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Does Habituation Differ in Chronic Low Back Pain Subjects Compared to Pain-Free Controls? A Cross-Sectional Pain Rating ERP Study Reanalyzed with the ERFIA Multilevel Method

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Abstract: The objective of the present study was to investigate cortical differences between chronic low back pain (CLBP) subjects and pain-free controls with respect to habituation and processing of stimulus intensity. The use of a novel event-related fixed-interval areas (ERFIA) multilevel technique enables the analysis of event-related electroencephalogram (EEG) of the whole post stimulus range at a single trial level. This technique makes it possible to disentangle the cortical processes of habituation and stimulus intensity.

In a cross-sectional study, 78 individuals with CLBP and 85 pain-free controls underwent a rating paradigm of 150 nonpainful and painful somatosensory electrical stimuli. For each trial, the entire epoch was partitioned into 20-ms ERFIAs, which acted as dependent variables in a multilevel analysis. The variability of each consecutive ERFIA period was modeled with a set of predictor variables, including 3 forms of habituation and stimulus intensity.

Seventy-six pain-free controls and 65 CLBP subjects were eligible for analysis. CLBP subjects showed a significantly decreased linear habituation at 340 to 460 ms in the midline electrodes and C3 ($Ps < .05$) and had a significantly more pronounced dishabituation for the regions of 400 to 460 ms and 800 to 820 ms for all electrodes, except for T3 and T4 ($Ps < .05$). No significant group differences for stimulus intensity processing were observed.

In this study, group differences with respect to linear habituation and dishabituation were demonstrated. By means of the ERFIA multilevel technique, habituation effects were found in a broad post stimulus range and were not solely limited to peaks. This study suggests that habituation may be a key mechanism involved in the transition process to chronic pain. Future studies with a longitudinal design are required to solve this issue.

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Abbreviations: CLBP = chronic low back pain, EEG = electroencephalogram, EOG = electrooculogram, ERFIA = event-related fixed-interval areas, ERP = event-related potentials, ISI = inter-stimulus interval.

INTRODUCTION

Chronic pain may be considered as a nosological entity in its own right, in which neuroplastic alterations in the central nervous system occur.[1–5] An alteration in habituation is proposed as one of the mechanisms involved in chronic pain. Habituation is the process that refers to a decrease in a behavioral response to a repeatedly presented stimulus.[6,7] Habituation can be observed and measured both at the subjective experiential level (pain report) as well as at the psychophysiological level. In this respect, event-related potentials (ERPs) may be a useful tool in studying the process of habituation. ERPs are time-locked responses to stimuli derived from an ongoing electroencephalogram (EEG). ERPs may contribute to the understanding of the cortical processing of pain and possible differences between subjects with chronic pain and healthy subjects. Commonly, the intensity of (non-) noxious stimuli (laser, heat, or electrical) is positively associated with the second negative and second positive ERP peak, termed N2 and P2.[8,9] In other words, higher stimulus intensities are typically accompanied by larger N2 and P2 peak amplitudes.[9–12] Because blocks of stimuli are generally averaged between conditions,[13,14] the possible influence of habituation may be disregarded in the case of averaging. It is therefore not clear if and how the process of habituation influences different conditions and group effects.

Two recent developments enable a more detailed study of the relationship between ERPs on the one hand and stimulus intensity and habituation to stimuli on the other. First, Vossen and colleagues proposed the use of a multilevel technique in the analysis of ERPs.[15] A multilevel approach has several advantages over commonly used ANOVA techniques, especially when studying the process of habituation. Not only does multilevel analysis consider the hierarchical nature of ERP data, in which trials are nested within subjects, but also person-by-time effects can be studied, the latter through the incorporation of random effects and nonlinear contrasts.[15,16] A second development was the recent introduction of event-related fixed-interval areas (ERFIAs) under the curve analyzed with multilevel analysis. This technique is based on the idea that not only maximized peaks may contain relevant information, but also fundamentally each

post stimulus latency point may carry relevant information, regardless of whether it is peak-related or not.

In the ERFIA multilevel technique, the entire post stimulus epoch is partitioned into 20-ms ERFIAs for each single trial.[17,18] The ERFIA multilevel technique enables the investigation of the influence of predictor variables of interest on the whole epoch at single trial level, and thereby taking person-by-time effects between epochs such as habituation into account. In a sample of (n = 76) pain-free subjects analyzed with the multilevel ERFIA method, the results showed that cortical processing of both habituation and stimulus intensity was associated with post stimulus areas broader than peaks. In addition, stimulus intensity processing was influenced by the previous stimulus intensity in a broad range.[17]

Altogether, the analytical developments and first results gave the impetus to reanalyze habituation processes in an existing ERP-dataset of a pain-rating paradigm of chronic low back pain (CLBP) subjects and pain-free controls.

The aim of the present study is to investigate whether the cortical processing of habituation, stimulus intensity, and the interaction of the previous intensity with the actual stimulus intensity differs between subjects with CLBP and pain-free controls, using the ERFIA multilevel technique. This study is mainly explorative in nature, because of the novelty of the ERFIA multilevel technique. Nevertheless, some a-priori hypotheses were postulated based on existing ERP literature.

First, with respect to habituation, Valeriani and colleagues reported a reduced habituation of the N2/P2 amplitudes in patients diagnosed with migraine compared to pain-free controls.[19] In addition, we found linear habituation effects in the ranges from 100 to 140 ms and 200 to 560 ms in healthy subjects.[17] Based on the finding of Valeriani and our previous results, a reduced linear habituation for CLBP subjects was postulated in the ranges from 100 to 140 and 200 to 560 ms. Habituation was modeled in 3 ways—linear habituation, fast habituation (inverse relationship), and dishabituation (quadratic relationship). However, no a-priori hypotheses were made for fast habituation and dishabituation.

