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Electrophysiological assessment of nociception in patients with Parkinson's disease: A multi-methods approach

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ABSTRACT

Objective: Nociceptive abnormalities indicating increased pain sensitivity have been reported in patients with Parkinson's disease (PD). The disturbances are mostly responsive to dopaminergic (DA) treatment; yet, there are conflicting results. The objective of the present study was to investigate pain processing and nociception in PD patients in a more comprehensive manner than previous studies. For this purpose, a multi-methods approach was used in order to monitor different levels of the central nervous system (spinal, subcortical-vegetative, cortical).
Methods: The heat-pain threshold, contact-heat evoked brain potentials (CHEPs) and sympathetic skin responses (SSR), nociceptive flexion responses (NFR) and subjective pain ratings were measured in 23 idiopathic PD patients both in the Off-phase (without DA medication) and On-phase (after DA medication intake) as well as in 23 healthy controls.
Results: Compared to controls, PD patients showed decreased heat-pain thresholds only in the Off and tentatively increased NFR amplitudes in both phases. We found no between-group differences for the CHEPs, the NFR threshold/latency or the pain ratings. Yet, SSR amplitudes/frequencies were decreased and latencies were increased in PD patients in both phases. Correlations between CHEPs amplitudes and pain ratings were found only in controls.
Discussion: Increased pain sensitivity (heat-pain threshold) in the Off which normalizes in the On argues for DA induced dysfunctions of the nigrostriatal pain loops with the basal ganglia as main circuit in our PD sample. Dysfunctions of the subcortical-vegetative parameters despite of inconspicuous cortical nociception suggest disturbances of the central or peripheral innervation of sympathetic branches with coincidently intact ascending pathways in the PD group.

1. Introduction

Chronic pain symptoms are common in PD which can roughly be classified in pain due to motor symptoms (musculoskeletal or dystonic pain), as well as central pain which may be caused by PD-induced dysfunctions in the nociceptive system [12,16,18,30,31,39]. Furthermore, evidence suggests abnormal processing of experimentally induced pain, indicating increased pain sensitivity in PD patients, which in turn may augment the occurrence of chronic pain. More precisely, diminished pain or nociceptive flexion reflex (NFR) thresholds and abnormalities in electrophysiological pain measures like alterations in the evoked brain potentials and sympathetic skin responses (SSR) were found in PD patients [7,12,20,30,31,39–41,47].

Since these abnormalities especially occur in the Off-phase (no sufficient dopaminergic (DA) release or substitute by medication) and often – but not always - improve or even disappear in the On-phase (after DA medication intake), PD specific DA deficiencies in the nociceptive basal ganglia loops also including the thalamus, the cingulate cortex (ACC), the amygdala and the somatosensory cortex have been discussed as the pathophysiological mechanism underlying the nociceptive abnormalities in PD [5,7,12,39]. Additionally, there is growing evidence that central vegetative centers are involved in the nociceptive disturbances [39].

Yet, the reported studies revealed inconsistencies. One group, for example, found nociceptive abnormalities only in PD patients with central pain but not in pain-free PD patients [39]. In other studies, the nociceptive abnormalities were not responsive to DA treatment [12,40,41].

One reason for the ambiguous results might be a lack of studies using multiple methods, which cover different nociceptive levels of the central nervous system (CNS)(i.e. spinal, subcortical-vegetative, cortical, subjective). So far, most studies focused on a single psychophysiological parameter of the nociceptive system, like the NFR (spinal level) [18,31], pain-evoked brain potentials (cortical) [40,41] or subjective ratings [12,27,43].

Using a multi methods approach enables to test different components of the afferent pain pathways concurrently. Additionally, such

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an approach may provide clues to differentiate between abnormalities of the afferent and efferent components of the nociceptive system.

One study with PD patients with central pain using a two-methods approach by combining laser-evoked brain potentials (LEP) with the sympathetic skin responses (SSR) found increased LEPs and a lack of habituation of the SSR in PD patients in the Off, while these abnormalities attenuated in the On. The authors discuss afferent sensitization (increased LEPs) and abnormalities of vegetative nociceptive processing in brainstem areas accounting for these results [39].

Another reason for inconsistent results might be the different disease stadiums of the patients participating in the studies. Between-level differences in the PD-induced nociceptive abnormalities may especially be detected in patients with comparably mild symptoms while in later disease stadiums the pathophysiology of PD has spread throughout all nociceptive levels [30].

In the present study, we aimed to investigate nociception in PD patients while covering three physiological levels of the CNS (cortical, subcortical-vegetative and – in addition to Schestasky and colleagues [39] – spinal) by using a multi-methods approach. This enables us to monitor the nociceptive signal on its way to the cortex and back to the peripheral effectors. Therefore, brain (cortical) and sympathetic skin potentials (subcortical-vegetative) in response to phasic heat-pain and the NFR (spinal) in response to electrical sural nerve stimulation as well as pain ratings (subjective) were collected in PD patients both in the Off and On and in matched controls and analyzed for differences (1) between PD patients and controls and (2) between the Off- and On-phase. Nociceptive coherence across the central neuroaxis was examined using correlation analyses between the single levels. Additionally we tested if more severe PD symptoms/longer disease durations were accompanied by stronger nociceptive responses [30].

2. Materials and methods

2.1. Subjects

Twenty-three patients with idiopathic Parkinson's disease (PD) (ICD-10: G20) (3 female, *mean age* = 67.1, *SD* = ±9.9, *mean disease duration* = 8.1 years, *SD* = ±5.7 years) and 23 age and sex matched controls (3 female, *mean age* = 68.2, *SD* = ±7.8 years) participated in the study. PD patients were outpatients addressed via regional self-help groups. Inclusion criteria for patients were (1) diagnosis of idiopathic PD (disease duration 1–25 years), which affected both body sides to a similar degree (no hemi-PD), (2) therapy with DA medication (levodopa and/or DA agonists) and (3) significant relief of PD symptoms one hour after DA medication intake without strong fluctuations, based on self-report. PD patients were excluded from the study if they suffered from (1) other neurological and psychiatric diseases (especially depression – self-report about psychiatric history), (2) somatosensory disorders which may affect pain perception (for example diabetic neuropathy or polyneuropathy excluded by sensory testing of epicritic and protopathic modalities), (3) further severe physical disorders (for example acute cardiovascular diseases), (4) severe pain which was not related to PD[1] or (5) indications of dementia (mini mental status examination (MMSE) < 24). The control group was recruited via advertisements in local newspapers. Control subjects had to meet the same criteria as the PD patients – except the diagnosis of PD. Moreover, healthy subjects who suffer from acute pain on the test day were not included.

The study was a cooperation of the Department of Neurology, Sozialstiftung Bamberg, and the Department of Physiological Psychology, University of Bamberg. The Department of Neurology was in charge of the medical, especially neurological diagnostics while the pain testing was conducted in the Department of Physiological Psychology.

[1] Pain was considered to be related to PD if it could not be attributed to another known somatic pathology.

All sessions took place in the morning, PD patients were asked to discontinue their dopaminergic medication for the 14–15 h leading up to the investigation. If treated with prolonged released medications, participants did not take these medications for the 24 h prior to the investigation in order to at least reduce retarded pharmacological activity. Likewise, other PD medications, like COMT or MAO inhibitors or NMDA receptor blockers, were also discontinued for 24 h.

On the test day between 8 and 9 am, PD patients in the Off were examined by experienced neurologists (PR or CM, who are chief or senior physicians) in the Department of Neurology, Sozialstiftung Bamberg, for inclusion and exclusion criteria as well as for the severity of the PD. For this purpose the 3rd scale (motor examination) of the Unified Parkinson's Disease Rating Scale (UPDRS) was used. Afterwards, the actual experiment was conducted in the Department of Physiological Psychology, University of Bamberg, starting between 9 and 10 am. The study protocol was approved by the ethics committee of the University of Erlangen. All participants gave their written informed consent. For their participation all participants received 60 Euros and travel expenses.

2.2. General procedure

The procedure with a time schedule of the study is illustrated in Fig. 1. The experimental session comprised two identical blocks. Each block consisted of 3 parts. First, heat stimuli, which were intended to evoke brain and skin potentials, were applied while EEG and SSR were recorded. Second, NFR threshold was determined and subsequently participants were stimulated with supra-NFR threshold stimuli while EMG of the biceps femoris was recorded. Third, the heat-pain threshold was determined. After threshold-determination facial pain expressions in response to heat-pain were recorded in a test of 15 min (data not reported here, see [37]).

