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Slowing in peak-alpha frequency recorded after experimentally-induced muscle pain is not significantly different between high and low pain-sensitive subjects

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Running title: Peak alpha frequency during prolonged muscle pain

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ABSTRACT

Peak alpha frequency (PAF) reduces during cutaneous pain, but no studies have investigated PAF during movement-related muscle pain. Whether high-pain sensitive (HPS) individuals exhibit a more pronounced PAF response to pain than low-pain sensitive (LPS) individuals is unclear. As a pain model, twenty-four participants received nerve growth factor injections into a wrist extensor muscle at Day0, Day2, and Day4. At Day4, a subgroup of twelve participants also undertook eccentric wrist exercise to induce additional pain. Pain numerical rating scale (NRS) scores and electroencephalography were recorded at Day0 (before injection), Day4, and Day6 for 3 minutes (eyes closed) with wrist at rest (Resting-state) and extension (Contraction-state). The average pain NRS scores in contraction-state across Days were used to divide participants into HPS (NRS-scores≥2) and LPS groups. PAF was calculated by frequency decomposition of electroencephalographic recordings. Compared with Day0, contraction NRS-scores only increased in HPS-group at Day4 and Day6 ($P<0.001$). PAF in Contraction-state decreased in both groups at Day6 compared with Day0 ($P=0.011$). Across days, HPS-group showed faster PAF than LPS-group during Resting-state and Contraction-state ($P<0.04$). Average pain NRS-scores across days during Contraction-states correlated with PAF at Day0 ($P=0.012$). Pain NRS-scores were associated with PAF during Contraction-state at Day4 and Day6 ($P<0.05$).

Perspective: PAF was slowed during long-lasting movement-related pain in both groups, suggesting a widespread change in cortical excitability independent of the pain sensitivity. Moreover, HPS individuals showed faster PAF than LPS individuals during muscle pain, which may reflect a different cognitive, emotional, or attentional response to muscle pain among individuals.

Keywords: Prolonged hyperalgesia, nerve-growth factor, oscillations, electroencephalography, muscle soreness

1. INTRODUCTION

Acute muscle pain serves an important protective function in preventing or limiting muscle damage. However, often in chronic musculoskeletal pain conditions, muscle pain no longer serves protective functions but may be associated with abnormal brain responses[20,54]. Functional and structural brain imaging studies have recently demonstrated that increased perception of chronic musculoskeletal pain is associated with altered activity of neural networks[3,4] and grey matter thickness of several cortical regions[1,51].

The activation of cortical networks during evoked pain results in changes of the neural oscillations at different frequencies, such as theta (4–8 Hz)[12,36,49], alpha (8–13 Hz)[2,8,9,11], beta (13–29 Hz)[8,36,49], and gamma (30–100 Hz)[36,43,49]. Within those frequencies, average power in the alpha-band is probably the most explored in pain research[2,8,9,11,36,49]. Recently, the peak alpha frequency (PAF), defined as the 'center frequency' of that bandwidth[21], has attracted attention due to the stability of the measure over time (months)[21–23]. While evidence on changes of the parasagittal PAF, also named central PAF, have been reported during cutaneous pain as the target tissue using heat-capsaicin[21,22] pain and thermal pain[22,40,41], deep somatic tissue pain has received less attention[23]. Classical methods to experimentally induce muscle pain include intramuscular injection of hypertonic saline[9] and capsaicin[10] characterized by the short-lasting activity of nociceptors. Contrarily, long-lasting muscle pain models, based on nerve growth factor (NGF) injections or unaccustomed exercise–inducing delayed-onset muscle soreness (DOMS), mimic typical behavior of myofascial pain syndrome by sensitizing nociceptors[30,35,44] up to 21 days[26,33]. Importantly, muscle pain induced by DOMS and NGF typically appears with some delay (around 6-12 hours), reaches the pain peak after 24-48 hours, and disappears within 3–7 days[6,52]. DOMS and NGF-induced pain models do not also produce any spontaneous muscle pain, but mechanical pressure to the muscle belly or muscle contraction excites the sensitized nociceptors[26,52] evoking pain. Although a recent study has found no central PAF changes during NGF-induced pain[23], this study had explored brain oscillation at rest when ongoing muscle pain was absent. Therefore, it is unknown whether contraction-induced muscle pain may have a suppressive effect on the central PAF, as shown after applying intramuscular injections of hypertonic saline[9] and capsaicin[10].

Individual differences in pain sensitivity obtained under identical instructions and conditions of stimulation have been reported in healthy participants[14]. Whereas some individuals perceive a sensory input as intensely painful, others perceive the same event as only slightly painful[12,15,39]. For this reason, pain is considered as a subjective experience more related to affective and cognitive factors[39] than linked to the peripheral nociceptive input. Although the activation of a diverse array of brain regions can predict perceived pain intensity within a given person[16,57], it is much more challenging to predict pain sensitivity across different individuals[27]. Several studies have explored brain patterns associated with individuals' pain sensitivity using brain imaging MRI[15,18] and electroencephalography (EEG)[12,21,27,28]. However, most of the previous findings were inconclusive, especially concerning alpha brain oscillations. Recently, depending on the analytical approach and the pain model, several researchers have shown that PAF was found to reflect[21,22,41] or not reflect[55] pain sensitivity across individuals. Moreover, both positive[41] and negative[21,22] correlations between PAF and pain intensity have been reported. Collectively, it is still unclear whether PAF can be considered a neural indicator of perceptual variability of pain across individuals.

The objectives of this study were to investigate whether high pain sensitive (HPS) individuals showed a more evident reduction of central PAF than low pain sensitive (LPS) individuals during days with contraction-evoked muscle pain and whether slower central PAF reflects higher pain sensitivity across individuals.

2. METHODS

2.1 Participants

This study is based on unpublished secondary data from a study in which the primary electrophysiological data has been published[33]. The recruitment and data collection have been conducted from November 2017 and January 2018 at the Center for Neuroplasticity and Pain (CNAP), Aalborg University (Denmark). Twenty-four healthy right-handed subjects (14 females) participated in the study, recruited through online advertising and flyers posted at Aalborg University. All subjects had no upper and lower limb pain conditions, spine pain, and neurological or other major medical disorders. Furthermore, exclusion criteria were any psychiatric disorders and a complaint of sleep disorders. The sample size estimates were based on primary outcomes (cortical motor map)[33,48]. The study was performed according to the Helsinki Declaration, approved by the local ethics committee (N-20160022), and registered at ClinicalTrials.gov (NCT03354624). Written informed consent was obtained before study commencement.

2.2 EEG data collection

The study comprised four sessions over six days (Figure 1). On Day0, Day4, and Day6, surface EEG was collected. The time of data collection was kept consistent across days since fluctuations in circadian rhythms could impact EEG recordings. On Day2, neurophysiological testing was not performed because no cortical excitability changes were found affected in a previous study[48]. Sixtytwo electrodes in an EEG-cap were used (g.GAMMA cap2, Schiedlberg, Austria), labeled according to a 10-20 system with Cz orientated to the vertex of the head[42]. The ground electrode was placed halfway between the eyebrows, and all electrodes were referred to as an electrode placed on the right earlobe. The impedance was maintained below five kΩ throughout the data collection. Unfiltered EEG signals were amplified (50000x) and sampled at 2400 Hz (g.HIamp biosignal amplifier; g.tec-medical engineering GmbH, Schiedlberg, Austria). Once the EEG set-up was complete, participants were seated in a comfortable armchair in a quiet, semi-darkened room. A pillow around the neck was used to minimize the contraction of the neck muscles. The participants were instructed to keep their eyes closed during the continuous EEG recording, remain still, and relax without falling asleep. Two tasks were sequentially recorded: 1) three minutes with the right hand and forearm in pronation supported on a platform (resting-state condition), 2) three minutes in maximal wrist extension, holding 1.3 kg weight with the forearm in pronation supported on a platform (contraction-state condition). EEG was recorded during muscle contraction causing wrist extension. Based on a previous study, a 1.3 Kg weight was selected because this load represented around ten percent of the MVC in a healthy young population (see De Martino et al.[32]). 10% of MVC was selected because it is similar to the amount of force needed for most of the daily activities of the hands[6], and previous studies indicated that this level of contraction of wrist extensors did not produce the onset of forearm muscle fatigue[6].