Second, regarding stimulus intensity, it has been suggested that the peak-to-peak amplitudes are larger in chronic pain patients compared to pain-free controls.[20] Higher vertex amplitudes were observed in fibromyalgia patients compared to controls.[20–22] Based on these studies, a group × stimulus intensity interaction was expected, in which the effect of stimulus intensity on ERFIAs in the N2 and P2 peak regions is more positive for CLBP subjects compared to pain-free controls.

Third, previously we demonstrated a strong and robust effect (range 380–660 ms) of the previous stimulus intensity on the processing of the actual stimulus intensity, which suggests a kind of ''stimulus-related memory process''.[17] Therefore, it was decided to investigate whether this phenomenon would be different between subjects with CLBP and pain-free controls. This hypothesis was tested by the inclusion of a 3-way interaction (group × actual stimulus intensity × previous stimulus intensity) in the model.

MATERIALS AND METHODS

Subjects

Eighty-five pain-free subjects and 78 CLBP subjects, ranging in age from 18 to 65 years, were enrolled in the study between November 2005 and April 2007. Subjects with low back pain were included in the study if they had an anamnestic history of nonmalignant low back pain for at least 6 months without other interfering pain complaints. Pain-free subjects were recruited if they had no chronic pain complaints during the past 6 months and did not use any analgesic or psychotropic medication during this period. For both groups exclusion criteria were the consumption of analgesics <8 h before the start of the experiment and/or the structural use of psychoactive drugs, such as antidepressants, antipsychotics, antiepileptics, and opioids. Participation was rewarded with 25€ upon completion of the study. Approval was obtained from the Medical Ethics Committee of Maastricht University Medical Centre. All subjects gave their verbal and written informed consent before the study. Subjects were recruited by means of a flyer from the general population of Maastricht.

Stimuli

Intracutaneous electrical pulse stimuli with a duration of 10 ms were administered on the left middle finger, per Bromm and Meier.[10] Using this method, a small lumen in the epidermis was prepared, using a dental gimlet, ensuring that the procedure was not painful. In the prepared lumen, a golden electrode was placed and fixed with tape. Two grounding copper laces were attached around the prepared finger and wrist. First, the sensation and pain thresholds were determined by gradually increasing the intensity of the stimulus, starting at zero intensity. The first intensity that was consciously experienced was defined as the sensation threshold. Next, the first intensity that was experienced as painful was defined as the pain threshold. This procedure was repeated 3 times to generate a reliable measurement. Based on the difference between a subject's sensation and pain thresholds, 5 stimulus intensities were presented in a rating paradigm. One of the 5 intensities was equal to the pain threshold, against which the other intensities were defined: −50%, −25%, +25%, and +50% of the difference between the sensation and pain thresholds (threshold range). The maximum stimulus intensity never exceeded 5 mA.

Paradigm

One hundred fifty stimuli were presented in a rating paradigm.[10] The 5 stimulus intensities were presented semi-randomly. Blocks of 15 stimuli were administered, in which each intensity occurred 3 times. Interstimulus intervals (ISIs) randomly varied between 9 and 11 s. Subjects were asked to rate the intensity of each stimulus on a scale from 0 (no sensation) to 100 (the most excruciating pain imaginable). The subject was told that the intensity of the first stimulus would be exactly equal to the calibrated pain threshold and should be rated as 60.

EEG Recording

All EEG recordings were conducted in an electrically- and sound-shielded cubicle ($3 \times 4\,m^2$). Ag/AgCl electrodes were placed on Fz, Cz, Pz, C3, C4, T3, and T4 using the international 10 to 20 system.[23] Impedances were maintained <5 kΩ. A reference electrode was placed on each ear lobe. In order to check for possible vertical eye movements, an electrooculogram (EOG) electrode was placed 1 cm under the midline of the right eye. A ground electrode was placed at Fpz. All electrodes were fixed using 10 to 20 conductive paste. Neuroscan 4.3 software was used to record EEGs.

Procedure

Before starting the experiment, subjects were informed about the purpose of the study. Subjects were told that they would undergo an EEG registration while receiving various

intensities of electric shocks—some painless and some painful. After completion of the informed consent forms, the SF-36 questionnaire was completed.[24,25] Next, EEG electrodes were placed on the subjects, and the shock electrode was attached to the top of the left middle finger, per Bromm and Meier.[10] Then, the sensory and pain thresholds were determined, after which the rating paradigm was initiated.

Data Reduction and Computation of ERFIAs

EEGs were recorded at a 1000-Hz sampling rate. Trials were segmented from the continuous EEG, from 200 ms before the stimulus to 1500 ms post stimulus. Data were offline band-pass filtered (0–50 Hz), and baseline-corrected (interval −200–0 ms) using BrainVision Analyser 2.0, Brain Products, München, Germany. The filtered data segments (per millisecond) were exported to Microsoft Office Excel 2007. Areas under the curve amplitude sum scores of 20 consecutive milliseconds were calculated from 0 to 1500 ms post stimulus, resulting in 75 ERFIAs per trial, per EEG electrode, and per subject. Additionally, maximum and minimum values of the EOG channel were selected per 20-ms ERFIA. Next, the ERFIAs of all 150 trials of all 7 electrodes of the subjects were imported into SPSS 20.0. Single ERFIAs with EOG activity that exceeded $\pm 25\ \mu V$ were excluded from the multilevel analyses.

Statistical Analyses

Subject characteristics were analyzed using independent *t* tests and χ^2 tests. Multilevel random regression analyses were performed separately for each EEG electrode. Trial number (1–150 stimuli) was considered the repeated measure. Subjects represented the highest level in the model, and the 20-ms ERFIAs served as the dependent variables. The dependent variables were assessed for normality.

