₅₀ in patients with homotopic pain reflect abnormal M1 SDTP specifically induced by the activation of the nociceptive system. Hence, we suggest that in PD, and specifically in patients with chronic pain, the reduced responses to Laser-PAS₅₀ reflect abnormal pain-motor integration processes in functionally connected brain regions involved in pain matrices. In the present study, we did not compare pain-motor integration and sensori-motor integration in patients with chronic pain in other body regions because this subgroup of patients was characterized by heterogeneous pain localization, thus precluding a precise evaluation of pain-motor integration processes. Accordingly, the possible effect of heterotopic pain on pain-motor integration processes in patients with PD remains to be clarified.

Brain networks underlying abnormal pain-motor integration in Parkinson's disease

In a previous study in healthy subjects [16], we have suggested that Laser-PAS₅₀ elicits STDP in M1 through mechanisms of pain-motor integration reflecting the functional connectivity between pain and motor areas involved in the pain matrix (ACC, insula, S2,

supplementary motor area and M1). The pain matrix is thought to be responsible for cortical processing and conscious experience of pain [12,64–67]. Experimental evidence in animals and also in humans have demonstrated that most of the cortical areas contributing to the pain matrix are also targeted by dopaminergic projections including the “mesolimbic” and “mesocortical” dopaminergic pathways and that those pathways play a role in nociception [68,69]. Previous studies in healthy subjects with positron emission tomography have demonstrated increased dopaminergic release following various types of experimental pain [70–74]. In patients with PD, a study with functional magnetic resonance imaging demonstrated increased pain-induced activation of the right insula, prefrontal and left ACC in patients off therapy, but reduced activation in the same cortical areas in the on state of therapy, compared with controls [75]. Overall these findings support the hypothesis that in PD, altered mesolimbic and mesocortical dopaminergic pathways influence the activation of the pain matrix possibly leading to abnormal cortical pain-motor integration. Whether one specific cortical area, among those contributing to the pain matrix is responsible of abnormal cortical pain-motor integration in PD, remains to be clarified. It remains also unclear whether abnormal cortical pain-motor integration might predispose or contribute to the pathophysiology of chronic pain in PD.

A further comment concerns the possibility that in PD, abnormal pain-motor integration may occur also in subcortical brain regions. Experimental studies in animals and in humans have demonstrated that dopamine exerts a key role in modulating the “brainstem descending pain-modulatory system” [70–74,76]. Hence, in PD, dopaminergic degeneration in the substantia nigra pars compacta might drive an altered activation of the “brainstem descending pain-modulatory system” leading to abnormal pain-motor integration in the spinal cord. Furthermore, in PD, abnormal pain motor integration might occur also at basal ganglia level since dopaminergic denervation changes the release of neuropeptides contributing to nociception. It is known that striatal medium spiny neurons that give rise to the “direct pathway” release substance P and dynorphin, whereas those involved in the “indirect pathway” release enkephalin [77–79]. Experimental studies in animals have demonstrated that neurons in the substantia nigra, caudate, putamen and globus pallidus clearly respond to noxious stimulation [9,80,81]. In addition, the basal ganglia receive nociceptive information also directly from the cortex, thalamus and amygdala [9,80,81]. The laser-PAS₅₀ technique however, has been designed to examine specifically cortical pain-motor integration processes. Future studies would clarify the possible pathophysiological role of abnormal pain-motor integration processes at brainstem and basal ganglia level.

When interpreting our neurophysiological observations in PD, with and without chronic pain, several limitations should be taken into account. Given that we recorded LEPs in all patients on therapy, we cannot exclude a possible effect of dopaminergic therapy on N1 and N2/P2 LEP components in patients with and without pain. This hypothesis however, seems unlikely as suggested by a previous LEP study [82]. Future studies however, will clarify possible changes in pain-motor integration in drug naïve patients with PD, thus excluding possible confounding factors secondary to dopaminergic treatments. In our cohort of patients with PD, we studied no patients with a neuropathic pain including the central parkinsonian pain [4]. Hence, we cannot clarify whether the response to Laser-PAS₅₀ may differ in patients with nociceptive or neuropathic pain. Future studies will clarify whether the nociceptive or neuropathic pain component predominantly contribute to the abnormal response to Laser-PAS₅₀ in PD.

Conclusions

In this study, we report abnormal responses to Laser-PAS₅₀ in patients with PD, and prominently in those with chronic pain, which point to abnormal pain-motor integration. In addition, given that a number of experimental and clinical studies have demonstrated that invasive as well as non-invasive motor cortex stimulation induce significant pain relief in patients with pain [83–87], our findings provide further information for designing new therapeutic strategies with non-invasive brain stimulating protocols for treating chronic pain in PD.

Conflict of interest

None.

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We have no acknowledgements to declare.

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