2.3 Muscle pain models

On Day0, Day2, and Day4, participants received an NGF injection (5µg/0.5 mL) into the right extensor carpi radialis brevis (ECRB) muscle to induce muscle hyperalgesia. On Day0 and Day4, NGF was injected 30 minutes after the EEG recording. On Day 4, eccentric exercise was performed after the EEG recording but before the injection of NGF. Sterile solutions of recombinant human Beta-NGF were prepared by the pharmacy (Skanderborg Apotek, Denmark) and injected into the muscle belly of ECRB under real-time ultrasound guidance (SonoSite M-Turbo, FUJIFILM SonoSite, USA). To induce additional muscle pain, on Day4, a subgroup of twelve randomly selected participants performed a high-intensity eccentric exercise to cause delayed onset muscle soreness (DOMS) on the right wrist extensor muscles before receiving the NGF injection. Eccentric contractions of the right hand were performed from a maximally extended wrist position to a maximally flexed wrist position with a duration of at least 4 seconds (max weight 25 kg). Sets of five repetitions were separated by an approximately 1-min rest period. The exercise was repeated until the participant could not control the eccentric contraction over 4 s (for more details about the pain models, see De Martino et al.[33]).

2.4 Peak alpha frequency

From each of the six EEG recordings (resting-state condition and contraction-state condition at Day0, Day4, and Day6), the PAF was extracted by a procedure described previously[21]. The main steps of the analysis are shown in Figure 2. The data processing of EEG data was done using EEGLAB 19.1[17] and FieldTrip[42]. First, band-pass filtering between 5 and 16 Hz (function 'eegnewfilt') was applied, after which the independent component analysis was applied[5], and 62 independent components (ICs) were obtained (square matrix), which were based on statistically independent sources, not single electrodes. The obtained matrix for each Day was then applied to the corresponding unfiltered EEG data, resulting in a component that retained broadband spectral content. The IC located in the central region was identified and stored for further analysis (Figure 3). The frequency-spectra of the selected IC was performed to confirm the presence of relevant brain activity. The data was segmented into 5-s epochs, and power spectral density in the 2–40 Hz range was derived for each epoch in 0.2 Hz bins using the 'ft_freqanalysis_mtmfft' function. For each 5-second epoch from the segmentation of the 3-minute EEG recording, the PAF was estimated using a center of gravity (CoG) method previously described[21]. Briefly, CoG was defined as follows:

$$CoG = \frac{\sum_{i=1}^{n} Fi * Ai}{\sum_{i=1}^{n} \mathrm{Ai}}$$

F_i is the ith frequency bin including and above 9 Hz, *n* is the number of frequency bins between 9 and 11 Hz, and A_i the spectral amplitude for F_i[21]. Peak alpha frequency was estimated for the central alpha components for every 5 s epoch and then averaged[21]. The frequency decomposition of the component data was performed using the routines in FieldTrip.

In addition to the central PAF, occipital PAF was also extracted to investigate whether the central PAF changes could represent a localized activity of the sensorimotor region or a widespread alpha-wave effect. The activity over the central cortex was previously characterized by combinations of two rhythms in an 8-12 Hz frequency band: a widespread rhythm alpha and a localized mu rhythm[38].

2.5 High and low pain-sensitive groups

The pain intensity was assessed on an 11-point numerical rating scale (NRS), where 0 defined 'no pain," and 10 was the 'most intense pain imaginable.' Immediately after resting-state and contraction-state EEG recordings, participants indicated the pain intensity on the 11-point NRS by being asked to: "Rate the average amount of pain in your forearm during the task." The participants were separated into LPS and HPS groups by performing a split based on the average pain NRS scores across Day0, Day4, and Day6 during the contraction-state condition. Participants below 2 on the average pain NRS were considered LPS, while equal and higher than 2 on the average NRS were considered HPS. The NRS score of 2 was based on the type of pain models used in the current study. NGF and eccentric contractions-inducing muscle pain in the wrist extensor muscles only produce moderate pain (although multiple NGF injections and eccentric contractions-inducing muscle pain), with an average between 2 and 4 (SD = 2)[6,48,52].

2.6 Statistical analysis

Statistical analysis was done in Statistical Package for Social Sciences (SPSS; Version 25, IBM, Chicago, IL, USA). All data are presented as the mean and standard deviation (SD). Statistical significance was set at $P < 0.05$. Measurements from all assessments were normality-tested using visual inspection (histograms and Q–Q plots). Accordingly, pain NRS scores, central and occipital PAF were analyzed by two-way repeated-measures analyses of variance (RM ANOVA) with Time (Day0, Day4, and Day6) as the within-subject factor, and Group (HPS and LPS) as the between-group factor. When necessary, the Greenhouse-Geisser correction was used to correct for non-sphericity. Post hoc analyses were performed using Bonferroni multiple comparison tests (with corresponding confidence intervals generated).

Spearman's rank correlation between the average pain NRS scores across days and the central PAF at Day0 was used to assess whether central PAFs recorded at Day0 (before pain model) correlated with pain intensity. Furthermore, to investigate the relationship between PAFs recorded at Day6 and the pain intensity reported by the participants at Day6, correlation analyses were applied. The significance of multiple correlation analyses was Bonferroni corrected by two comparisons.

3. RESULTS

3.1 Muscle pain intensity in LPS and HPS groups

The application of DOMS at Day4 on a sensitize muscle in 12 individuals did not provoke any additional muscle pain during the 3-minute muscle contraction, and it was not considered in the statistical model. Ten participants fulfilled the criteria being included in the LPS group (six subjects received only NGF and did not perform the eccentric exercise to induce DOMS) and fourteen participants in the HPS group (six subjects received only NGF). Demographics of the two groups is shown in Table 1. The average pain NRS score across days was 0.8±0.5 in the LPS group and 3.1±0.8 in the HPS group (Figure 4). During resting-state condition, none of the participants reported any pain NRS scores above 0. During contraction-state condition, the ANOVA revealed a main effect of Time ($F_{2,44}$ = 16.59, P < 0.001, η^2 = 0.43), Group ($F_{2,22}$ = 58.15, P < 0.001, η^2 = 0.73) and an interaction ($F_{2,44}$ = 3.67, P = 0.034; η^2 = 0.14). Pairwise contrasts showed an increase of 1.9 (CI 95% [1.0 2.9], P < 0.001) in pain NRS scores in the HPS group between Day0 and Day4, and of 2.3 (CI 95% [1.3 3.2], P < 0.001) between Day0 and Day6 (Figure 4). LPS did not show any significant increase in pain intensity between Day0 and Day4 (CI 95% [-0.3 1.9], P = 0.179) and between Day0 and Day6 (CI 95% [-0.4 1.9], P = 0.296). Moreover, higher pain NRS scores were found in the HPS group compared with LPS group at Day0 (CI 95% = 0.5 2.3], P = 0.003), Day4 (CI 95% [1.4 3.5], P < 0.001), and Day6 (CI 95% [2.1 3.8], P < 0.001).

