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Research Report

Pain assessment by continuous EEG: Association between subjective perception of tonic pain and peak frequency of alpha oscillations during stimulation and at rest

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ABSTRACT

Recordings of neurophysiological brain responses to noxious stimuli have been traditionally based on short stimuli, in the order of milliseconds, which induce distinct event-related potentials (ERPs). However, using such stimuli in the experimental setting is disadvantageous as they are too brief to faithfully simulate clinical pain. We aimed at utilizing continuous EEG to investigate the properties of peak alpha frequency (PAF) as an objective cortical measure associated with subjective perception of tonic pain. Five minute long continuous EEG was recorded in 18 healthy volunteers under: (i) resting-state; (ii) innocuous temperature; and (iii) psychophysically-anchored noxious temperature. Numerical pain scores (NPSs) collected during the application of tonic noxious stimuli were tested for correlation with peak frequencies of alpha power-curves derived from central, temporal and frontal electrodes. NPSs and PAFs remained stable throughout the recording conditions (RM-ANOVAs; $Ps > 0.51$). In the noxious condition, PAFs obtained at the bilateral temporal scalp were correlated with NPSs ($Ps < 0.001$). Moreover, resting-state PAFs recorded at the bilateral temporal scalp were correlated with NPSs reported during the noxious condition ($Ps < 0.01$). These psychophysical-neurophysiological relations attest to the properties of PAF as a novel cortical objective measure of subjective perception of tonic pain. Moreover, resting-state PAFs might hold inherent pain modulation attributes, possibly enabling the prediction of individual responsiveness to prolonged pain. The relevance of PAF to the neural processing of tonic pain may indicate its potential to advance pain research as well as clinical pain characterization.

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1. Introduction

Neurophysiological studies of experimental pain, which aim at revealing indirect indices of neural activity through time-locked event-related potentials (ERPs), employ phasic, brief stimuli, in the order of milliseconds, which are too short to faithfully simulate the experience of clinical pain. Experimental tonic pain, on the other hand, is a stimulus which better resembles the sensory experience in the clinical setting (Huber et al., 2006). While extensive functional imaging-based data have been gathered regarding experimental tonic noxious stimuli (Peyron et al., 2000; Ringler et al., 2003; Schreckenberger et al., 2005; Owen et al., 2010), little is known about the changes in the EEG frequency content during the processing of such stimuli. Recording and electrophysiological characterization of cortical responses to tonic pain require methods other than ERPs, namely continuous EEG.

Continuous EEG is commonly analyzed by transforming data from the time domain to the frequency domain. The transformed data is characterized by a curve of the amplitude of power–density plotted against the frequency range of interest. The area under this curve is the frequency content of the observed activity. A significant measure derived from such analysis is the highest power–density point within the investigated frequency range. This peak-point is identified by two parameters: (i) the frequency at which it occurs on the frequency axis, and (ii) its amplitude on the power–density axis.

Within the frequency domain, alpha-band oscillations (7.5–12 Hz) are the most explored frequencies. These oscillations are widely distributed in the cerebral cortex, and their peak amplitude was found to decrease in the corresponding cortical regions during somatosensory stimuli and voluntary movements (Pfurtscheller and Lopes da Silva, 1999; Fu et al., 2001; Cheyne et al., 2003; Stančák et al., 2003; Feige et al., 2005). Similar decreases in alpha amplitudes were also reported to be induced by experimental pain (Chen and Rappelsberger, 1994; Chen et al., 1998; Chang et al., 2001a,b, 2002a,b; Ploner et al., 2006; Dowman et al., 2008). While alpha oscillations may conjointly represent cognitive performance, lower and higher frequencies have been associated with attentional processes and specific task requirements, respectively (Klimesch, 1999).

The measure of peak alpha frequency (PAF) has not yet been explored in the context of tonic pain processing. In other experimental paradigms, PAF has been described repeatedly as a stable measure (Poulos et al., 2002; Maltez et al., 2004; Vuga et al., 2006), increasing with cognitive processing, attentional demands and arousal (Köpruner et al., 1984; Klimesch et al., 1990; Li et al., 1996). A large study of 688 twins found PAF to be highly heritable (Posthuma et al., 2001), and the intra-individual stability of PAF was suggested to qualify this parameter as a 'personal signature' due to its high reproducibility (Näpflin et al., 2007). Moreover, a study of 550 normal subjects aged 11–70 revealed that PAF was positively correlated with working memory performance across the lifespan (Clark et al., 2004).

These advantages of the PAF measure, which are lacking in the peak alpha amplitude, together with the novelty of associating PAF with processing of tonic pain, motivated us to focus on this measure. Specifically, the present study aimed at investigating whether PAF values induced by tonic noxious thermal stimuli could serve as an objective cortical measure associated with the subjective perception of tonic heat pain.