After the first block, a break of one hour was taken, in which no pain measurements were conducted, neither in the PD patients nor in the controls. At the beginning of the break, PD patients took their individual DA medication. During the break, PD patients completed a set of questionnaires (cognitive functioning, non-motor PD symptoms in the last 4 weeks, see below).

Prior to the second experimental block, patients were asked to rate their motor performance. The On-phase was assumed to be reached during the first hour after medication intake, which is good practice in comparable studies investigating pain processing in PD patients both in the Off and On [7,18,20,39]. In addition, PD patients were asked to estimate whether the severity of the potentially persisting PD-symptoms after medication intake was of the usual degree. After the break, PD patients and controls completed the second experimental block. For simplifying the terminology, “Off” always refers to the first test block in PD patients and in controls, while “On” refers to the second block both in PD patients and in controls throughout the paper, although the latter did of course not receive any medication. Duration of the experimental session was about 5 h (2 h for each block and 1 h break). Participants were carefully familiarized with the methods. For the measurements participants were comfortably seated on a chair.

2.3. Stimulators

Heat stimuli were generated and applied by a contact-heat evoked potential sensory and pain evaluation system (CHEPS, Medoc, Israel) with a round 27 mm-diameter surface stimulator.

Electrical stimuli, which were intended to elicit the nociceptive flexion reflex (NFR), were generated by a bipolar constant current stimulator (Digitimer DS5, Hertfordshire, AL7 3BE, England) and applied via two round 5 mm diameter surface electrodes.

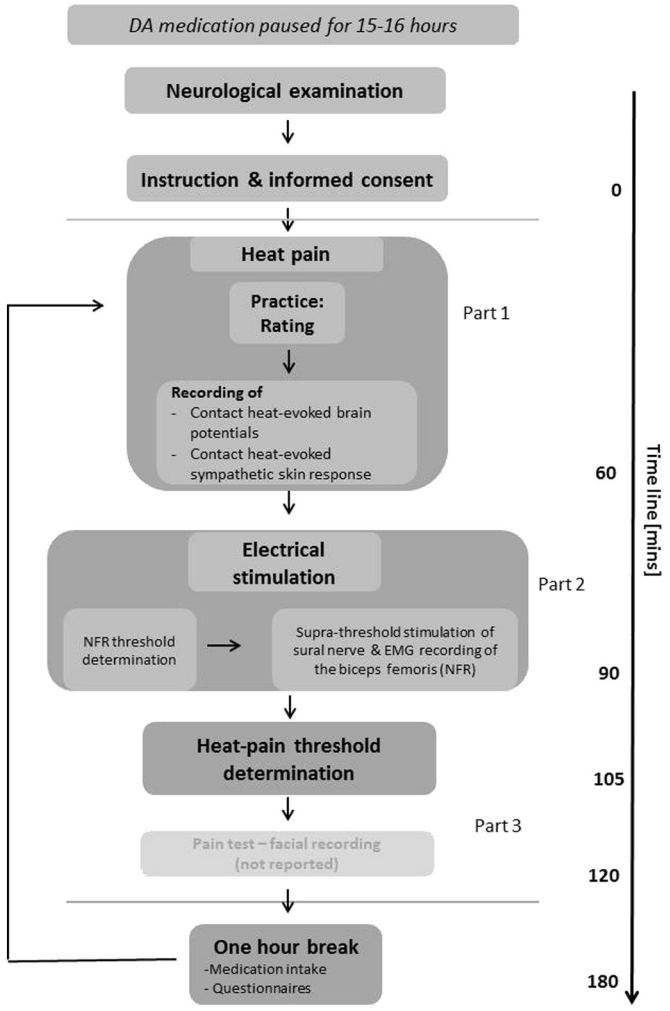

Fig. 1. Schematic illustration of the experimental protocol.

2.4. Heat-stimulation and pain-evoked brain and skin potentials

2.4.1. Stimuli

In order to elicit contact-heat-evoked brain potentials (CHEPs) and skin potentials (SSR), participants were exposed to 20 phasic heat stimuli, ten of which had a fixed temperature of 51 °C or alternatively 45 °C (baseline temperature of 35 °C, plateau duration of 10 ms, rate of rise/fall of 70 °C/40 °C per second). The sequence of the 45 °C and 51 °C stimuli was randomized once and then set for all participants. All stimuli were applied to participants' volar site of the left forearm[2] in an about 7×7 cm^2 area located 2–4 cm beneath the cubital joint. The thermode was held by an experimenter to allow for flexible placement and slightly moved after each stimulus to prevent receptor fatigue and cortical habituation [19]. Participants were instructed to rate the painfulness of each stimulus using a 11 points numeric rating scale (NRS) ranging from 0 ("not painful at all") to 10 ("highest imaginable pain") after they had felt the stimulus.

2.4.2. EEG and SSR recording

EEG-recording was accomplished by a DC Brain Amp amplifier (Brain Products GmbH, Germany) with a sampling rate of 1024 Hz and a recording bandwidth from 0.1 Hz to 300 Hz. For electrode placement, a commercial cap realizing the international 10–20 system was used. Cz served as reference. The impedances of all electrodes were kept below 5 kΩ. Furthermore, tin electrodes were placed on the mastoids for offline re-referencing the data in order to regain Cz. In addition, an electro-oculogram (EOG) was recorded.

Sympathetic skin responses were assessed by use of the SUEmpathy100 (SUESS Medizintechnik, Germany). The differential surface electrodes were fixed at the palm of the right hand with the reference electrode fixed on the proximal third of the right forearm. The biosignal was sampled at a rate of 512 Hz.

2.4.3. EEG parametrization

EEG data from Cz were analyzed offline (Brain Vision analyzer, Brain Products, Germany) in order to determine N2 and P2 amplitudes and latencies as well as N2P2 peak-to-peak amplitudes following the protocol described by Granovsky and colleagues [19].

The ten potentials evoked by the 45 °C stimuli were averaged in the Off and On, so was done with the ten potentials evoked by the 51 °C stimuli. The averaged signals were used to determine two components: N2 was defined as the most negative peak in a time window from 200 to 500 ms, P2 was defined as the most positive peak in a time window from 400 to 650 ms. Thereby, we used more liberal criteria (wider time windows) for peak detection than Granovsky and colleagues [19] did in their study on healthy individuals (N2: 300–500 ms, P2: 400–600 ms). We did so because latencies of the CHEPs might be changed in the PD patients (increased due to the potentially down-regulated cortical system or decreased due to increased pain sensitivity) and in the aged participants (increased due to age-related deceleration of the neuronal transmission).

For further analysis, the peak-to-peak N2P2 amplitude, i.e. the absolute difference between the voltage of the N2 and the P2, was calculated. Consequently four N2P2-complex amplitude scores resulted for each subject, one for the 45 °C and one for the 51 °C stimulus both in the Off and On. Additionally, N2 and P2 amplitudes and latencies both for the 45 °C and for the 51 °C stimulus both in the Off and On were kept for further analysis.

2.4.4. SSR parametrization

Following the protocol described elsewhere [11], SSR data were analyzed in order to determine the peak-to-peak amplitude of the first negative peak (N1) and the subsequent positive peak (P2), i.e. the N1P2-complex amplitude. For non-responses in the SSRs zero values were subscribed and kept as such in the analyses. Additionally, the response latency, which is defined as time from trial onset to the onset of the negative deflection, was determined for the trials in which a response occurred. Then, the amplitudes and the latencies were averaged for the 45 °C-trials in the Off and On, so was done for the 51 °C-trials. Four N1P2-complex amplitude and latency values resulted for each subject, one for the 45 °C and one for the 51 °C stimulus both in the Off and On. Since the SSR might not occur in every trial due to habituation processes, which might differ between groups, the frequency of responses was parameterized per subject both for the 45 °C and for the 51 °C stimulus in the Off and On.

2.5. Electrical stimulation and NFR

2.5.1. NFR threshold determination and EMG recording

Trains of five rectangular pulses with 1 ms duration each at a frequency of 250 Hz served as stimuli for NFR threshold determination and NFR supra-threshold stimulation. For the purpose of eliciting the NFR, the sural nerve of the left leg[2] was stimulated in its retromalleolar pathway. For NFR threshold determination the up-down staircase method was used (6 turning points, EMG amplitude at least 50 μV, time window 70–200 ms after stimulus onset; see [31–33] for a detailed description).

After NFR threshold determination participants were exposed to 10 supra-threshold stimuli (threshold + 3 mA, ISI = 20 s) which should reliably elicit the NFR. Comparable to the first part of the session (heat-

[2] The stimulation side was held constant across participants since slight between-side differences regarding the NFR may even occur in our non-hemi PD patients. The left side was chosen because of the right hemispheric dominance of pain perception in the brain [28].

pain), participants were instructed to rate the painfulness of each stimulus using the already familiar 11 points NRS ranging from 0 ("not painful at all") to 10 ("highest imaginable pain").