3.2 Central and occipital PAF over days in LPS and HPS groups

During the resting-state condition, main effects of Time (Figure 5A; $F_{2,44}$ = 3.27, P = 0.047, η^2 = 0.13) and Group ($F_{1,22}$ = 4.76, P = 0.040, η^2 = 0.18) were found for the central PAF. By contrast, a significant Time x Group interaction was not found ($F_{2,44}$ = 2.04, P = 0.142, η^2 = 0.14). However, pairwise contrasts did not show any significant change in the resting-state central PAF from Day0 to Day4 (CI 95% [-0.09 0.01], P = 0.078) and from Day0 to Day6 (CI 95% [-0.09 0.02], P = 0.285). In contrast, the resting-state central PAF across all days was faster in the HPS group compared with the LPS group (CI 95% [0.01 0.23]).

During the contraction-state condition, main effects of Time (Figure 5B; $F_{2,44}$ = 6.61, P = 0.007, η^2 = 0.20) and Group ($F_{1,22}$ = 17.90, P < 0.001, η^2 = 0.45) were found for central PAF. By contrast, a significant Time x Group interaction was not found ($F_{2,44}$ = 0.99, P = 0.377, η^2 = 0.04). Pairwise contrasts in the contraction-state central PAF showed a decrease from Day0 to Day6 (CI 95% [-0.01 -0.12], P = 0.011) and faster PAF in the HPS group compared with LPS group across time points (CI 95% [0.11 0.31]).

3.3 Occipital PAF over days in LPS and HPS groups

During the resting-state condition, the ANOVA showed a main effect of Group (Figure 6A; $F_{1,22}$ = 7.76, P = 0.011, η2 = 0.26) for the occipital PAF, without any main effects of Time ($F_{2,44}$ = 1.03, P = 0.366, η2 = 0.05) and interaction ($F_{2,44}$ = 1.96, P = 0.153, η2 = 0.08). The resting-state occipital PAF across all days was faster in the HPS group compared with the LPS group (CI 95% [0.05 0.31).

During the contraction-state condition, the ANOVA revealed a main effects of Time (Figure 6B; $F_{2,44} = 3.98$, $P = 0.026$, $\eta^2 = 0.15$) and Group ($F_{1,22} = 14.99$, $P < 0.001$, $\eta^2 = 0.41$) for the occipital PAF, without a significant interaction ($F_{2,44} = 0.09$, $P = 0.991$, $\eta^2 = 0.00$). Pairwise contrasts in the contraction-state occipital PAF showed a decrease from Day0 to Day6 (CI 95% [-0.00 -0.15], P = 0.040) and faster PAF in the HPS group compared with LPS group across time points (CI 95% [0.12 0.39]).

3.4 Correlation between central PAF and pain

The average pain NRS score during the contraction-state condition was associated with central PAF during contractions at Day0 (Figure 7A; Spearman R = 0.544; P = 0.012; Bonferroni corrected). Similarly, at Day4 and Day6, pain NRS scores during the contraction-state condition were associated with central PAF during the contraction-state condition (Figure 7B, Day4 Spearman R = 0.487; P = 0.032; Figure 7C, Day6 Spearman R = 0.494; P = 0.028; both Bonferroni corrected). By contrast, no correlations were found during resting-state condition between average pain NRS scores central PAF at Day0 (Spearman R = 0.147; P = 1.00; Bonferroni corrected), at Day4 (Spearman R = 0.231; P = 0.556; Bonferroni corrected), at Day6 (Spearman R = 0.225; P = 0.580; Bonferroni corrected).

4. DISCUSSION

The present study investigated how the central PAF adaptations were associated with prolonged muscle pain in HPS and LPS individuals during resting-state and contraction-state conditions. The central PAF was slowed during the contractions causing muscle pain across days, but no difference was detected between more or less pain-sensitive individuals. As the central PAF, occipital PAF was slowed during contraction-state conditions on Day6, suggesting a widespread alpha-wave effect. Surprisingly, HPS individuals showed faster central and occipital PAF than LPS individuals during resting-state and contraction-state conditions. Furthermore, a positive correlation between pain intensity and central PAF was also detected during contraction-state conditions either before or during muscle pain.

4.1 Reduced PAF during ongoing muscle pain

During the contraction-state condition (ongoing muscle pain), this study demonstrated that central PAF slowed by 0.07±0.10 Hz after six days of muscle pain. However, no interactions were found during muscle pain, suggesting that this brain oscillation reduction does not reflect the increased subjective report of pain during contraction-evoking muscle pain. The PAF reduction during pain in the current study agrees with some of the previous experimental pain studies, which mostly showed decreased amplitude or peak frequency slowing of alpha oscillations during cutaneous tonic pain[13,19,21,40,43,49]. However, a few studies failed to show any alpha band changes[12,41,55], or they found an increased power[2], probably due to methodological differences, such as EEG data recordings or data processing.

Compared to other experimental pain models, an essential feature of the present study is NGF- and DOMS-induced muscle pain reflects manifestations also seen in clinical musculoskeletal pain. Both pain models induce clinical characteristics of myofascial pain syndrome, and identical neurotrophic substances are likely involved in this syndrome[26,34,37]. Although speculative, we can hypothesize that people affected by myofascial pain syndrome may have temporary PAF slowing during ongoing muscle pain, similar to what has been described in the current study. Whether prolonged myofascial pain (months or years) may provoke some maladaptive neuroplastic changes in the cortical area remains unknown. Based on cross-sectional studies, patients suffering from chronic neuropathic pain conditions showed PAF slowing relative to matched healthy individuals[47,56], and it has been hypothesized that PAF slowing contributes to the generation of pathological pain, perhaps reflecting thalamocortical dysrhythmia[46,47]. Although the underlying neural structure generating the widespread alpha-wave rhythm is controversial, the alpha waves seem to act within the nervous system by propagating from higher-order to lower-order cortical areas (i.e., in the somatosensory cortex, alpha waves propagate from associative regions toward the primary cortex), and from the cortex to the thalamus[25].

A second feature in the current study compared to previous studies is the long-lasting muscle pain duration. While short-lasting pain models have shown increased functional activity in the sensorimotor area during tonic[24,45] and phasic pain[7], eight consecutive days of thermode-induced heat pain have demonstrated an increased grey matter volume in regions involved in processing nociceptive information, including midcingulate or somatosensory cortex[53]. These differences were no longer detectable one year after, indicating that pain-related structural changes can be experimentally induced in a few days, and they reversed after noxious stimulation[53]. Considering that central PAF slowing in the present study was only detected six days after the NGF injection, this may indicate that alpha oscillation changes may underpin some structural reorganization in the sensorimotor cortex due to muscle pain over several days. However, this hypothesis requires an appropriate investigation.

Finally, during the resting-state condition, the present central PAF showed a reduction of 0.03±0.10Hz, which was insufficient to reach a statistical difference. These findings agree with a previous study using a similar muscle pain model, which did not reveal any significant PAF changes[23]. The absence of ongoing pain during the resting-state condition may explain the absence of a robust reduction in central PAF since ongoing nociceptive inputs are likely needed to reveal brain excitability adaptations. Alternatively, prolonged duration of muscle pain is required to detect PAF changes at rest.

4.2 Faster PAF in HPS individuals

HPS individuals showed faster central PAF than LPS individuals before and during muscle pain in the current study. A similar correlation was found on day 4 and day 6, suggesting that the application of exercise-inducing DOMS on day 4 did not modify these associations. Several studies have investigated PAF and subjective perception of pain, reporting contrasting results. A previous study described a positive correlation between PAF and pain NRS score[41] as confirmed by the current research, while others found a negative correlation[21,22] or no correlation[55]. In addition to several differences between the studies (i.e., different pain modalities, a diverse range of self-reported pain, reliability of self-reported pain across days), EEG data processing and alpha wave characteristics may help explain these partially divergent findings. Furman et al.[22] presented the relationship between pain sensitivity and power at smaller frequency bins within the alpha range (8-12Hz). They demonstrated that slower (8–9.5 Hz) components were positively associated with pain sensitivity, while faster (10.5–12 Hz) components were negatively associated with pain sensitivity. These results may indicate that minor differences in frequency elements within the alpha range can produce apparent opposite results. This observation has an important practical implication for future study design. If central PAF will be proposed as a reliable biomarker of prolonged pain sensitivity with the potential for prospectively identifying pain sensitivity in clinic settings[22], there is a need for unified methods.