Habituation was modeled in 3 ways, namely linear habituation, fast habituation, and dishabituation. First, linear habituation was modeled as trial number ($trial_{linear}$), assuming a linear decrease or increase of the dependent variable (of a particular ERFIA) over time (trial number). Second, fast habituation was modeled as an inverse relationship ($trial_{inverse}$), representing a rapid decline, followed by a gradual decline or plateau phase—that is, habituation of the initial trials is more pronounced than later in the experiment. The inverse relationship was computed as 1 divided by trial number (1/trial number). Third, dishabituation was modeled as a quadratic function, representing a sensitization process (or dishabituation) after an initial habituation. This parabolic relationship was computed as trial number × trial number ($trail_{quadratic}$).[15,17]

The full multilevel model comprised the following independent variables (fixed factors): actual stimulus intensity, $trial_{linear}$, $trial_{inverse}$, $trial_{quadratic}$, age, gender, previous stimulus intensity, difference between pain and sensory thresholds (diff_pain_sens), and group (CLBP, coded as 1 vs pain-free coded as 0). The following 2-way interactions were modeled (Table 1): actual stimulus intensity × previous stimulus intensity, group × actual stimulus intensity, group × $trial_{linear}$, group × $trial_{inverse}$, group × $trial_{quadratic}$, and group × diff_pain_sens. Finally, one 3-way interaction (group × actual stimulus intensity × previous stimulus intensity) was incorporated.

With respect to random effects in the model, we made the assumption that subjects differ from one another in their response to the 5 intensities and with regard to habituation. Consequently, random effects, such as a random intercept and a random slope for intensity and trial number, were also included. The scaled identity covariance structure was used in the multilevel analyses.

The analyses were performed separately for each 20-ms ERFIA period for all 7 cranial sites, resulting in 75 (1500 ms/20 ms) × 7 (cranial locations) = 525 multilevel models. For this large number of statistical tests, a correction for multiple testing should be performed. We chose not to define a specific *P*-value for statistical significance, due to the partially explorative aspect of the analyses. Instead, we considered relatively long-lasting effects (3 or consecutive >20-ms ERFIAs) with *P*-values ≤.05 as significant. Single ERFIAs were considered significant when the *P*-value was ≤.0007 (with a corresponding *T*-value of 3.43), based on Bonferroni correction for the complete epoch, obtained by dividing a significance level of .05 by the number of ERFIAs (n = 75).[17] The full multilevel model is described in Appendix A. All statistical analyses were performed with SPSS 20.0.

Because the present study pertains a reanalysis of a dataset, no a-priori study size calculation was made.

To visualize and create an overview of the results, so-called ERFIA predictor blots were constructed. ''Blots'' are the tables in which the columns represent the 75 consecutive 20-ms ERFIAs, and the rows represent the EEG electrodes of a given predictor. In each row, cells were given a color when *T*-values were < −2 or >2. Additionally, a plus or minus sign was added to each cell to indicate whether the *T*-value was positive or negative.

RESULTS

Sample Characteristics

From the 85 pain-free subjects, 9 were excluded for one of the following reasons: relevant pain complaints in the past week, consumption of more than 5 units of alcohol on the evening before the experiment or EEG-related technical errors. Thus, 76 pain-free subjects were analyzed: 26 men (34.2%) and 50 women (65.8%). Of the 78 CLBP subjects, 65 patients were analyzable. Thirteen individuals were excluded because of a variety of reasons: technical problems in the EEG measurement, use of painkillers or sleep medication the day before the experiment, other accompanying pain complaints, and excessive structural alcohol use. Table 2 summarizes the group characteristics. Pain-free controls were on average 6.1 years younger ($P = .01$). In addition, the CLBP group showed a statistically significant higher score on the items ''pain magnitude'' and ''pain interference'' of the SF-36 scale (Table 2). Grand averages of ERP differences between CLBP and pain-free subjects are presented with respect to overall (Figure 1A), stimulus intensity (Figure 1B), and habituation (Figure 1C) differences between the groups.

Habituation

The main effects of linear habituation were observed in 3 latency ranges: an early positive effect between 100 and 140 ms, a second opposite (negative) effect between 200 and 320 ms, and a third negative effect at 580 to 640 ms. These effects could be observed at all electrodes except T4, in which the third region of linear habituation was located at 940 to 1220 ms (Figure 2A). Interaction effects between group and linear habituation were mainly seen in the region of 340 to 460 ms in the midline (Fz, Cz, and Pz) and C3 (Figure 2B).

Fast habituation effects (inverse function) were rather scattered in the blot. However, clustered effects were seen at

TABLE 1. Summary of the main, interaction and random factors of the multilevel model

Main (fixed) Factors	Interaction Factors	Random Factors
Age		
Gender		
Group (CLBP* vs pain-free)		
Linear habituation (trial number)	Linear habituation × group	Trial number
Fast habituation (1/trial number)	Fast habituation × group	
Dishabituation (trial × trial)	Dishabituation × group	
Sensory and pain threshold difference (Diff_pain_sens)	Diff_pain_sens × group	
Actual stimulus intensity	Actual Stimulus intensity × group	Actual stimulus intensity
Previous stimulus intensity	Previous stimulus intensity × group	
	Actual stimulus intensity × previous stimulus intensity	
	Actual stimulus intensity × previous stimulus intensity × group	

* CLBP = chronic low back pain.

approximately 1220 to 1440 ms for most electrodes (Figure 2C). Interaction effects between fast habituation and group were mainly located at Cz, C3, and T3 (Figure 2D).