For quantifying the NFR activity of the left biceps femoris in response to the electrical stimulation, two surface recording electrodes were used, one of which was placed over the short head of the biceps femoris and one of which was placed over the tendon of the biceps femoris at the head of the fibula. EMG recording was accomplished by the device SIGMA PlPro/Type Databox DB 36 (Sigma Medizintechnik, Gelenau, Germany). The EMG signal was sampled at a rate of 512 Hz.

2.5.2. EMG parametrization

EMG data analysis (conducted with Brain Vision analyzer, Brain Products, Germany) referred both to the amplitude and latency of the NFR. Raw data were filtered offline with a high pass filter of 20 Hz and a low pass filter of 250 Hz as well as a 50 Hz notch filter. Next, signal segments of 300 ms duration starting with stimulus onset were marked. Afterwards, the signal was rectified and integrated. Segments were visually inspected for artifacts and NFR-like events, which occurred outside the time window of 70–200 ms after stimulus onset, and, if necessary, excluded from further analysis. Remaining segments were averaged (at least 5 valid NFR responses were required for calculating mean values). The most negative data point within the 70–200 ms time window in the averaged signal was defined as the NFR amplitude.

2.6. Heat-pain threshold determination[3]

Participants' heat-pain threshold was determined using the method of limits. Thereby, the thermode temperature increased from a baseline of 35 °C at a rate of 0.7 °C/s until the participants felt a first pain sensation and responded by pressing a button. Overall, there were 8 trials and pain thresholds were determined as the average of the last 5 trials. Please see [37] for a more detailed description.

2.7. Self-report of pain

PD patients rated their highest, lowest and average PD-related pain intensity during the last 4 weeks and their current pain intensity each by using the already familiar 11 points NRS (0 = "no pain", 10 = "highest pain imaginable") which results in a sum score between 0 and 40. In order to support PD patients to differentiate between PD related pain and pain stemming from further pathologies, they were instructed to consider only those pain symptoms which may be some kind of "unexplainable", i.e. which cannot be attributed to another known somatic pathology and which may improve after dopaminergic medication.

2.8. Neurological examination and questionnaires

PD patients' motor symptoms were quantified by experienced neurologists (PR and CM) using the third scale of the Unified Parkinson Disease Rating Scale (UPDRS). Additionally, the Mini-Mental Status Examination (MMSE) [15] and the German version of the Non-Motor Symptoms Questionnaire for PD patients (NMSQuest) (self-report) were applied [9,23].

2.9. Statistical analysis

2.9.1. Biosignals and rating data

EEG data (N2P2-complex amplitudes, N2 and P2 amplitudes as well as N2 and P2 latencies) and SSR data (N1P2-complex amplitudes, latencies and response frequencies) as well as the pain ratings of the heat stimuli were subjected to separate split-plot ANOVAs with the between-factor group (PD vs. controls) and the within-factors phase (Off vs. On) and stimulus intensity (45 °C vs. 51 °C). NFR data (threshold, amplitude and latency) as well as the pain ratings of the electrical stimuli were subjected to separate split-plot ANOVAs with the between-factor group (PD vs. controls) and the within-factor phase (Off vs. On). The expected pattern (between-group differences in the Off, not in the On) should manifest itself in an interaction of group and phase.

If sphericity could not be assumed in ANOVAs a Greenhouse-Geisser adjustment dfs was accomplished. Post-hoc tests were generally two-tailed. If we had a specific hypothesis about the direction of an effect, one-tailed tests were computed, which are clearly denoted.

2.9.2. Heat-pain threshold

Since the pain thresholds were not normally distributed (Kolmogorov-Smirnov-test: p's < 0.01) non-parametric tests (Mann-Whitney-U-tests and Wilcoxon-Rank-tests) were used for between-group (PD vs. controls) and within-group (PD Off vs. PD On) comparisons.

2.9.3. Correlations among and between physiological and psychological variables

We considered deficient nociceptive coherence in PD patients especially in the Off which should manifest itself in missing/smaller correlations between the responses of the different nociceptive levels. Therefore, two correlation analyses were run separately for both groups in the Off and On. In the first analysis, the most prominent physiological parameters of the different levels of the nociceptive system, i.e. the NFR amplitude, the SSR N1P2-complex amplitude and the EEG N2P2-complex amplitude, were correlated. In the second analysis, the physiological parameters were correlated with the psychological parameters.

For the correlation analysis, EEG and SSR responses as well as the heat-pain ratings were combined across stimulus intensities (45 °C and 51 °C stimuli) both for the Off and On in order to get more general measures for nociception. Correlations were tested for between-group differences using the Fisher-Z-test. Thereby, only those correlations were tested which differed significantly from zero in at least one group (PD/controls).

2.9.4. Correlations between clinical variables and nociceptive parameters

For testing if more severe PD symptoms and a longer disease duration are related to higher nociceptive responses, the UPDRS score and disease duration were correlated with the EEG N2P2-complex amplitude, the SSR N1P2-complex amplitude, the heat-pain ratings, the NFR threshold, the NFR amplitude, the NFR pain ratings and the heat-pain threshold in PD patients.

Significance level was set $\alpha = 5\%$. Statistical Package for the Social Sciences version 20 (IBMSPSS, Chicago, IL, USA) was used for all analyses.

3. Results

3.1. Sample characteristics

Table 1 provides an overview over the sample characteristics. Mean age of the 23 PD patients (3 female) was 67.1 years ($SD = 9.90$), mean age of the 23 controls was 68.2 years ($SD = 7.78$). The difference regarding age between the groups was not significant ($t < 1$). Additionally, groups did not differ in height and weight (all t's < 1). Mean disease duration of PD patients was 8.1 years ($SD = 5.65$). In the PD patients, the mean UPDRS score was 18.4 ($SD = 9.06$) and the mean NMSQuest score was 8.8 ($SD = 4.47$), which both are in line with the assumption of rather mild symptoms [9].

Five PD patients took dopaminergic agonists alone, one took levodopa supplements alone and 17 took a combination of both. In addition, 9 patients were treated with MAO inhibitors and 2 with COMT inhibitors, while 5 PD patients were treated with NMDA receptor blockers. Sixteen of the 23 PD patients reported clinical pain which was related to PD

[3] The heat-pain threshold data have already been published in a paper issuing part 3 of the experimental blocks (see Fig. 1) [37]. In this study, the heat-pain thresholds were used in order to tailor pain stimuli which were supposed to elicit the facial expression of pain to the individual pain sensitivity.

Table 1
Means (*M*) and standard deviations (*SD*) of the sample characteristics for PD patients and controls. UPDRS = 3rd scale of the Unified Parkinson's Disease Rating Scale, NMSQuest = Non-motor symptoms Questionnaire Score, MMSE = Mini-mental status examination score.

	PD patients ($n = 23$, 3 female)		Controls ($n = 23$, 3 female)	
	M	*SD*	*M*	*SD*
Age (years)	67.09	9.90	68.21	7.78
Height (cm)	174.17	8.81	175.17	6.90
Weight (kg)	83.09	13.76	80.22	8.21
Disease duration (years)	8.09	5.65		
NRS pain rating (0–40)	9.17	6.98		
UPDRS (Off) (0–108)	18.39	9.06		
NMSQuest (0–30)	8.82	4.47		
MMSE (0–30)	28.96	1.19		

(musculoskeletal pain alone: 13, central pain alone: 1, musculoskeletal and central pain: 2) [16] while seven did not report pain. The clinical pain occurring in patients was rather moderate (~9/40 scores on the NRS, see Table 1) and always responsive to dopaminergic treatment.

3.2. Non-responders and drop-outs

Unfortunately we lost some data regarding the NFR: First, 4 four PD patients and one control subject showed no NFR in response to the electrical stimulation both in the Off and On. Second, 3 PD patients and one control subject were NFR responders in the Off but not in the On. Consequently, NFR threshold and rating data could be collected in 16 PD patients and 21 controls in both phases. Furthermore, unfortunately we lost the EMG data of 6 controls and 2 PD patients during the supra-threshold stimulation due to technical problems (deficient adherence of electrodes, disturbed signal), and – after normal threshold determination – due to non-responding to supra-threshold stimulation. Consequently, the NFR amplitudes and latencies of 15 controls and 14 PD patients in both phases could be determined.