The present results confirmed that HPS and LPS showed a different brain response to the same nociceptive stimuli during muscle pain. A similar dichotomy response to experimental pain has been observed in several studies by applying fMRI and EEG[12,15,18,27,28,50]. Although still unclear, this dichotomic difference in brain activity among individuals before inducing pain may indicate cognitive self-regulation or anxiety/fear response to pain.

It is important to note that several individuals, particularly in the HPS group, reported muscle pain at Day0 after the 3 min contraction before receiving the first injection of NGF. Although the weight selected in the current study was light (~10% MVC) to avoid muscle pain or fatigue at day0, a 3-minute tonic contraction may be sufficient to decrease the intramuscular pH and, consequently, produced acidification of the muscle environment. Tissue acidosis may activate chemo-sensitive channels located on the nociceptors[29,58], resulting in mild muscle pain in pain-sensitive individuals. Considering that the alpha responses are easily influenced by attention[43], it is also possible that higher muscle pain on the right forearm before and during muscle pain was provoked by attention changes towards the stimulated territory.

4.3 Limitations

There are several limitations to the current study. A heavier load (>10% MVC) likely increases the muscle pain intensity resulting in a more evident PAF slowing. However, considering that DOMS on wrist extensor muscles provokes a reduction of 15-25% MVC[31–33], it was predicted that a heavier load could interfere with the 3-minute EEG recording. Furthermore, facial muscle contractions, typically associated with intense efforts, could alter our EEG recording. A second limitation is the co-contraction of the flexor digitorum muscles, recruited to hold the weight during wrist extension (finger flexion for gripping). A third limitation is the absence of a control group. However, the study aimed to investigate the PAF changes during movement-evoked pain in a sensitized muscle and the difference across individuals. The LPS group may also be regarded as an even better control condition since they are exposed to similar experimental provocations. The absence of pain-free muscle contraction is also a limitation, but we did not expect that high-sensitive pain participants reported muscle pain during a steady contraction at 10% of the MVC for 3 minutes before receiving the first injection of NGF.

Although the current study selected to focus on the central and occipital regions given previous results[21–23], PAF is a stable measure over days or weeks[22], and it is not restricted to this region but could be observed at almost all EEG sensors [23]. Based on previous findings[21–23], the current study only focused on the central and occipital PAF analysis. However, the amplitude of the alpha wave has also been associated with skin pain intensity[40], and future studies should also investigate whether alpha power is affected by muscle pain. Moreover, IC's selection was restricted to single components, whereas the widespread alpha frequency may be made up of several different ICs. Importantly, results for both analyses were unchanged when PAF was calculated using the occipital IC. However, caution is recommended when comparing the current results with published literature applying different IC calculations. Finally, PAF can be affected by several factors (e.g., age, gender, mood, sleep quality). Although the present study was designed to limit confounding factors by recruiting homogenous participants, these cannot be excluded entirely.

4.4 Conclusion

This study provides new evidence of central PAF alteration associated with ongoing muscle pain. The reduction of central PAF induced by muscle pain over several days could be interpreted as an adapted cortical integration of nociceptive inputs from the sensitized tissue. More pain-sensitive individuals showed faster central PAF than less pain-sensitive individuals during muscle pain, which may reflect a different cognitive or emotional response to muscle pain across individuals.

FIGURE LEGENDS

Figure 1: Electrophysiological outcome measures were assessed at the beginning of each experimental session on Day 0, Day 4, and Day 6 during two conditions: Resting-state and contraction-state. On Day 0, Day 2, and Day 4, these measures were followed by injection of NGF to the right extensor carpi radialis brevis. On Day 4, a subgroup of twelve randomly selected participants performed eccentric exercise before receiving the NGF injection.

Figure 2: Diagram of the PAF extraction. After filtering raw data, an independent component analysis (ICA) was performed, followed by the extraction of the corresponding IC weights.

Figure 3: The figure shows the main steps of analysis with ICs recorded during EEG from a representative participant. The IC localized in the central (A) and occipital (B) regions were visually selected.

Figure 4: Pain numerical rating scale (NRS) scores at Day0, Day4, Day6, and average over days (Day0, Day4, and Day6) for participants in the high pain sensitive (HPS, N = 14) or low pain sensitive (LPS, N = 10) group. Open circles represent an individual NRS score, the group mean is a filled square, and the standard deviation is vertical lines. Significantly higher pain NRS scores in the HPS compared with LPS group or compared with Day0 (*, P<0.05).

Figure 5: Central PAF (peak alpha frequency) at Day0, Day4, and Day6 for participants in the high pain sensitive (HPS, N = 14) or low pain sensitive (LPS, N = 10) group in the resting-state condition (A) and contraction-state condition (B). Open circles represent individual PAF results, the group mean is a filled square, and the standard deviation is vertical lines. Significantly higher PAF in the HPS compared with LPS group (#, P<0.05) or compared with Day0 (*, P<0.05).

Figure 6: Occipital PAF (peak alpha frequency) at Day0, Day4, and Day6 for participants in the high pain sensitive (HPS, N = 14) or low pain sensitive (LPS, N = 10) group in the resting-state condition (A) and contraction-state condition (B). Open circles represent individual PAF results, the group mean is a filled square, and the standard deviation is vertical lines. Significantly higher PAF in the HPS compared with LPS group (#, P<0.05) or compared with Day0 (*, P<0.05).

Figure 7: A) Correlations between average pain NRS scores (across Day0, Day4, and Day6) and central PAF during contraction-state condition on Day0. B) Correlations between pain NRS scores at Day4 and central PAF during contraction-state condition at Day4. C) Correlations between pain NRS scores at Day6 and central PAF during contraction-state condition at Day6. Grey shaded area indicates 95% confidence intervals, and the dashed line is the linear trendline.

TABLE

Table 1: Demographics of the High-Pain Sensitive (HPS) and Low-Pain Sensitive (LPS) groups.