The main effects of dishabituation (quadratic function) were situated in the same regions as linear habituation. However, the direction of effects was the opposite and lasted only until 280 ms (Figure 2E). Again, in the region of T4, a late effect was observed at 940 to 1220 ms. Interactions of dishabituation with group were observed in the regions of 400 to 460 ms and 800 to 820 ms for all electrodes, except for T3 and T4 (Figure 2F).

As an example, the model of 320 ms at Cz was chosen to visualize the 3 habituation interaction effects. Based on the model regression estimates, the habituation effects at Cz were calculated for the CLBP group and the pain-free controls (Figure 3). These figures illustrate that pain-free subjects show a faster linear habituation, have a faster inverse habituation, and display less dishabituation compared to the CLBP group. For dishabituation, the top of the parabola was calculated for both groups. This top was located at trial number 22 for the CLBP group and on trial number 34 for the pain-free control group.

Stimulus Intensity

There were no statistically significant effects for either the interaction between group and actual stimulus intensity or for the interaction between group and previous stimulus intensity, or for the 3-way interaction of group × actual stimulus intensity × previous stimulus intensity. Consequently, these interaction terms were removed from the model, resulting in a reduced model (Appendix A, model 2).

The main effects of the variable stimulus intensity are depicted in Figure 2G. Significant effects were found in several latency ranges but were especially pronounced between 100 to 340 ms and 1040 to 1500 ms. ERFIAs were not marked in case significant stimulus intensity interaction effects with previous stimulus intensity occurred (Figure 2H).

The interaction between actual stimulus intensity times previous stimulus intensity was statistically significant in the latency range 400 to 680 ms for all electrodes (Figure 2H).

TABLE 2. Characteristics of Chronic Low Back Pain Subjects and Pain-Free Controls Eligible for Analysis

	Pain-free Controls	CLBP* Subjects	*P*
N	76	65	
Gender male/female (n)	26/50	32/33	0.07
Age (years, sd)	34.8 (13.7)	40.9 (15.3)	0.01
Pain threshold (mA)	1.1 (.9)	1.2 (1.1)	0.40
Pain magnitude (SF-36† item, sd)	1.7 (.9)	3.5 (.9)	<0.001
Pain interference (SF-36 item, sd)	1.2 (.5)	2.2 (.9)	<0.001

* CLBP: chronic low back pain.
† SF-36: short-form, 36 items.

Difference between Pain and Sensory Threshold

The main effects of the pain and sensory threshold difference were observed in the early latencies until 260 to 280 ms (Figure 2I). In these latency ranges, an increase in the ''pain-sensory gap'' corresponded with more positive ERFIAs. An interaction effect with group was found between 260 and 320 ms at T4, 360 to 500 ms at T3, 400 to 560 at C3, and from 440 to 560 ms at Cz, respectively (Figure 2J).

Random Effects

All random intercepts were significant in all models, indicating that intercepts varied significantly between subjects ($P < .001$). Slopes were significant ($P < .05$) in the majority of models, indicating that slopes varied significantly between subjects. Significant slopes were found for 72% of the models for stimulus intensity and 76% of the models for habituation. Nonsignificant random effects for the slopes were only found for ERFIAs after 700 ms post stimulus.

DISCUSSION

Differences in cortical processing pertaining to a rating paradigm using electrical stimuli were explored between subjects with CLBP and pain-free controls. The results suggested that CLBP subjects habituated to a lesser extent to repetitive stimuli than pain-free controls for linear habituation in the region of 340 to 460 ms, cortical processing of different stimulus intensities, ranging from 50% below the pain threshold