3.3. Impact of PD on the specific nociceptive levels[4]

Figs. 2 and 3 provide an overview over the descriptive statistics underlying the upcoming analyses.

The expected pattern with between-group differences in the Off which attenuate or disappear in the On (interaction group*phase) was only found for the heat-pain thresholds. Compared to the controls, PD patients showed significantly reduced heat-pain thresholds in the Off ($U = 1.80$, $p = 0.036$, one-tailed); in the On heat-pain thresholds of PD patients substantially increased ($U = 2.44$, $p = 0.008$, one-tailed) to a level which did not significantly differ from the controls ($U = 1.46$; $p = 0.073$, one-tailed). For all other parameters (EEG, SSR, NFR and rating data) the interaction group*phase tested by means of ANOVAs missed significance (all p's > 0.05).

Yet, we found a main effect of group for the SSR parameters with overall lower N1P2 amplitudes ($F(1,44) = 13.556$; $p = 0.001$; $\eta^2 = 0.470$), lower SSR frequencies ($F(1,44) = 4.354$; $p = 0.043$; $\eta^2 = 0.090$) and longer SSR latencies ($F(1,44) = 8.375$; $p = 0.007$; $\eta^2 = 0.207$) in PD patients compared to controls. Additionally, PD patients showed marginally higher NFR amplitudes than controls in both phases ($F(1,27) = 3.075$; $p = 0.091$; $\eta^2 = 0.102$), while no between-group differences were found for the NFR threshold or NFR latency, neither for the EEG parameters nor the rating data (all p's > 0.10).

Thus, PD patients showed an overall reduced subcortical-vegetative activity (less frequent and weaker SSRs) and – as the median effect size [$\eta^2 = 0.102$] indicates – a clear tendency of increased spinal activity (increased NFR amplitudes) in response to pain in both phases, while the cortical (EEG) and subjective parameters (ratings) were not affected by PD neither in the Off nor in the On.

3.3.1. Correlations among and between physiological and psychological variables

Correlation analyses were run separately for both groups in order to test for relationships between the parameters representing the different levels of the nociceptive system. For this purpose, correlations were calculated among the physiological parameters and between the physiological and physiological parameters. When looking at Table 2a it becomes obvious that the pattern of significant correlation differed between the two groups; thus, significant correlations in the controls did not reach significance for the PD patients and vice versa.

In both phases, we found a significant correlation between the EEG N2P2-complex amplitude and the heat-pain ratings only in controls (Off: CG: $r = 0.427$, $p = 0.042$; PD: $r = -0.026$, $p = 0.907$; On: CG: $r = 0.636$, $p = 0.001$; PD: $r = 0.296$, $p = 0.171$), with significant between-group differences in both phases (Off: $Z = 1.54$, $p = 0.031$; On: $Z = 1.42$, $p = 0.039$; one-tailed). Nevertheless this correlation marginally significantly increased for PD patients in the On relative to the Off ($Z = 1.05$, $p = 0.074$; one-tailed).

Moreover, in the Off significant correlations between the EEG N2P2-complex amplitude and the SSR N1P2-complex amplitude ($r = 0.454$, $p = 0.030$) alternatively the NFR amplitude occurred only in controls ($r = 0.771$, $p < 0.001$), but not in the PD patients ($r = 0.024$, $p = 0.915$; $r = 0.074$, $p = 0.762$) with significant between-group differences ($Z = 1.46$, $p = 0.037$; $Z = 3.00$, $p = 0.005$; one-tailed). In contrast, none of these correlations were found to be significant in the On neither in PD patients nor in the controls (p's > 0.05).

3.4. Correlations between clinical variables and nociceptive parameters

Table 2b indicates widely missing significant correlations between UPDRS score/disease duration and the nociceptive parameters in the PD patients. Merely the correlation between the UPDRS score and the EEG N2P2 complex amplitude (Off) ($r = 0.441$, $p = 0.035$) and the UPDRS-score and the NFR threshold (On) ($r = 0.535$, $p = 0.033$) reached significance.

4. Summary

Taken together, our analyses revealed the following results. (1) The expected pattern, namely between-group differences indicating higher nociceptive responses in PD patients only in the Off, not in the On, was found only for the heat-pain threshold while it was missed for all other parameters. (2) Compared to controls, PD patients showed lower SSR amplitudes/frequencies and longer SSR latencies as well as a clear tendency of increased NFR amplitudes in both phases. (3) No between-group differences were found regarding the EEG parameters or rating data neither in the Off nor in the On. (4a) In contrast to the controls, no substantial correlations between the EEG N2P2-complex amplitudes and the heat-pain ratings were found in PD patients. (4b) Correlations of the nociceptive parameters and the disease duration/UPDRS score were largely non-significant.

5. Discussion

The objective of the present study was to investigate pain processing and nociception in PD patients with mild symptoms on the spinal, subcortical-vegetative and cortical level using a multi-methods approach. While we found increased pain sensitivity in the lower end of the pain range (heat-pain threshold) only in the Off (not in the On), diminished activity of the vegetative parameters occurred in both phases. In contrast only minor dysfunctions could be detected on the cortical or spinal

[4] In the running text we focus on the main results, namely the main effect of group and the interaction group * phase. All other effects are illustrated in the Tables A1 and A2 in the appendix section.

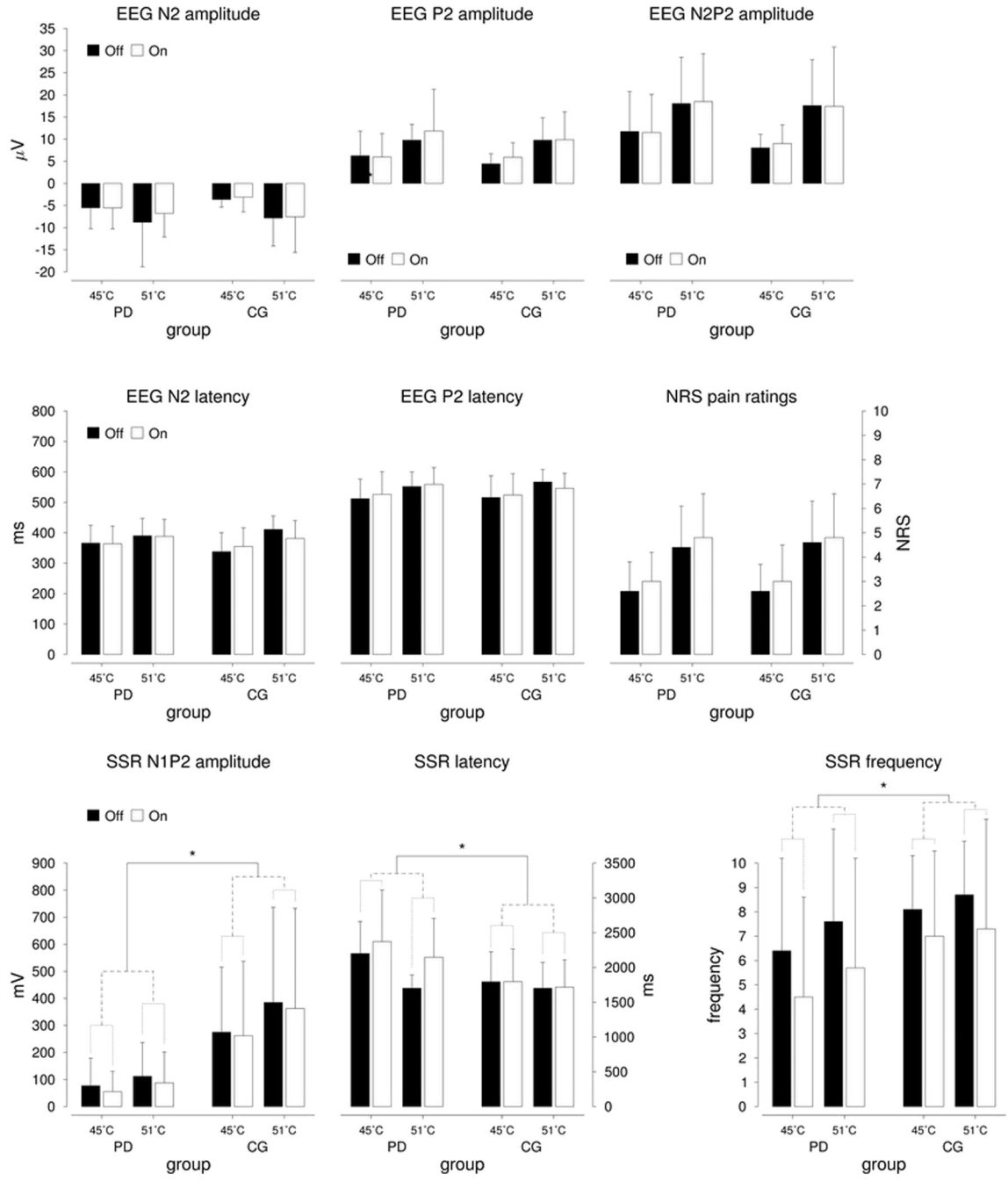

Fig. 2. Means and standard deviations of the EEG (first and second row) and SSR measurements (third raw). "+" = $p < 0.1$; "*" = $p < 0.05$. Referring to our hypotheses, only the main effects of group and the interactions of group and phase are plotted for the reason of clarity.

level, namely disturbances of the nociceptive integration (cortical: missing relationship between N2P2-complex amplitude and pain ratings) and a trend towards increased NFR activity (spinal) in PD patients in both phases. Interestingly, there were hardly any substantial relationships between disease duration or motor symptom severity (UPDRS) and the experimental parameters.