REFERENCES

1. Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR: Chronic Back Pain Is Associated with Decreased Prefrontal and Thalamic Gray Matter Density. 24:10410–5, 2004.
2. Backonja M, Howland EW, Wang J, Smith J, Salinsky M, Cleeland CS: Tonic changes in alpha power during immersion of the hand in cold water. Electroencephalogr Clin Neurophysiol 79:192–203, 1991.
3. Baliki MN, Apkarian AV: Nociception, pain, negative moods and behavior selection. Neuron 87:474–91, 2015.
4. Baliki MN, Chialvo DR, Geha PY, Levy RM, Harden RN, Parrish TB, Apkarian AV: Chronic Pain and the Emotional Brain: Specific Brain Activity Associated with Spontaneous Fluctuations of Intensity of Chronic Back Pain. J Neurosci 22:12165–12173, 2006.
5. Bell AJ, Sejnowski TI: An Information-Maximization Approach to Blind Separation and Blind Deconvolution. Neural Comput 7:1129–59, 1995.
6. Bergin MJG, Hirata R, Mista C, Christensen SW, Tucker K, Vicenzino B, Hodges P, Graven-Nielsen T: Movement Evoked Pain and Mechanical Hyperalgesia after Intramuscular Injection of Nerve Growth Factor: A Model of Sustained Elbow Pain. Pain Med (United States) 16:2180–91, 2015.
7. Bornhövd K, Quante M, Glauche V, Bromm B, Weiller C, Büchel C: Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: A single-trial fMRI study. Brain 125:1326–36, 2002.
8. Chang PF, Arendt-Nielsen L, Chen ACN: Dynamic changes and spatial correlation of EEG activities during cold pressor test in man. Brain Res Bull 57:667–75, 2002.
9. Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Chen ACN: Psychophysical and EEG responses to repeated experimental muscle pain in humans: Pain intensity encodes EEG activity. Brain Res Bull 59:533–43, 2003.
10. Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN: Different EEG topographic effects of painful and non-painful intramuscular stimulation in man. Exp Brain Res 141:195–203, 2001.
11. Chang PF, Arendt-Nielsen L, Graven-Nielsen T, Svensson P, Chen ACN: Topographic effects of tonic cutaneous nociceptive stimulation on human electroencephalograph. Neurosci Lett 305:49–52, 2001.
12. Chen ACN, Dworkin SF, Haug J, Gehrig J: Topographic brain measures of human pain and pain responsivity. Pain 37:129–41, 1989.
13. Chen ACN, Rappelsberger P: Brain and Human pain: Topographic EEG amplitude and coherence mapping. Brain Topogr 7:129–40, 1994.
14. Clark JW, Bindra D: Individual differences in pain thresholds. Can J Psychol 10:69–76, 1956.
15. Coghill RC, McHaffie JG, Yen YF: Neural correlates of interindividual differences in the subjective experience of pain. Proc Natl Acad Sci U S A 100:8538–42, 2003.
16. Coghill RC, Sang CN, Maisog JMA, Iadarola MJ, Robert C, Sang CN, Maisog JM: Pain Intensity Processing Within the Human Brain : A Bilateral , Distributed Mechanism. J Neurophysiol 82:1932–43, 1999.
17. Delorme A, Makeig S: EEGLAB : an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 134:9–21, 2004.
18. Emerson NM, Zeidan F, Lobanov O V., Hadsel MS, Martucci KT, Quevedo AS, Starr CJ, Nahman-Averbuch H, Weissman-Fogel I, Granovsky Y, Yarnitsky D, Coghill RC: Pain sensitivity is inversely related to regional grey matter density in the brain. Pain [Internet] International Association for the Study of Pain; 155:566–73, 2014. Available from: http://dx.doi.org/10.1016/j.pain.2013.12.004

19. Ferracuti S, Seri S, Mattia D, Cruccu G: Quantitative EEG modifications during the cold water pressor test: hemispheric and hand differences. Int J Psychophysiol 17:261–8, 1994.
20. Flor H, Braun C, Elbert T, Birbaumer N: Extensive reorganization of primary somatosensory cortex in chronic back pain patients. Neurosci Lett 224:5–8, 1997.
21. Furman AJ, Meeker TJ, Rietschel JC, Yoo S, Muthulingam J, Prokhorenko M, Keaser ML, Goodman RN, Mazaheri A, Seminowicz DA: Cerebral peak alpha frequency predicts individual differences in pain sensitivity. Neuroimage [Internet] Elsevier Ltd; 167:203–10, 2018. Available from: https://doi.org/10.1016/j.neuroimage.2017.11.042
22. Furman AJ, Prokhorenko M, Keaser ML, Zhang J, Chen S, Mazaheri A, Seminowicz DA: Sensorimotor Peak Alpha Frequency Is a Reliable Biomarker of Prolonged Pain Sensitivity. Cereb Cortex :1–14, 2020.
23. Furman AJ, Thapa T, Summers SJ, Cavaleri R, Fogarty JS, Steiner GZ, Schabrun SM, Seminowicz DA: Cerebral peak alpha frequency reflects average pain severity in a human model of sustained, musculoskeletal pain. J Neurophysiol 122:1784–93, 2019.
24. Gelnar PA, Krauss BR, Sheehe PR, Szeverenyi NM, Apkarian AV: A comparative fMRI study of cortical representations for thermal painful, vibrotactile, and motor performance tasks. Neuroimage 10:460–82, 1999.
25. Halgren M, Ulbert I, Bastuji H, Fabó D, Eross L, Rey M, Devinsky O, Doyle WK, Mak-McCully R, Halgren E, Wittner L, Chauvel P, Heit G, Eskandar E, Mandell A, Cash SS: The generation and propagation of the human alpha rhythm. Proc Natl Acad Sci U S A 116:23772–82, 2019.
26. Hayashi K, Shiozawa S, Ozaki N, Mizumura K, Graven-Nielsen T: Repeated intramuscular injections of nerve growth factor induced progressive muscle hyperalgesia, facilitated temporal summation, and expanded pain areas. Pain [Internet] International Association for the Study of Pain; 154:2344–52, 2013. Available from: http://dx.doi.org/10.1016/j.pain.2013.07.007
27. Hu L, Iannetti GD: Neural indicators of perceptual variability of pain across species. PNAS 29:1782–91, 2018.
28. Huang G, Xiao P, Hung YS, Zhang ZG, Hu L: A novel approach to predict subjective pain perception from single-trial laser-evoked potentials. Neuroimage 81:283–93, 2013.
29. Ikeuchi M, Kolker SJ, Burnes LA, Walder RY, Sluka KA: Role of ASIC3 in the primary and secondary hyperalgesia produced by joint inflammation in mice. Pain 137:662–9, 2008.
30. Inoue A, Iwasa M, Nishikura Y, Ogawa S, Nakasuka A, Nakata Y: The long-term exposure of rat cultured dorsal root ganglion cells to bradykinin induced the release of prostaglandin E2 by the activation of cyclooxygenase-2. Neurosci Lett 401:242–7, 2006.
31. Leger a B, Milner TE: Muscle function at the wrist after eccentric exercise. Med Sci Sports Exerc [Internet] 33:612–20, 2001. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11283438
32. De Martino E, Petrini L, Schabrun SM, Graven-Nielsen T: Cortical Somatosensory Excitability Is Modulated in Response To Several Days of Muscle Soreness. J Pain [Internet] 19:1296–307, 2018. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1526590018301925
33. De Martino E, Zandalasini M, Schabrun S, Petrini L, Graven-Nielsen T: Experimental Muscle Hyperalgesia Modulates Sensorimotor Cortical Excitability, Which is Partially Altered by Unaccustomed Exercise. Pain [Internet] :1, 2018. Available from: http://insights.ovid.com/crossref?an=00006396-900000000-98889
34. Murase S, Terazawa E, Hirate K, Yamanaka H, Kanda H, Noguchi K, Ota H, Queme F, Taguchi T, Mizumura K: Upregulated glial cell line-derived neurotrophic factor through cyclooxygenase-2 activation in the muscle is required for mechanical hyperalgesia after exercise in rats. J Physiol 591:3035–48, 2013.