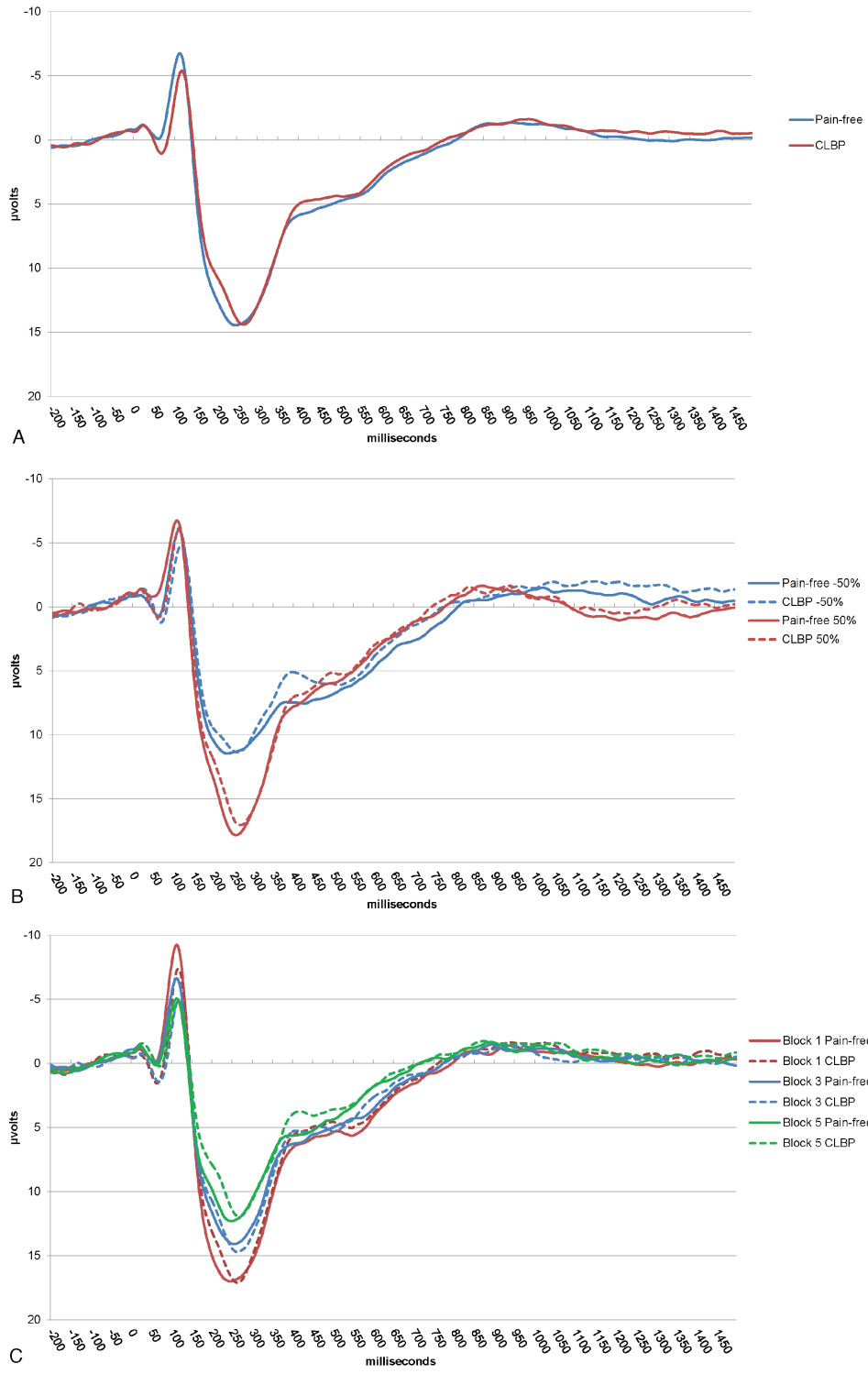

FIGURE 1. Grand averages for chronic low back pain subjects versus pain-free controls at Cz of 150 intracutaneous electrical stimuli. (A) Overall differences, (B) grand averages of 2 stimulus intensities (50% below and 50% above pain threshold), and (C) grand averages for habituation at Cz of 150 intracutaneous electrical stimuli. Of the 5 blocks, only blocks 1, 3, and 5 are shown (30 stimuli for each block).

to 50% above, was equal between CLBP subjects and pain-free controls, and the influence of the previous stimulus intensity on the actual stimulus intensity did not differ between CLBP subjects and pain-free controls. These group interaction effects are discussed consecutively in the light of available literature below.

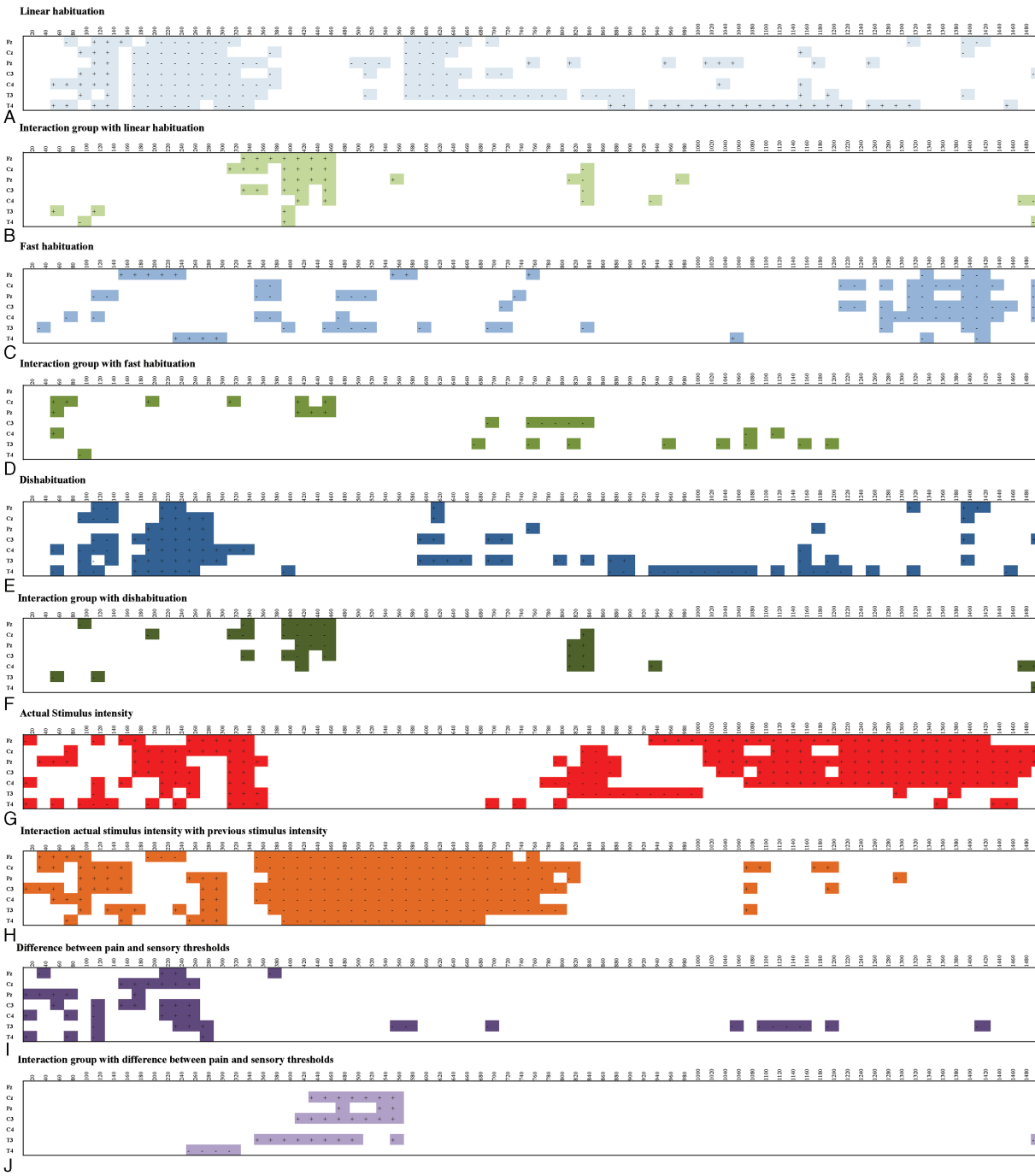

FIGURE 2. (A to J) Twenty-millisecond ERFIA predictor blot of the model variables. Columns represent consecutive 20-ms ERFIAs whereas rows display cranial locations. Cells with significant results are colored ($P < .05$) and the plus or minus sign expresses the direction of the relationship.