5.1. Intact excitatory ascending pain pathways in PD patients

The occurrence of heat-evoked brain potentials, SSRs and NFRs in our study indicates intact peripheral Aδ-fibers and central ascending nociceptive pathways (spinothalamic tract) in PD patients, which has already been shown by other research groups [18,39–41]. Yet, in contrast to our data, the previous studies reported either increased (in PD patients with central pain) [39] or decreased [40, 41] amplitudes of the pain-evoked brain potentials indicating disturbances of the cortical processing of nociceptive input [39–41]. In our study the lack of abnormalities in the pain-evoked brain potentials might result from preserved functionality of the pain-signaling and pain-modulating pathways, including thalamus, ACC, somatosensory cortex and insula [40,41] along with the basal ganglia, the substantia nigra and further cortical circuits [5,10] in our PD sample due to their relatively mild symptoms. In line with this, as the correlation analysis indicates, more severe motor symptoms and an advanced stage of PD were accompanied by stronger nociceptive cortical responses in our study.

Yet, our data also provide indication for abnormal processing of nociceptive input in PD patients at higher CNS levels. The well-established

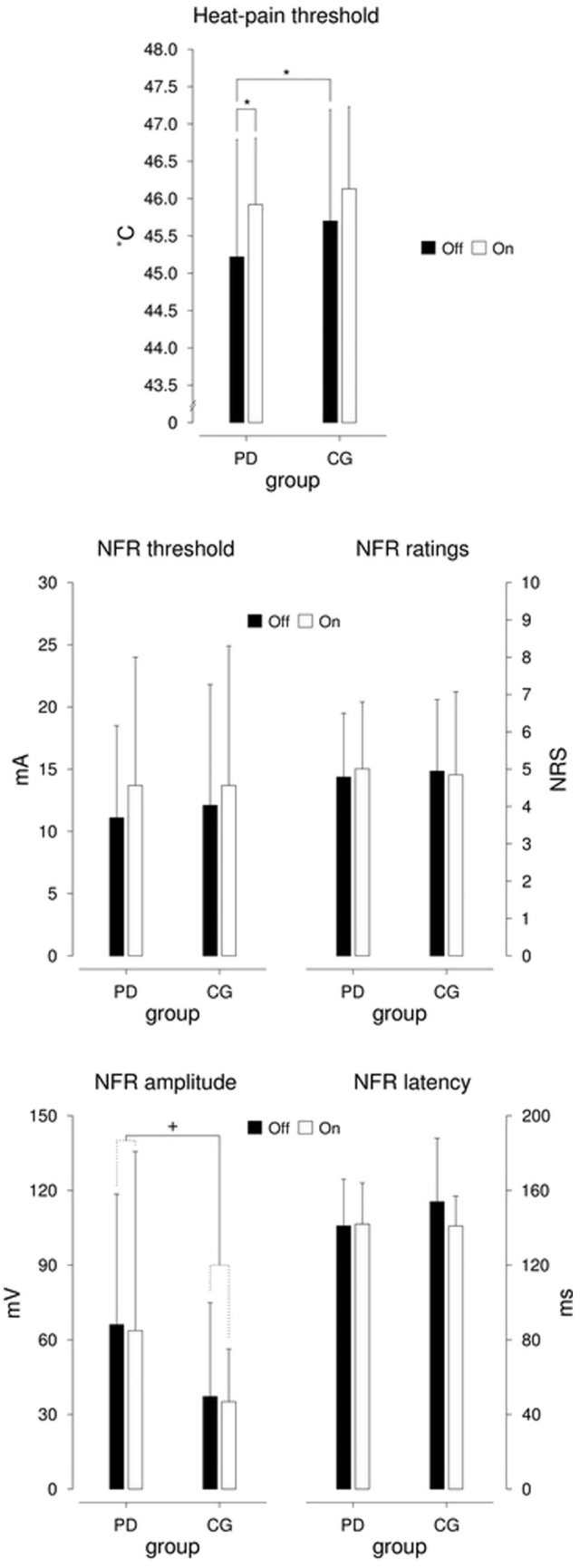

Fig. 3. Means and standard deviations of the heat-pain threshold, the NFR threshold, the pain rating auf the NFR eliciting stimulus the NFR amplitude and the NFR latency. "+" = $p < 0.1$; "*" = $p < 0.05$.

relationship between the amplitude of the pain-evoked brain potentials and the pain ratings of the eliciting stimulus (for example [19]), which was replicated in our control group, did not occur in PD patients. This missing relationship may derive from DA induced disturbances of the pain-integrative pathways including the basal ganglia, especially the cortico-basal ganglia-thalamic-cortical loop with input from the ACC and the insula [5,39–41].

5.2. Efferent vs. afferent disturbances on the subcortical-vegetative and spinal level

Whereas the activation of various indicators of afferent nociceptive processes could be reliably achieved in the present study, the degree of activation differed (normal CHEPs vs. reduced SSRs and a tendency towards increased NFR amplitudes). We claim that the efferent pathways triggering the SSR and the NFR which both can be seen as nocifensive actions [32] should be focused on in order to explain this pattern.

5.2.1. Reduction of the sympathetic skin response (SSR)

The SSR in response to pain is mainly driven by the excitation of the medial pain system. Peripheral and central Aδ-fibers (spinothalamic tract) transmit the signal to higher areas which leads – both via spinoreticular projections and medial thalamic nuclei – to an activation of the anterior cingulate cortex (ACC). While one afferent branch carries the signal to higher cortical areas, like the insula and the somatosensory cortex (brain potentials), an efferent branch leads from the ACC to the anterior hypothalamus which produces the SSR via further relay centers like the brainstem, vegetative ganglions and the postganglionic C-fibers [3,32,35,44].

In our PD sample the efferent pathways might be disturbed due to PD related degenerations of vegetative nuclei in the brainstem. Especially, the periaqueductal grey (PAG), which contains some dopaminergic neurons and receives input from the substantia nigra, should be focused on [14]. Since the neural degeneration in PD begins in lower medullar areas and in the course of the disease moves upwards through the brainstem to cortical areas, it is understandable that vegetative abnormalities occur already early in the course of disease, like in our PD sample [4,6,48]. Additionally, referring to authors who found a reduced SSR activity in response to non-painful stimuli in PD patients, disturbances of the peripheral branches of the vegetative nervous system, especially a reduced postganglionic sympathetic cholinergic activation, should be considered also in our PD sample [1,17,22,45].

5.2.2. Tendency towards increased spinal pain reactivity (NFR)

In our study, we observed between-group differences neither in the NFR threshold nor the NFR latency. Yet, there was a tendency of increased motor activity in response to painful stimulation in PD patients (increased NFR amplitudes).

The widely unaltered NFR parameters can be explained by intact afferent Aδ-pathways carrying the nociceptive input to the dorsal horn and intact spinal NFR circuits. Yet, we have to point out that a reduced NFR threshold in PD patients has already been shown which was responsive to DA treatment [18]. PD related insufficiency of descending dopaminergic pain-inhibiting input to the dorsal horn [5,10,36], might account for the finding reported by Gerdelat-Mas and colleagues [18]. The lack of clear differences between PD patients and controls on the spinal level in our study might be explained by the mild symptomatology in our PD sample, in which the DA induced pain inhibition might widely be preserved.

Yet, the slightly increased NFR amplitudes provide at least an indication of spinal disturbances of nociception. On a speculative level, the latter might result from disturbances of the efferent pathway including top-down NFR control which descends from the thalamus via brainstem areas [29] with a decrease of descending inhibitory motor inputs from the substantia nigra to the thalamus and brainstem [2,29].