35. Murase S, Terazawa E, Queme F, Ota H, Matsuda T, Hirate K, Kozaki Y, Katanosaka K, Taguchi T, Urai H, Mizumura K: Bradykinin and Nerve Growth Factor Play Pivotal Roles in Muscular Mechanical Hyperalgesia after Exercise (Delayed-Onset Muscle Soreness). J Neurosci [Internet] 30:3752–61, 2010. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.3803-09.2010
36. Nickel MM, Ta Dinh S, May ES, Tiemann L, Hohn VD, Gross J, Ploner M: Neural oscillations and connectivity characterizing the state of tonic experimental pain in humans. Hum Brain Mapp 41:17–29, 2020.
37. Nie H, Madeleine P, Arendt-Nielsen L, Graven-Nielsen T: Temporal summation of pressure pain during muscle hyperalgesia evoked by nerve growth factor and eccentric contractions. Eur J Pain [Internet] European Federation of Chapters of the International Association for the Study of Pain; 13:704–10, 2009. Available from: http://dx.doi.org/10.1016/j.ejpain.2008.06.015
38. Niedermeyer E: The Normal EEG of the Waking Adult. Eds EN& FL da S, editor. Baltimore: Electroencephal- ography: Basic Principles, Clinical Applications and Related Fields. Lippincott Williams & Wilkins,; 1999.
39. Nielsen CS, Staud R, Price DD: Individual Differences in Pain Sensitivity: Measurement, Causation, and Consequences. J Pain [Internet] Elsevier Ltd; 10:231–7, 2009. Available from: http://dx.doi.org/10.1016/j.jpain.2008.09.010
40. Nir RR, Sinai A, Moont R, Harari E, Yarnitsky D: Tonic pain and continuous EEG: Prediction of subjective pain perception by alpha-1 power during stimulation and at rest. Clin Neurophysiol [Internet] International Federation of Clinical Neurophysiology; 123:605–12, 2012. Available from: http://dx.doi.org/10.1016/j.clinph.2011.08.006
41. Nir RR, Sinai A, Raz E, Sprecher E, Yarnitsky D: Pain assessment by continuous EEG: Association between subjective perception of tonic pain and peak frequency of alpha oscillations during stimulation and at rest. Brain Res [Internet] Elsevier B.V.; 1344:77–86, 2010. Available from: http://dx.doi.org/10.1016/j.brainres.2010.05.004
42. Oostenveld R, Praamstra P: The five percent electrode system for high-resolution EEG and ERP measurements. Clin Neurophysiol 112:713–9, 2001.
43. Peng W, Hu L, Zhang Z, Hu Y: Changes of spontaneous oscillatory activity to tonic heat pain. PLoS One 9:1–11, 2014.
44. Pethö G, Reeh PW: Sensory and signaling mechanisms of bradykinin, eicosanoids, platelet-activating factor, and nitric oxide in peripheral nociceptors. Physiol Rev 92:1699–775, 2012.
45. Di Piero V, Ferracuti S, Sabatini U, Pantano P, Cruccu G, Lenzi GL: A cerebral blood flow study on tonic pain activation in man. Pain 56:167–73, 1994.
46. Ploner M, Sorg C, Gross J: Brain Rhythms of Pain. Trends Cogn Sci [Internet] Elsevier Ltd; 21:100–10, 2017. Available from: http://dx.doi.org/10.1016/j.tics.2016.12.001
47. Sarnthein J, Stern J, Aufenberg C, Rousson V, Jeanmonod D: Increased EEG power and slowed dominant frequency in patients with neurogenic pain. Brain 129:55–64, 2006.
48. Schabrun SM, Christensen SW, Mrachacz-Kersting N, Graven-Nielsen T: Motor Cortex Reorganization and Impaired Function in the Transition to Sustained Muscle Pain. Cereb Cortex 26:1878–90, 2016.
49. Schulz E, May ES, Postorino M, Tiemann L, Nickel MM, Witkovsky V, Schmidt P, Gross J, Ploner M: Prefrontal gamma oscillations encode tonic pain in humans. Cereb Cortex 25:4407–14, 2015.
50. Schulz E, Tiemann L, Schuster T, Gross J, Ploner M: Neurophysiological coding of traits and states in the perception of pain. Cereb Cortex 21:2408–14, 2011.
51. Seminowicz, Wideman TH, Naso L, Hatami-Khoroushahi Z, Fallatah S, Ware MA, Jarzem P, Bushnell MC, Shir Y, Ouellet JA, Stone LS: Effective Treatment of Chronic Low Back Pain in

Humans Reverses Abnormal Brain Anatomy and Function. J Neurosci [Internet] 31:7540–50, 2011. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.5280-10.2011
52. Slater H, Arendt-Nielsen L, Wright A, Graven-Nielsen T: Experimental deep tissue pain in wrist extensors - A model of lateral epicondylalgia. Eur J Pain 7:277–88, 2003.
53. Teutsch S, Herken W, Bingel U, Schoell E, May A: Changes in brain gray matter due to repetitive painful stimulation. Neuroimage 42:845–9, 2008.
54. Tsao H, Danneels L, Hodges P: ISSLS Prize Winner: Smudging the Motor Brain in Young Adults With Recurrent Low Back Pain. Spine (Phila Pa 1976) 36:1721–1727, 2011.
55. Valentini E, Halder S, McInnersey D, Cooke J, Romei V: Assessing the specificity of the relationship between brain alpha oscillations and tonic pain. bioRxiv [Internet] :787283, 2019. Available from: https://www.biorxiv.org/content/10.1101/787283v1?rss=1
56. de Vries M, Wilder-Smith OHG, Jongsma MLA, van den Broeke EN, Arns M, van Goor H, van Rijn CM: Altered resting state EEG in chronic pancreatitis patients: Toward a marker for chronic pain. J Pain Res 6:815–24, 2013.
57. Weger T, Atlas L, Lindquist M, Roy M, Woo C, Kross E: An fMRI-Based Neurologic Signature of Physical Pain. N Engl J Med 368:1388–97, 2013.
58. Wemmie JA, Taugher RJ, Kreple CJ: Acid-sensing ion channels in pain and disease. Nat Rev Neurosci [Internet] Nature Publishing Group; 14:461–71, 2013. Available from: http://dx.doi.org/10.1038/nrn3529

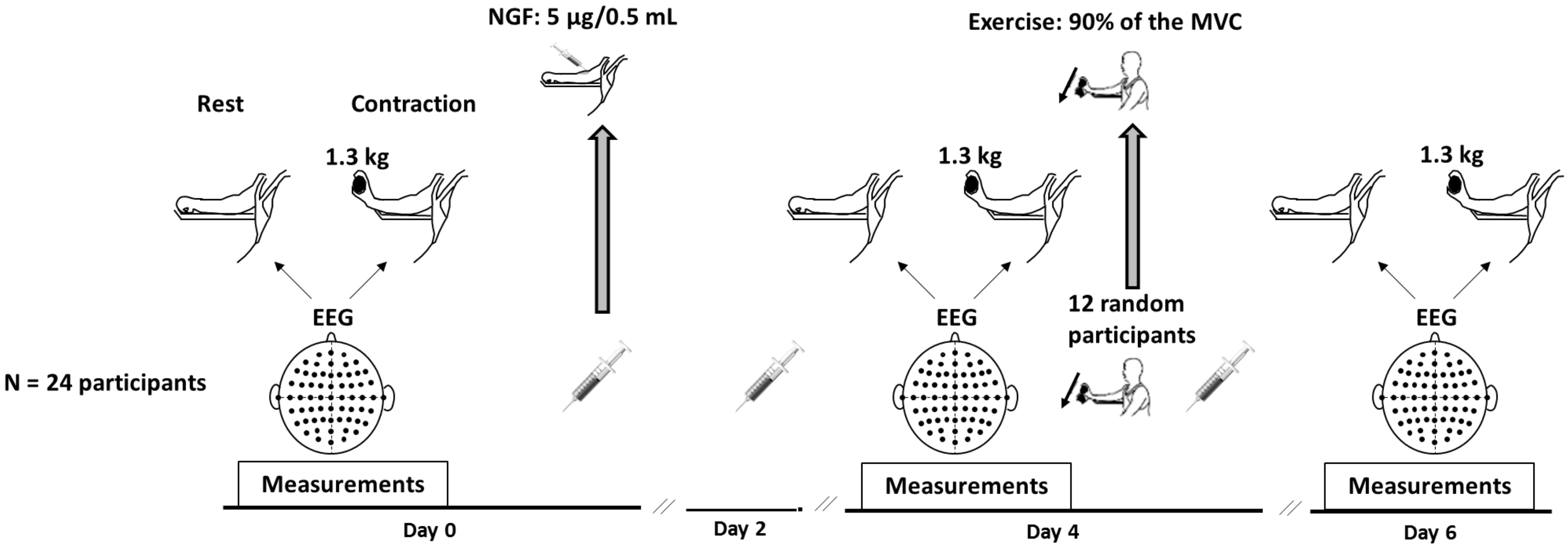

Figure 1: Electrophysiological outcome measures were assessed at the beginning of each experimental session on Day 0, Day 4, and Day 6 during two conditions: Resting-state and contraction-state. On Day 0, Day 2, and Day 4, these measures were followed by injection of NGF to the right extensor carpi radialis brevis. On Day 4, a subgroup of twelve randomly selected participants performed eccentric exercise before receiving the NGF injection.