Habituation

Three types of habituation were investigated, namely linear habituation (modeled with trial number), fast habituation (modeled with 1/trial number), and dishabituation (modeled with trial number × trial number). The results showed that linear habituation and dishabituation are influenced by CLBP status (Figure 2B, D, and F). To gain more insight into these different habituation processes, the course of habituation for CLBP subjects and pain-free controls was calculated, based on the model parameters (Figure 3). Overall, CLBP patients habituated less than pain-free controls in all 3 mathematically modeled forms of habituation. The differences of the top (trial number 22 vs 34) of the parabola (Figure 3C) indicate that the habituation process of the CLBP group is shorter compared to the pain-free controls.

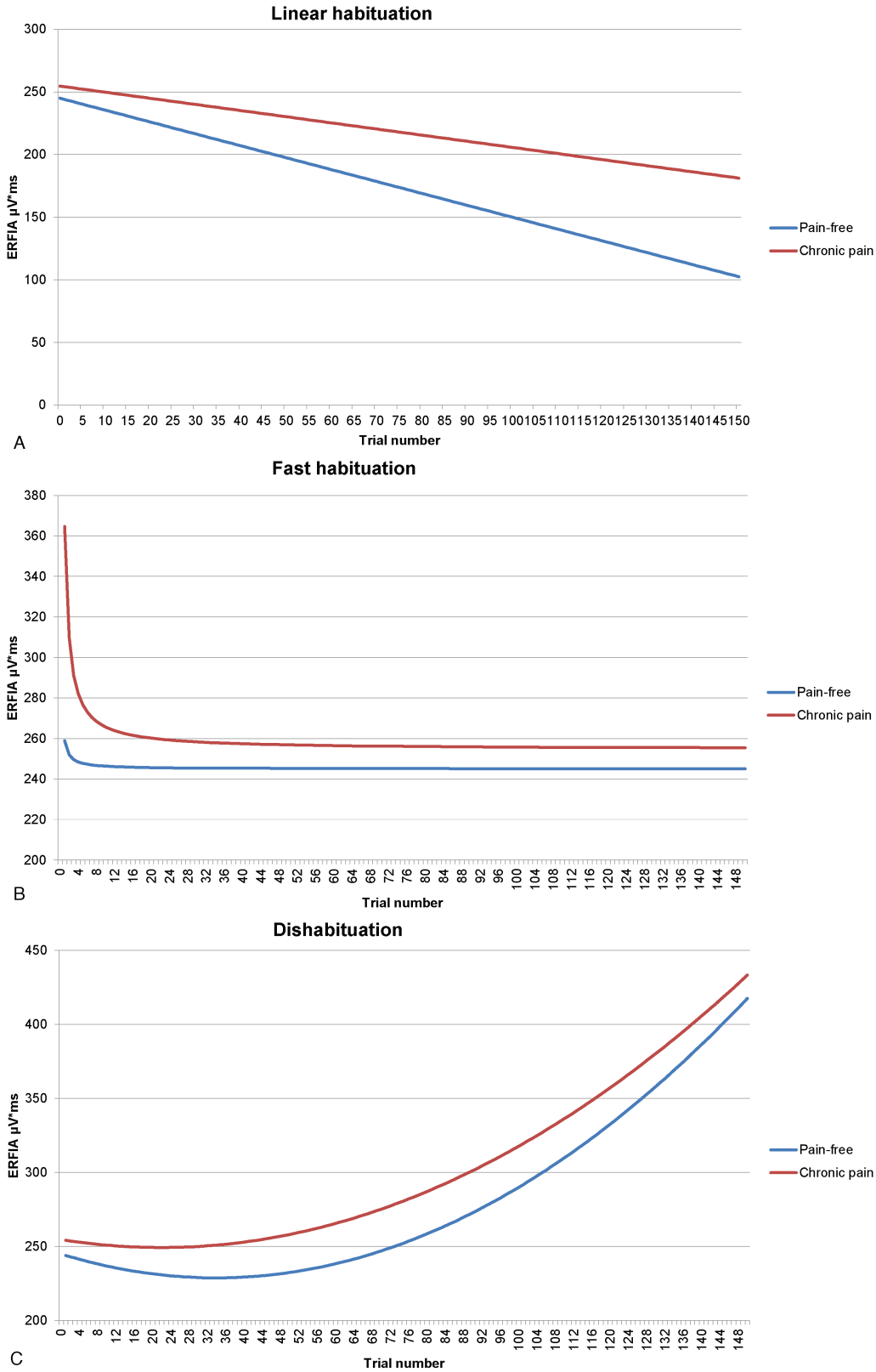

FIGURE 3. Habituation differences for chronic low back pain subjects versus pain-free controls, based on model estimates for the 20-ms event-related fixed-interval area at 320 ms on Cz. (A) Linear habituation (trial number), (B) fast habituation (1/trial number), and (C) dishabituation (trial number × trial number).