Table 2a
Correlations among the electrophysiological parameters of the different levels of the nociceptive system and correlations between the pain ratings and the corresponding electrophysiological parameters for PD patients and controls both in the Off and On. Correlations which differ significantly from a zero-correlation are marked: "*" = $p < 0.05$. "**" = $p < 0.01$. Additionally, the columns "between test" illustrate the results of the Fisher-Z-tests which test correlations for between-group differences.

	Parameters		Off				On			
			Group		Between-test		Group		Between-test	
			PD	CG	Z	p	PD	CG	Z	p
Electrophysiological parameters	EEG N2P2	SSR N1P2	0.024	0.454*	1.46	0.037	−0.080	0.154	–	–
	EEG N2P2	NFR amplitude	0.074	0.771**	3.00	0.005	−0.229	0.045	–	–
	SSR N1P2	NFR amplitude	0.645**	0.065	2.22	0.007	0.377	0.273	–	–
Pain ratings and electrophysiological parameters	Heat-pain rating	EEG N2P2	−0.026	0.427*	1.54	0.031	0.296	0.636**	1.42	0.039
	Heat-pain rating	SSR N1P2	−0.024	0.297	–	–	−0.283	0.221	–	–
	NFR pain rating	NFR amplitude	−0.352	0.267	–	–	−0.290	0.000	–	–

5.3. Pain hypersensitivity in PD patients and further data collected in the sample [37]

At least the reduced heat-pain thresholds in PD patients in the Off normalizing in the On argue for increased pain sensitivity due to DA-dysfunctions in our PD sample which has been shown by other authors [40,41,46]. Since the descending pain inhibition seemed to be intact in our patients (normal NFR threshold) (see also [31]), disturbances of nociceptive basal ganglia loops including the thalamus, the cingulate cortex (ACC), the amygdala and the somatosensory cortex in the Off which are restored after DA medication intake may underlie this pattern [39].

It is further interesting that another study on the same PD sample investigating the facial expression of pain [37] revealed an overall reduced facial expression in response to pain in the Off, not in the On. Thus, the facial data which actually underpin alterations in pain sensitivity [24,25], seemingly contradict the assumption of increased pain sensitivity which was actually reflected by the heat-pain threshold data. Thus, even if there might be more pain to be expressed in PD patients, the motor problems prevent its expression which may lead to an underestimation of PD patients' pain in the Off by observers. In the On this contradiction dissolves with the decrease in pain sensitivity and the increase in facial responses to pain [37].

5.4. The role of the phase (Off vs. On)

Since most of our parameters have shown no substantial abnormalities neither in the Off nor in the On (CHEPs, ratings, NFR) conclusions about the impact of medication on nociception in our PD sample are difficult to draw. Only the heat-pain threshold revealed the expected pattern namely higher sensitivity in PD patients in the Off which attenuated in the On. This might be due to an improvement of nigrostriatal DA activity in the On [7].

Yet, the substantially down-regulated SSR occurred both in the Off- and the On-phase in our study. Three explanations might account for this finding. First, the functionality of the nigrostriatal DA system, especially the loops, cannot be sufficiently reestablished so that the activation of the PAG via basal ganglia is not sufficient to elicit normal SSR. Second, peripheral dysfunctions of the VNS, especially a reduction in the cholinergic activation of the postganglionic sympathetic fibers, may contribute to the weak sympathetic activity in PD patients [1,17,22,45]. Third, other transmitter systems, especially the serotonergic raphe nuclei and the noradrenergic locus coeruleus, might be affected in PD due to degenerations in brainstem areas and may consequently be involved in nociceptive abnormalities especially on the vegetative level in PD [8,21,38,39]. Yet, missing differences in nociceptive parameters between the Off and On in PD patients have already been reported [12,40].

Table 2b
Correlations between disease duration/the UPDRS score and the electrophysiological and subjective parameters of the nociceptive system for PD patients in both phases. Correlations which differ significantly from a zero-correlation are marked: "*" = $p < 0.05$. "**" = $p < 0.01$.

		Off	On
Disease duration	EEG N2P2	0.091	0.140
	SSR N1P2	−0.405	−0.384
	Heat-pain rating	0.305	0.393
	NFR threshold	−0.040	0.288
	NFR amplitude	0.079	0.300
	NFR pain rating	0.100	0.296
	Heat-pain threshold	−0.023	−0.105
UPDRS	EEG N2P2	0.441*	0.362
	SSR N1P2	−0.085	−0.142
	Heat-pain rating	0.108	0.161
	NFR threshold	0.280	0.535*
	NFR amplitude	0.175	0.047
	NFR pain rating	−0.247	−0.078
	Heat-pain threshold	0.169	−0.061

5.5. Limitations

First, we have to point out that in spite of testing three levels of the CNS there was a confounding with changes in the physical modality of the stimulus (spinal: electrical; subcortical-vegetative and cortical: heat). The most self-evident way might be to elicit the responses of all investigated levels without varying the physical features which enables to monitor all levels at the same time. Yet, we aimed to choose the optimal stimulus configuration for eliciting the particular physiological response. Second, only 3 female patients participated in our study although the sex ratio of PD prevalence is 1.6 with men predominating [49]. Since sex has been shown to affect nociception (for example [13,26,33]) future studies should include more female patients. Third, although the On-phase should be reached one hour after medication intake by all PD patients, the degree of nigrostriatal DA functioning at this time point might differ between patients due to different types of medication. Fourth, a randomization of the sequence of the phases was omitted (all PD patients were first tested in the Off and then in the On). Yet, in order to control for possible order effects, the control group was also tested two times. Additionally, we spared by that an extra session for the patients. Fifth, although sensory testing of epicritic and protopathic modalities revealed no abnormal findings, slightest forms of subclinical polyneuropathy, as described by Toth and colleagues (2010) [42], cannot be completely ruled out in our PD sample which may in turn contribute to the alterations in nociception [34].

6. Conclusions

The aim of the present study was to investigate nociception in patients with mild PD on different levels of the nervous system. Partially in line with previous studies, a clear indication of PD associated hyperalgesia was only given by the reduced heat-pain threshold in the Off, which normalized in the On. The latter pattern might be due to

DA induced disturbances of nociceptive basal ganglia loops in the Off which dissolved after DA-medication intake.

Furthermore, while the CHEPs parameters of the cortical nociception were widely inconspicuous, substantially decreased subcortical-vegetative activity (decreased and slower SSRs) and at least tentatively increased spinal activity (increased NFR activity) in response to pain were found in PD patients in both phases. Since the afferent nociceptive pathways seem to be intact (undisturbed signals reach the cortex), central or peripheral dysfunctions of the efferent nocifensive system may account for this result pattern. Since these abnormalities persisted in the On, the impact of other transmitters, like serotonin, noradrenaline and acetylcholine as well as non-synaptic mechanisms, like central or peripheral lesions of the efferent pathways, on PD-related nociceptive dysfunctions should be clarified in future studies.

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Appendix A. Appendix

For completeness, we elaborate the effects of the ANOVAs here, which were not focussed by our hypotheses (see Section 3.3).

EEG, SSR, heat-pain ratings

Main effect of phase

The significant main effects of phase for the heat-pain ratings refer to higher pain ratings in the On compared to the Off in both groups. For the SSR frequencies, the main effect of phase indicates a reduced amount of SSRs in the On relative to the Off both in PD patients and controls. Thus, the pattern of the heat-pain ratings (Off < On) suggest sensitization while the SSR-frequency pattern (Off > On) rather argues for habituation in the On compared to the Off. Since the phase effect occurred in both groups (main effect) it rather seems to result from repeated testing than from a pharmacological impact.

Main effect of stimulus intensity

The main effect of stimulus intensity, which was found for all parameters, resulted from generally higher EEG N2P2-complex, N2 and P2 amplitudes and longer N2 and P2 latencies in response to the 51 °C stimulus compared to the 45 °C stimulus. Furthermore, higher SSR N1P2-complex amplitudes and SSR frequencies as well as shorter SSR latencies and higher heat-pain ratings were found for the 51 °C stimulus compared to the 45 °C stimulus. Thus, the stronger stimuli led differentially to stronger responses according to all parameters.

Interactions of group and stimulus intensity

For post-hoc testing the (marginally) significant two-way interaction group * stimulus intensity for the SSR parameters (N1P2-complex amplitude, latency and frequency), data were combined across phases (Off/On) for each parameter.