Figure 2: Diagram of the PAF extraction. After filtering raw data, an independent component analysis (ICA) was performed, followed by the extraction of the corresponding IC weights.

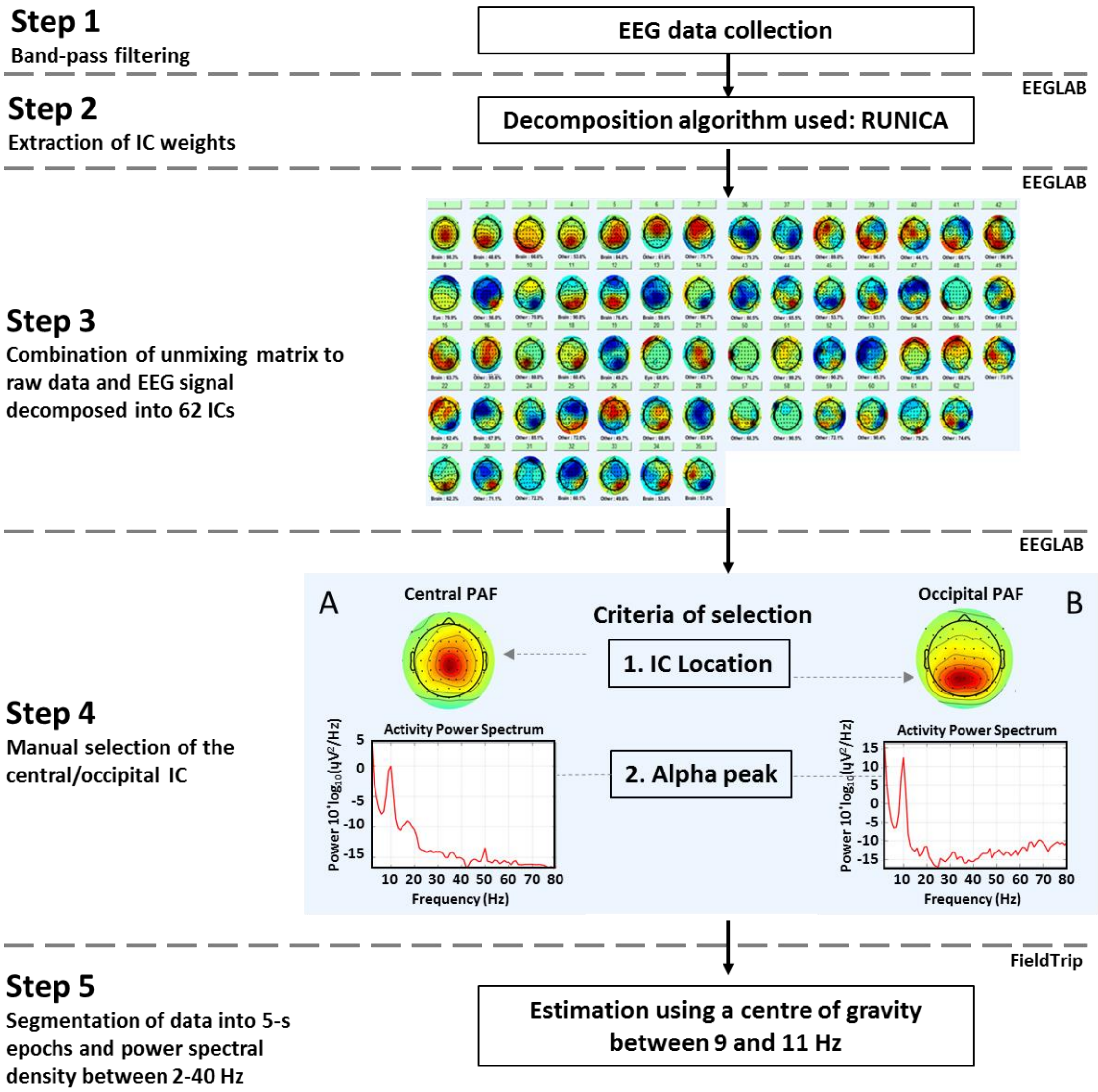

Figure 3: The figure shows the main steps of analysis with ICs recorded during EEG from a representative participant. The IC localized in the central (A) and occipital (B) regions were visually selected.

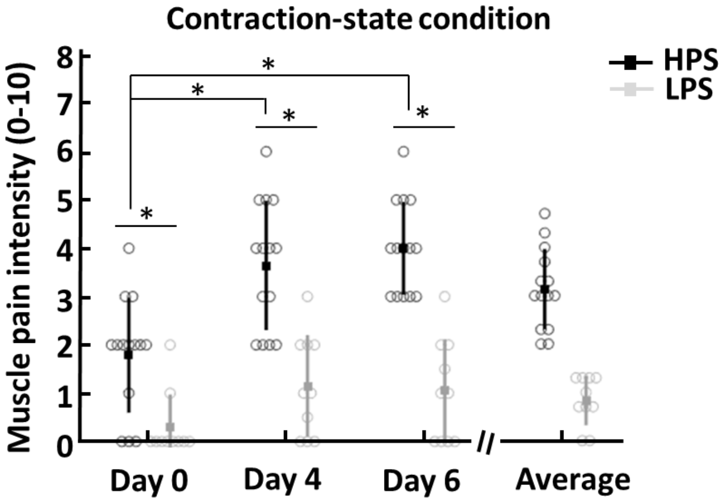

Figure 4: Pain numerical rating scale (NRS) scores at Day0, Day4, Day6, and average over days (Day0, Day4, and Day6) for participants in the high pain sensitive (HPS, N = 14) or low pain sensitive (LPS, N = 10) group. Open circles represent an individual NRS score, the group mean is a filled square, and the standard deviation is vertical lines. Significantly higher pain NRS scores in the HPS compared with LPS group or compared with Day0 (*, P<0.05).

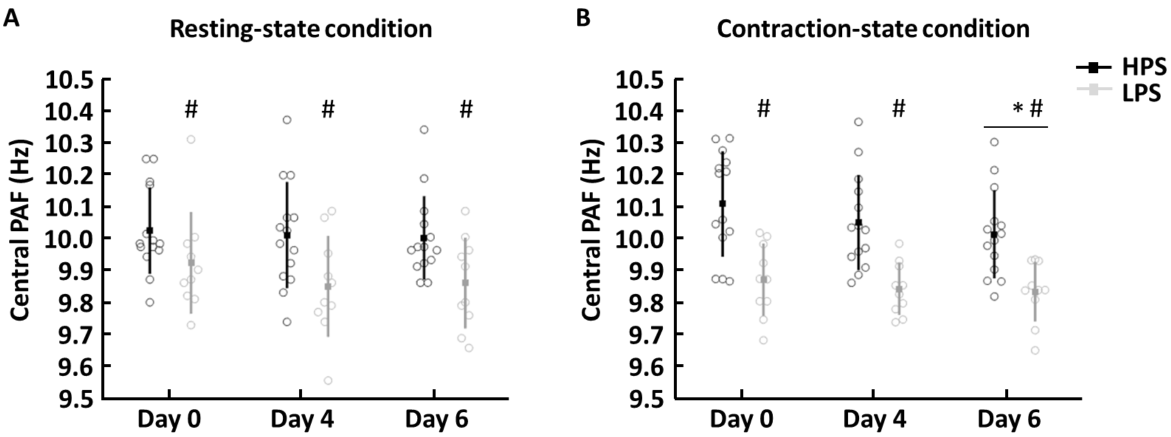

Figure 5: Central PAF (peak alpha frequency) at Day0, Day4, and Day6 for participants in the high pain sensitive (HPS, N = 14) or low pain sensitive (LPS, N = 10) group in the resting-state condition (**A**) and contraction-state condition (**B**). Open circles represent individual PAF results, the group mean is a filled square, and the standard deviation is vertical lines. Significantly higher PAF in the HPS compared with LPS group (#, P<0.05) or compared with Day0 (*, P<0.05).