Although comparisons of the present results with those of other studies are difficult to make (different populations, different stimulation paradigms, and different analysis techniques), some similarities can be observed. Valeriani and colleagues found reduced habituation of ERP amplitudes in migraine compared to pain-free controls in response to painful CO_2 laser stimulation.[19] Research from de Tommaso and coworkers also showed a decreased habituation in the peak-to peak amplitude in migraine patients and a decreased habituation in the N1, N2, and P2 peaks in fibromyalgia patients in a next study.[20,26]

The question arises why habituation may be different in CLBP subjects. One of the explanations is the idea of a ''windup'' mechanism and central sensitization.[21,27] The disability to habituate may be the result of changes in the modulation of nociceptive input, associated with central sensitization.[28–30] The present results may support this hypothesis.

Furthermore, the fact that significant effects also appear on electrodes such as Fz and Pz suggests that higher cortical processes, such as affective, evaluative, and cognitive processes, may play a role in the relationship between habituation and pain, which is in line with the notion of multidimensionality of the pain experience. In this perspective, investigation of the influence of coping, pain vigilance, pain catastrophizing, and mood on the cortical processing of pain is essential in future research.

Stimulus Intensity

Inconsistent reports exist regarding the comparison of peak-to-peak amplitudes between chronic pain populations and pain-free controls. Several studies reported higher amplitudes (N2–P2 component) in fibromyalgia patients and tension-type headache patients compared to pain-free controls.[20,21,31] On the contrary, Diers and colleagues found a lower P260 component in CLBP patients compared to pain-free controls.[30] Valeriani and co-workers did not find any amplitude differences between patients with migraine, tension-type headache patients, and a control group.[19] Also in chronic neuropathic pain Noteworthy, all these studies applied only a single intensity level. In the present study, we did not observe a difference between the groups with respect to the cortical processing of 5 different stimulus intensities. Obvious explanations would be the different chronic pain populations between studies and the difference in number of stimulus intensities applied. However, the most plausible explanation may be related to the fact that only in the present study, the estimates for stimulus intensity were corrected for the influence of habituation. Not taking into account habituation may have confounded the results of previous studies. In addition, in a multilevel analysis, within-subject variability (random intercepts and slopes) is modeled, estimating fixed effects more precisely than analyses ignoring random effects. Given the highly significant random effects in the present study, the intensity effects may be considered more accurate.

The 3-way interaction group × previous stimulus intensity × actual stimulus intensity was not significant. This finding suggests that a ''stimulus intensity-related memory process'' is not different between CLBP subjects and pain-free controls. The 2-way interaction previous stimulus intensity × actual stimulus intensity, however, remained highly significant in the present analyses, in which CLBP subjects were added to the dataset, compared to the results of pain-free subjects alone in the previous study.[17] These robust effects contribute to the notion that the short-term ''stimulus-intensity-related memory process'' reflects a basic phenomenon in stimulus processing. In other words, the previous stimulus intensity may ''resonate'' within the brain, thereby affecting the processing of the present intensity. Hence, we suggest the effect of the previous stimulus intensity upon the processing of the present intensity should be taken into account in future analyses of intensity-related information.

Pain–Sensory Threshold

With respect to possible group differences in sensory and pain thresholds, the literature is limited and inconsistent, if only because different pain populations have been included across studies. Peters and colleagues noted a higher pain threshold for experimental pain in CLBP patients as compared to control subjects.[32] Other studies, however, reported significantly lower pain thresholds in fibromyalgia patients, regional pain syndrome patients, and chronic back pain patients.[21,33] A main effect related to the difference between the sensory and the pain threshold was observed at 360 to 420 ms post stimulus, in a recent study of cortical processing of electrical noxious stimuli, performed in healthy subjects.[17] This suggests that a relationship exists between the extent of the interval ''sensory–pain gap'' between these 2 stimulus thresholds on the one hand, and the cortical processing of (non-)painful stimuli on the other. Present results indicate that the effect of this ''sensory–pain gap'' differs between the 2 groups notably around 400 to 560 ms post stimulus for Cz, C3, and T3. A clear explanation for these findings cannot be given, Because the experiment was not designed to investigate this issue. Nevertheless, it seems to be worthwhile to include the ''sensory–pain gap'' in future analyses.

Discrepancies between Multilevel Model Outcomes and Grand Averaged ERP Graphs

Inspecting the grand averaged graphs (Figure 1), compared to the results of the multilevel analysis, may lead to different conclusions. For example, no main effect for group was found in the multilevel models, whereas a difference between the groups seems apparent in the grand averaged graph (Figure 1). However, the present study shows that the ERP needs to be statistically corrected for the influence of several variables such as habituation, stimulus intensity, and the influence of previous stimulus intensity on present stimulus intensity processing. In conclusion, grand averaged ERPs cannot adequately express the complexity of the cortical processing of (non-)painful stimuli.

Limitations

A cross-sectional design does not allow conclusions on causal interferences. Therefore, the results of the present study should be interpreted with caution. Although the present study population was relatively large in view of an ERP study, some considerations need to be taken into account. First, the CLBP sample was selected from the general population. Group interaction effects with the variables of interest may have been stronger in a purely clinical CLBP population. On the other hand, the heterogenous CLBP group of this study may reflect chronic pain complaints of the general population more accurately.

Second, the age distribution differed significantly between the CLBP group and pain-free group. The CLBP group was on average 6 years older than the pain-free controls and this difference could have influenced the results. In recent literature, aging has been reported to reduce N2 to P2 amplitudes, and to

increase latencies in laser and electrical ERPs.[34,35] The mean age difference between the studied groups was, compared to the difference in our study, much larger, 27.8 and 40.6 years, respectively. To correct for the potential age influences on habituation, age was added as a covariate in the analyses in the present study. Furthermore, it could be questioned whether this statistically significant group difference of 6 years is clinically relevant.