For the SSR N1P2 complex amplitudes, the two-way-interaction group * stimulus intensity results from bigger between group differences (PD < CG) for the 45 °C stimulus compared to the 51 °C stimulus

Table A1
Statistics of the ANOVAs regarding the EEG, SSR and heat-pain rating data. G = (Effect of) group, P = phase, I = (stimulus) Intensity, G * P = interaction group * phase […]. Bold numbers and letters mark significant effects ($p < 0.05$), cursive numbers and letters mark marginally significant effects ($p < 0.10$). When *F*-values were below 1 (significance is impossible), only $F < 1$ is reported.

EEG/ratings		Effect	*F*	*p*	η^2		Effect	*F*	*p*	η^2
	N2P2 amplitude	G	<1			Heat-pain ratings	G	<1		
		P	0.067	0.797	0.002		**P**	**7.152**	**0.010**	**0.140**
		I	**40.755**	**<0.001**	**0.481**		**I**	**189.629**	**<0.001**	**0.812**
		G * P	<1				G * P	<1		
		G * I	<1				G * I	<1		
		P * I	<1				P * Í	<1		
		G * P * I	<1				G * P * I	<1		
	N2 amplitude	G	<1			N2 latency	G	<1		
		P	1.089	0.302	0.024		P	<1		
		I	**10.674**	**0.002**	**0.195**		**I**	**21.566**	**<0.001**	**0.334**
		G * P	<1				G * P	<1		
		G * I	1.128	0.294	0.025		G * I	2.662	0.110	0.058
		P * I	<1				**P * I**	**4.586**	**0.038**	**0.096**
		G * P * I	<1				**G * P * I**	**4.920**	**0.032**	**0.103**
	P2 amplitude	G	<1			P2 latency	G	<1		
		P	2.687	0.108	0.058		P	<1		
		I	**68.879**	**<0.001**	**0.610**		**I**	**17.385**	**<0.001**	**0.288**
		G * P	<1				G * P	<1		
		G * I	<1				G * I	<1		
		P * I	<1				P * I	<1		
		*G * P * I*	*3.494*	*0.068*	*0.074*		G * P * I	<1		
SSR	N1P2 amplitude	**G**	**13.556**	**0.001**	**0.470**	Latency	**G**	**8.375**	**0.007**	**0.207**
		P	1.072	0.306	0.024		P	2.761	0.106	0.079
		I	**23.716**	**<0.001**	**0.350**		**I**	**14.658**	**0.001**	**0.314**
		G * P	<1				G * P	2.284	0.141	0.067
		G * I	**6.066**	**0.018**	**0.121**		*G * I*	*3.458*	*0.072*	*0.098*
		P * I	<1				P * I	<1		
		G * P * I	<1				G * P * I	<1		
	SSR frequency	**G**	**4.354**	**0.043**	**0.090**					
		P	**8.283**	**0.006**	**0.158**					
		I	**13.616**	**0.001**	**0.236**					
		G * P	<1							
		*G * I*	*3.036*	*0.088*	*0.065*					
		P * I	<1							
		G * P * I	<1							

(Cohen's $d = 1.11$ vs. Cohens $d = 0.21$). For the SSR frequencies, the interaction group*stimulus intensity, resulted from lower SSR frequencies in PD patients compared to controls for the 45 °C stimulus, $t(44) = 2.50$, $p = 0.016$, not for the 51 °C stimulus, $t(44) = 1.57$, $p = 0.125$, and higher SSR frequencies for the 51 °C stimulus compared to the 45 °C stimulus in PD patients, $t(22) = 3.81$, $p = 0.001$, not in controls, $t(22) = 1.39$, $p = 0.179$. Last, compared to controls, the SSR latencies in PD patients were longer for the 51 °C but not for the 45 °C stimulus, $t(42) = 2.11$, $p = 0.041$. Additionally, latencies were shorter for the 51 °C stimulus compared to the 45 °C stimulus only in PD patients, $t(20) = 3.93$, $p = 0.001$, not in controls, $t(22) = 1.37$, $p = 0.186$.

Interaction of group, phase and stimulus intensity

Indeed, there was a three-way interaction group * phase * stimulus intensity for the EEG P2 amplitudes and the EEG N2 latencies which might be driven by the expected pattern (between-group differences in the Off, not in the On) mediated by the stimulus intensity. Yet, these interactions result from higher P2 amplitudes in the On relative to the Off only in controls for the 45 °C stimulus, $t(22) = 2.12$, $p = 0.045$, alternatively from a marginally significant drop of the N2 latency for the 51 °C stimulus in the On relative to the Off only in the control group, $t(21) = 1.89$, $p = 0.072$. All other comparisons were non-significant (p's > 0.10).

NFR

Although the marginally significant interaction group * phase for the NFR latency is consistent with our hypothesis, the pattern differed from the expected one: The interaction resulted from a marginally significant drop of the NFR latency in the On compared to the Off in controls ($t(14) = 1.85$, $p = 0.085$), while there was no Off—On difference in PD patients $t < 1$.

Table A2
Statistics of the ANOVAs regarding the NFR data. G = (Effect of) group, P = phase, G*P = interaction group*phase. Bold numbers and letters mark significant effects ($p < 0.05$), cursive numbers and letters mark marginally significant effects ($p < 0.10$). When F-values were below 1 (significance is impossible), only $F < 1$ is reported.

	Effect	F	p	η^2
NFR threshold	G	<1		
	P	<1		
	G * P	<1		
NFR amplitude	*G*	*3.075*	*0.091*	*0.102*
	P	<1		
	G * P	<1		
NFR latency	G	<1		
	P	2.138	0.155	0.073
	*G * P*	*3.187*	*0.085*	*0.106*
NFR ratings	G	<1		
	P	<1		
	G * P	1.330	0.257	0.037