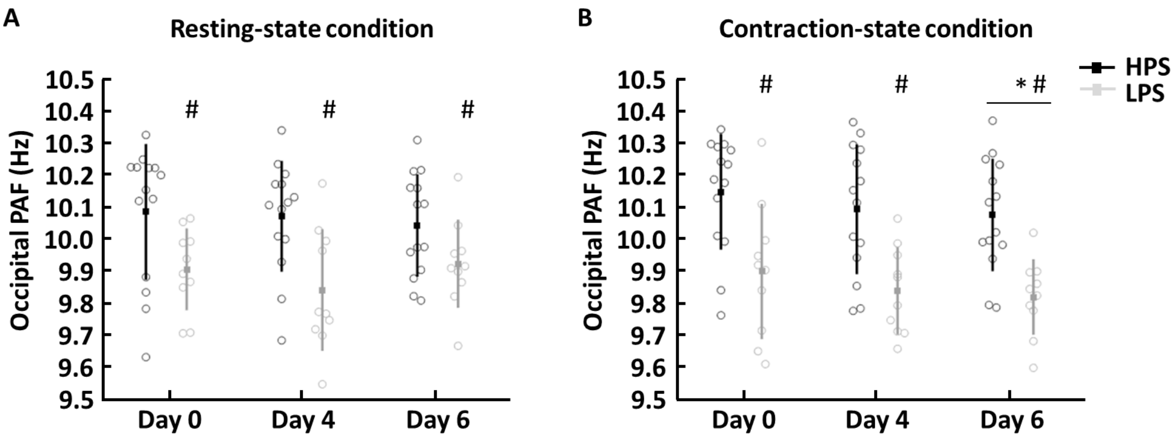

Figure 6: Occipital PAF (peak alpha frequency) at Day0, Day4, and Day6 for participants in the high pain sensitive (HPS, N = 14) or low pain sensitive (LPS, N = 10) group in the resting-state condition (**A**) and contraction-state condition (**B**). Open circles represent individual PAF results, the group mean is a filled square, and the standard deviation is vertical lines. Significantly higher PAF in the HPS compared with LPS group (#, P<0.05) or compared with Day0 (*, P<0.05).

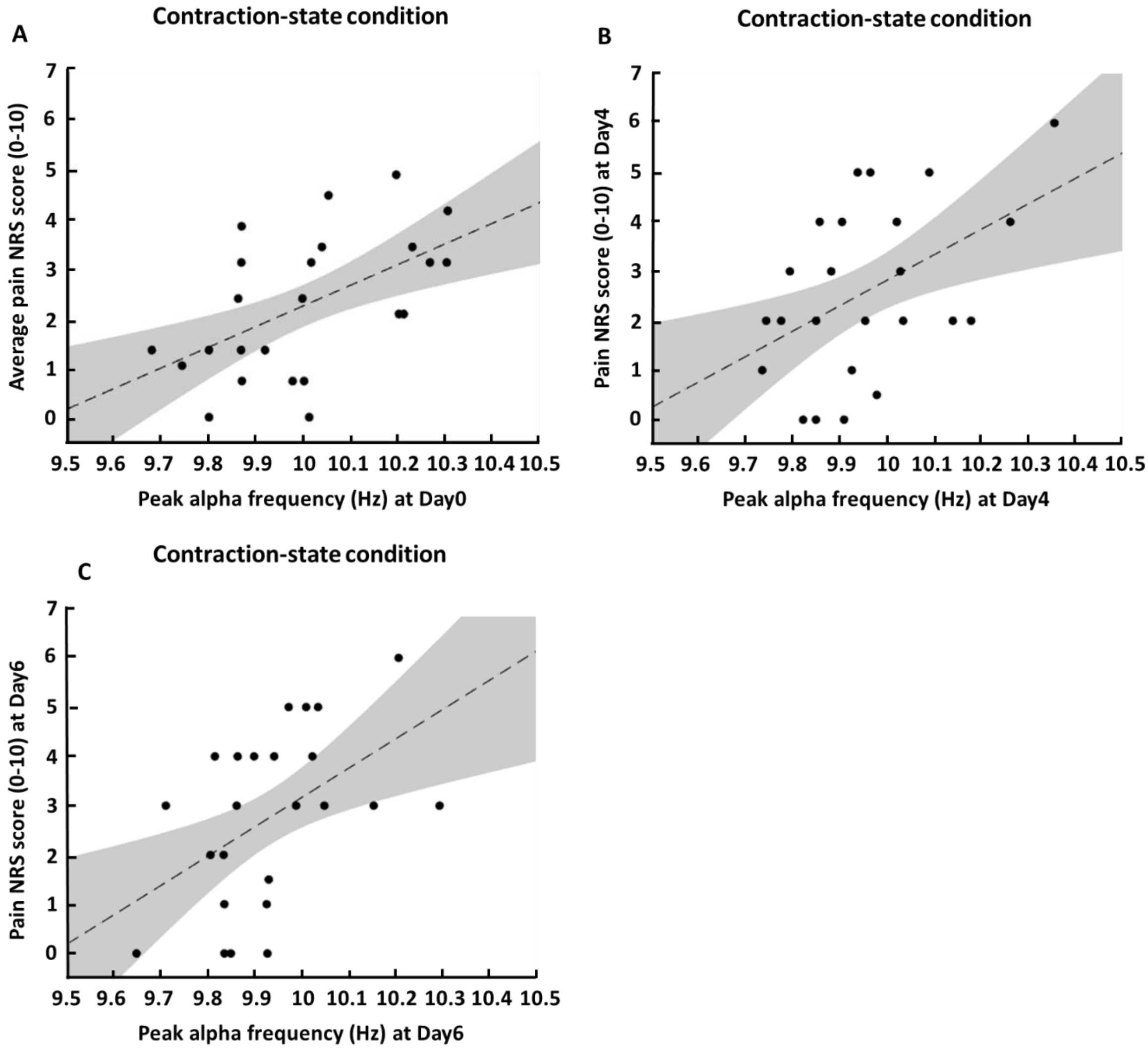

Figure 7: **A**) Correlations between average pain NRS scores (across Day0, Day4, and Day6) and central PAF during contraction-state condition on Day0. **B**) Correlations between pain NRS scores at Day4 and central PAF during contraction-state condition at Day4. **C**) Correlations between pain NRS scores at Day6 and central PAF during contraction-state condition at Day6. Grey shaded area indicates 95% confidence intervals, and the dashed line is the linear trendline.

TABLE

Variable	HPS-group	LPS-group
N	14	10
Sex (F)	8	6
Height (cm)	170.6±9.9	171.9±10.0
Weight (kg)	73.8±18.2	67.6±10.7
Age (years)	25±4	27±6

Table 1. Demographics of the High-Pain Sensitive (HPS) and Low-Pain Sensitive (LPS) groups.