Third, this study investigated the cortical differences between the 2 groups based on 7, mainly central, cranial locations. In future research, the number of EEG electrodes should be enlarged, especially in the frontal and parietal areas (F3–F4/P3–P4). This would allow further investigation of potential lateral effects.

A fourth possible limitation of our study concerns the correction for multiple testing. Because of the large number of statistical tests performed in this study, we used the strict Bonferroni criterion for single isolated ERFIAs. A less stringent cutoff point for significance was applied in the case of 3 or more consecutive ERFIAs with a *P*-value <.05. In this way, an attempt was made to find a balance between rejecting too many relevant influences, on the one hand, and the risk of accepting too many ''small'' and clinically nonrelevant but significant effects, on the other. Inspection of the ERFIA predictor blots (Fig. 2) show several ''long-lasting'' effects (ranging 60–140 ms) in the interaction (group × habituation) analyses. Without doubt, future research is needed to replicate the explorative interaction findings of the present study.

In conclusion, to our knowledge, this is the first study to investigate possible group differences between CLBP subjects and pain-free controls with respect to habituation and stimulus intensity processing, using the ERFIA multilevel technique. In contrast to stimulus intensity, group differences were found in 3 types of habituation (linear, fast, and dishabituation). Hence, habituation may be a promising key variable to gain more insight into the chronification mechanisms of pain. Future experimental studies with a longitudinal design are undoubtedly needed.

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APPENDIX A

Model 1: The Full Multilevel Model

$$Y_{ti} = ß_0 + ß_1 \times intensity_{lin} + ß_2 \times trial_{lin} + ß_3 \times trial_{quadratic} + ß_4 \times trial_{inverse} + ß_5 \times age + ß_6 \times \cdot gender + ß_7 \times difference\_pain\_and\_sensory\_threshold + ß_8 \times intensity_{lin}\_previous\_trial + + ß_9 \times intensity_{lin} \times intensity_{lin}\_previous\_trial + ß_{10} \times group \times trial_{lin} + ß_{11} \times group \times trial_{inverse} + ß_{12} \times group \times trial_{quadratic} + ß_{13} \times group \times difference\_pain\_and\_sensory\_threshold \quad ß_{14} \times group \times intensity_{lin} + ß_{15} \times group \times intensity_{lin}\_previous\_trial + ß_{16} \times group \times intensity_{lin} \times intensity_{lin}\_previous\_trial + e_{ti} + u_{0i} + u_1 \times intensity_{lin} + u_2 \times trial_{lin}$$

Model 2: The Reduced Multilevel Model

$$Y_{ti} = ß_0 + ß_1 \times intensity_{lin} + ß_2 \times trial_{lin} + ß_3 \times trial_{quadratic} + ß_4 \times trial_{inverse} + ß_5 \times age + ß_6 \times \cdot gender + ß_7 \times difference\_pain\_and\_sensory\_threshold + ß_8 \times intensity_{lin}\_previous\_trial + ß_9 \times intensity_{lin} \times intensity_{lin}\_previous\_trial + ß_{10} \times group \times trial_{lin} + ß_{11} \times group \times trial_{inverse} + ß_{12} \times group \times trial_{quadratic} + ß_{13} \times group \times difference\_pain\_and\_sensory\_threshold + e_{ti} + u_{0i} + u_1 \times intensity_{lin} + u_2 \times trial_{lin}$$

where t = time point (1–150); I = subject; intensity = −2 = −50%, −1 = −25%, 0 = 0%, 1 = 25%, and 2 = 50%; trial = 150 trial numbers, centered from −75 to +75; age = centered continuous variable in years;

gender = dichotomous variable, −1 = man, and 1 = woman; difference pain and sensory threshold = absolute pain threshold − absolute sensory threshold; intensity previous trial = −2 = −50%, −1 = −25%, 0 = 0%, 1 = 25%, and 2 = 50%; e_{ti} = error variance for subject i at time point t. This error term is subdivided into a random intercept, a random slope for $intensity_{lin}$, and a random slope for $trial_{lin}$.

The model must be interpreted as follows:

$ß_0$ = the outcome mean (amplitude) for the intensity equal to the pain threshold (intensity = 0) at trial number 75 for a male (gender = 0) subject with a mean age.

$ß_1$ = the mean difference between intensities

$ß_2$ = the mean change in linear contrast over the trial

$ß_3$ = the mean change in quadratic contrast over the trial

$ß_4$ = the mean change in inverse contrast over the trial

$ß_5$ = the mean change in amplitude per year

$ß_6$ = the mean difference between men and women

$ß_7$ = the mean pain and sensory threshold difference

$ß_8$ = the relationship between the intensity of the previous trial and the amplitude of the present trial

$ß_9$ = the interaction between the effect of the intensity of the previous trial and the intensity of the present trial

$ß_{10}$ = the interaction between group and the linear component of habituation

$ß_{11}$ = the interaction between group and the inverse component of habituation

$ß_{12}$ = the interaction between group and the quadratic component of habituation

$ß_{13}$ = the interaction between group and the pain-sensory threshold gap

$ß_{14}$ = the interaction between group and actual stimulus intensity

$ß_{15}$ = the interaction between group and previous stimulus intensity

$ß_{16}$ = the 3-way interaction between group, actual stimulus intensity and previous stimulus intensity.

u_{0i} = Individual variance from the average intercept

u_1 = individual variance from the average slope $ß_1$ of $intensity_{lin}$

u_2 = individual variance from the average slope $ß_2$ of $trial_{lin}$.

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