References

[1] G. Arunodaya, A. Taly, Sympathetic skin response: a decade later, J. Neurol. Sci. 129 (2) (1995) 81–89.
[2] M. Basso, A. Powers, C. Evinger, An explanation for reflex blink hyperexcitability in Parkinson's disease, I. Superior colliculus. J. Neurosci. 16 (22) (1996) 7308–7317.
[3] P. Zis, E. Sokolov, K.R. Chaudhury, An overview of pain in Parkinson's disease, in: A. Battaglia (Ed.), An Introduction to Pain and Its Relation to Nervous System Disorders, Wiley Blackwell, Oxford, 2016.
[4] E. Benarroch, Pain-autonomic interactions, Neurol. Sci. 27 (S2) (2006) s130.
[5] D. Borsook, J. Upadhyay, E. Chudler, L. Becerra, A key role of the basal ganglia in pain and analgesia - insights gained through human functional imaging, Mol. Pain 6 (1) (2010) 27.
[6] Braak H, Tredici K, Rüb U, Vos R de, Jansen Steur E, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol. Aging 2003;24(2):197–211.
[7] C. Brefel-Courbon, P. Payoux, C. Thalamas, F. Ory, I. Quelven, F. Chollet, J. Montastruc, O. Rascol, Effect of levodopa on pain threshold in Parkinson's disease: a clinical and positron emission tomography study, Mov. Disord. 20 (12) (2005) 1557–1563.
[8] B. Buzas, M. Max, Pain in Parkinson disease, Neurology 62 (12) (2004) 2156–2157.
[9] K. Chaudhuri, P. Martinez-Martin, A. Schapira, F. Stocchi, K. Sethi, P. Odin, R. Brown, W. Koller, P. Barone, G. MacPhee, L. Kelly, M. Rabey, D. MacMahon, S. Thomas, W. Ondo, D. Rye, A. Forbes, S. Tluk, V. Dhawan, A. Bowron, A.J. Williams, C.W. Olanow, International multicenter pilot study of the first comprehensive self-completed nonmotor symptoms questionnaire for Parkinson's disease: the NMSQuest study, Mov. Disord. 21 (7) (2006) 916–923.
[10] E. Chudler, W. Dong, The role of the basal ganglia in nociception and pain, Pain 60 (1) (1995) 3–38.
[11] O. Dittmar, C. Baum, R. Schneider, S. Lautenbacher, Effects of context and individual predispositions on hypervigilance to pain-cues: an ERP study, JPR 8 (2015) 507–521.
[12] R. Djaldetti, A. Shifrin, Z. Rogowski, E. Sprecher, E. Melamed, D. Yarnitsky, Quantitative measurement of pain sensation in patients with Parkinson disease, Neurology 62 (12) (2004) 2171–2175.
[13] R. Fillingim, Sex, gender, and pain: women and men really are different, Curr Rev Pain 4 (1) (2000) 24–30.
[14] J. Flores, F. El Banoua, B. Galán-Rodríguez, E. Fernandez-Espejo, Opiate antinociception is attenuated following lesion of large dopamine neurons of the periaqueductal grey: critical role for D1 (not D2) dopamine receptors, Pain 110 (1–2) (2004) 205–214.
[15] M. Folstein, S. Folstein, P. McHugh, "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician, J. Psychiatr. Res. 12 (3) (1975) 189–198.
[16] B. Ford, Pain in Parkinson's disease, Mov. Disord. 25 (1) (2010) 98–103.
[17] S. Fusina, S. Conte, L. Bertolasi, E. Fincati, E. Nardelli, L. Bongiovanni, Sympathetic skin response asymmetry in early stage idiopathic Parkinson's disease, Clin. Neurophysiol. 110 (2) (1999) 358–366.
[18] A. Gerdelat-Mas, M. Simonetta-Moreau, C. Thalamas, F. Ory-Magne, T. Slaoui, O. Rascol, C. Brefel-Courbon, Levodopa raises objective pain threshold in Parkinson's disease: a RIII reflex study, J. Neurol. Neurosurg. Psychiatry 78 (10) (2007) 1140–1142.
[19] Y. Granovsky, M. Granot, R. Nir, D. Yarnitsky, Objective correlate of subjective pain perception by contact heat-evoked potentials, J. Pain 9 (1) (2008) 53–63.
[20] Y. Granovsky, I. Schlesinger, S. Fadel, I. Erikh, E. Sprecher, D. Yarnitsky, Asymmetric pain processing in Parkinson's disease, Eur. J. Neurol. 20 (10) (2013) 1375–1382.
[21] G. Halliday, P. Blumbergs, R. Cotton, W. Blessing, L. Geffen, Loss of brainstem serotonin- and substance P-containing neurons in Parkinson's disease, Brain Res. 510 (1) (1990) 104–107.
[22] F. Hirashima, T. Yokota, M. Hayashi, Sympathetic skin response in Parkinson's disease, Acta Neurol. Scand. 93 (2–3) (1996) 127–132.
[23] W. Jost, P. Odin, I. Trender-Gerhard, A. Storch, Validierung des NMSQuest in deutscher Sprache, Akt Neurol 36 (S 02) (2009).
[24] M. Kunz, S. Lautenbacher, N. LeBlanc, P. Rainville, Are both the sensory and the affective dimensions of pain encoded in the face? Pain 153 (2) (2012) 350–358.
[25] M. Kunz, V. Mylius, K. Schepelmann, S. Lautenbacher, On the relationship between self-report and facial expression of pain, J. Pain 5 (7) (2004) 368–376.
[26] S. Lautenbacher, G. Rollman, Sex differences in responsiveness to painful and non-painful stimuli are dependent upon the stimulation method, Pain 53 (3) (1993) 255–264.
[27] S. Lim, M. Farrell, S. Gibson, R. Helme, A. Lang, A. Evans, Do dyskinesia and pain share common pathophysiological mechanisms in Parkinson's disease? Mov. Disord. 23 (12) (2008) 1689–1695.
[28] M. Lugo, G. Istúriz, C. Lara, N. García, A. Eblen-Zajjur, Sensory lateralization in pain subjective perception for noxious heat stimulus, Somatosensory & Motor Research 19 (3) (2009) 207–212.
[29] J. Miller, F. Boureau, D. Albe-Fessard, Supraspinal influences on nociceptive flexion reflex and pain sensation in man, Brain Res. 179 (1) (1979) 61–68.
[30] V. Mylius, J. Brebbermann, H. Dohmann, I. Engau, W. Oertel, J. Möller, Pain sensitivity and clinical progression in Parkinson's disease, Mov. Disord. 26 (12) (2011) 2220–2225.
[31] V. Mylius, I. Engau, M. Teepker, K. Stiasny-Kolster, K. Schepelmann, W. Oertel, S. Lautenbacher, J. Moller, Pain sensitivity and descending inhibition of pain in Parkinson's disease, J. Neurol. Neurosurg. Psychiatry 80 (1) (2009) 24–28.
[32] V. Mylius, M. Kunz, E. Hennighausen, S. Lautenbacher, K. Schepelmann, Effects of ageing on spinal motor and autonomic pain responses, Neurosci. Lett. 446 (2–3) (2008) 129–132.
[33] V. Mylius, M. Kunz, K. Schepelmann, S. Lautenbacher, Sex differences in nociceptive withdrawal reflex and pain perception, Somatosens Mot Res 22 (3) (2005) 207–211.
[34] M. Nolano, V. Provitera, A. Estraneo, M. Selim, G. Caporaso, A. Stancanelli, A. Saltalamacchia, B. Lanzillo, L. Santoro, Sensory deficit in Parkinson's disease: evidence of a cutaneous denervation, Brain 131 (7) (2008) 1903–1911.
[35] S. Pan, Y. Wang, W. Hou, C. Wang, T. Huang, Reduced sympathetic skin response in the isolated spinal cord of subjects with spinal cord injury, Arch. Phys. Med. Rehabil. 87 (9) (2006) 1201–1206.
[36] A. Pertovaara, I. Martikainen, N. Hagelberg, H. Mansikka, K. Nagren, J. Hietala, H. Scheinin, Striatal dopamine D2/D3 receptor availability correlates with individual response characteristics to pain, Eur. J. Neurosci. 20 (6) (2004) 1587–1592.
[37] J. Priebe, M. Kunz, C. Morcinek, P. Rieckmann, S. Lautenbacher, Does Parkinson's disease lead to alterations in the facial expression of pain? J. Neurol. Sci. 359 (1–2) (2015) 226–235.

[38] B. Scatton, F. Javoy-Agid, L. Rouquier, B. Dubois, Y. Agid, Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease, Brain Res. 275 (2) (1983) 321–328.
[39] P. Schestasky, H. Kumru, J. Valls-Solé, F. Valldeoriola, E. Marti M-J Tolosa, Neurophysiologic study of central pain in patients with Parkinson's disease, Neurology 69 (60) (2007) 2162–2169.
[40] M. Tinazzi, C. Del Vesco, G. Defazio, E. Fincati, N. Smania, G. Moretto, A. Fiaschi, D. Le Pera, M. Valeriani, Abnormal processing of the nociceptive input in Parkinson's disease: a study with CO_2 laser evoked potentials, Pain 136 (1–2) (2008) 117–124.
[41] M. Tinazzi, S. Recchia, S. Simonetto, G. Defazio, S. Tamburin, G. Moretto, A. Fiaschi, R. Miliucci, M. Valeriani, Hyperalgesia and laser evoked potentials alterations in hemiparkinson: evidence for an abnormal nociceptive processing, J. Neurol. Sci. 276 (1–2) (2009) 153–158.
[42] C. Toth, K. Breithaupt, S. Ge, Y. Duan, J. Terris, A. Thiessen, S. Wiebe, D. Zochodne, O. Suchowersky, Levodopa, methylmalonic acid, and neuropathy in idiopathic Parkinson disease, Ann. Neurol. 68 (1) (2010) 28–36.
[43] L. Vela, K. Lyons, C. Singer, A. Lieberman, Pain–pressure threshold in patients with Parkinson's disease with and without dyskinesia, Parkinsonism Relat. Disord. 13 (3) (2007) 189–192.
[44] R. Vetrugno, R. Liguori, P. Cortelli, P. Montagna, Sympathetic skin response, Clin. Auton. Res. 13 (4) (2003) 256–270.
[45] S. Wang, J. Fuh, D. Shan, K. Liao, K. Lin, C. Tsai, Z.A. Wu, Sympathetic skin response and R-R interval variation in parkinson's disease, Mov. Disord. 8 (2) (1993) 151–157.
[46] Q. Wu, L. Garciaa-Larrea, P. Mertens, A. Beschet, M. Sindoub, F. Mauguiere, Hyperalgesia with reduced laser evoked potentials in neuropathic pain, Pain 80 (1–2) (1999) 209–214.
[47] S. Zambito Marsala, M. Tinazzi, R. Vitaliani, S. Recchia, F. Fabris, C. Marchini, A. Fiaschi, G. Moretto, B. Giometto, A. Macerollo, G. Defazio, Spontaneous pain, pain threshold, and pain tolerance in Parkinson's disease, J. Neurol. 258 (4) (2011) 627–633.
[48] L. Zambreanu, R. Wise, J. Brooks, G. Iannetti, I. Tracey, A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging, Pain 114 (3) (2005) 397–407.
[49] W. Zetusky, J. Jankovic, F. Pirozzolo, The heterogeneity of Parkinson's disease: clinical and prognostic implications, Neurology 35 (4) (1985) 522–526.