2. Results

2.1. Psychophysics

In the present study, subjects were stimulated on the left, non-dominant volar forearm, using a contact-heat thermode for 5 min at each of two intensities: (i) 32 °C and (ii) 'pain-60' temperature, which had been determined in a preliminary session for each individual as the temperature that induced a pain experience at a magnitude of 60 on a 0–100 numerical pain scale during a 30-second stimulus (Granot et al., 2006, 2008; Weissman-Fogel et al., 2009).

The mean 'pain-60' temperature was 45.15±1.19 °C. The mean numerical pain scores (NPSs) obtained at 100 s, 200 s, and 300 s for the tonic stimuli of the noxious condition were 60.91±15.59; 54.27±13.39; and 55.39±18.21, respectively. An insignificant one-way RM-ANOVA ($P=0.68$) indicated no significant differences between the three NPSs which were reported during the application of the tonic noxious stimulation. This affirms the steady perception of a painful sensory experience throughout the noxious condition, and justifies the averaging of the three NPSs for further analysis. The grand-average of NPSs collected during tonic stimulation application was 56.33±14.03. The average of the fourth NPS, reflecting the overall pain experience during the tonic noxious stimulation, was 56.17±10.88. A paired two-tailed *t*-test revealed no significant difference between the grand-average of the NPSs collected during the tonic noxious 'pain-60' intensity and the fourth overall NPS collected after the stimulus ending ($P=0.87$).

2.2. PAFs in the EEG recording conditions

An insignificant two-way RM-ANOVA for each recording condition indicated no significant differences between the three averaged PAFs attributed to each third of the recorded data at the examined electrodes (resting-state: $P=0.518$; innocuous condition: $P=0.641$; noxious condition: $P=0.708$). These analyses attest to the stability of the PAF measure throughout the recording conditions and justify the averaging of the PAF values of the three epochs for further analysis. A significant two-way RM-ANOVA analysis ($F_{12,340}=6.577$; $P<0.001$) pointed to a statistical interaction among the factors Condition and Recording electrode location. A statistically significant change in PAFs between conditions (resting-state, innocuous and noxious) was indicated by the post-hoc Tukey–Kramer test only at the temporal electrodes ipsilateral ($P=0.028$) and contralateral ($P=0.015$) to the applied stimulation, namely T_7 and T_8, respectively. At these electrode positions, mean PAF values increased from 9.19±0.89 and 9.49±0.98 Hz under the resting-state condition, to 9.56±0.81 and 9.93±0.87 Hz under the innocuous condition, and to 10.07±1.05 and 10.49±1.12 Hz under the noxious condition, respectively. Table 1 describes the linear associations between PAFs of the continuous EEG recorded at the C_z, C_3, C_4, T_7, T_8, F_{p1} and F_{p2} electrodes during the resting-state, innocuous, and noxious conditions (presented *P*-values are post Bonferroni corrections

Table 1 – Regression analyses of peak alpha frequencies (PAFs) in the EEG recording conditions. *P-values are Bonferroni corrected for multiple comparisons.

Conditions	Electrode	R	*P	F (1,16)
Rest vs. innocuous	C_z	0.560	0.038	7.31
	C_3	0.816	0.0039	31.90
	C_4	0.896	0.0005	64.81
	T_7	0.690	0.009	14.53
	T_8	0.641	0.019	11.12
	F_{p1}	0.470	0.038	4.78
	F_{p2}	0.481	0.041	4.81
Rest vs. noxious	C_z	0.659	0.014	12.35
	C_3	0.612	0.047	9.60
	C_4	0.806	0.005	29.71
	T_7	0.787	0.001	26.57
	T_8	0.859	0.0001	45.44
	F_{p1}	0.508	0.029	5.62
	F_{p2}	0.524	0.04	6.06
Innocuous vs. noxious	C_z	0.870	0.0001	49.82
	C_3	0.538	0.040	6.52
	C_4	0.843	0.002	39.36
	T_7	0.878	<0.0001	60.96
	T_8	0.700	0.007	15.37
	F_{p1}	0.515	0.038	5.77
	F_{p2}	0.482	0.045	4.83

for multiple comparisons). The PAFs that were analyzed on the basis of the data recorded at the C_4, T_7 and T_8 electrodes during the noxious condition were positively correlated with the NPSs induced by the noxious condition (Fig. 1A). PAFs derived from resting-state EEG recordings were correlated with the NPSs induced by the noxious condition at the C_4, T_7 and T_8 electrodes (Fig. 1B). Fig. 1 portrays NPSs vs. PAF values only for the temporal electrodes since they solely demonstrated significant pain-induced changes in PAFs, as detailed in the aforementioned RM-ANOVA analysis incorporating Condition and Recording electrode location. Fig. 2 exemplifies increasing NPSs of tonic pain as well as the corresponding PAFs derived from the noxious and resting-state conditions.

2.3. *Association between 'pain-60' temperatures and parameters of the noxious condition*

In order to rule out the possibility that EEG changes were induced by intensity coding of the applied tonic noxious stimuli rather than by subjective perception of tonic pain, correlations between the individual 'pain-60' temperatures were examined with NPSs and PAF values of the noxious condition. None of these tests yielded statistical significance (Table 2).

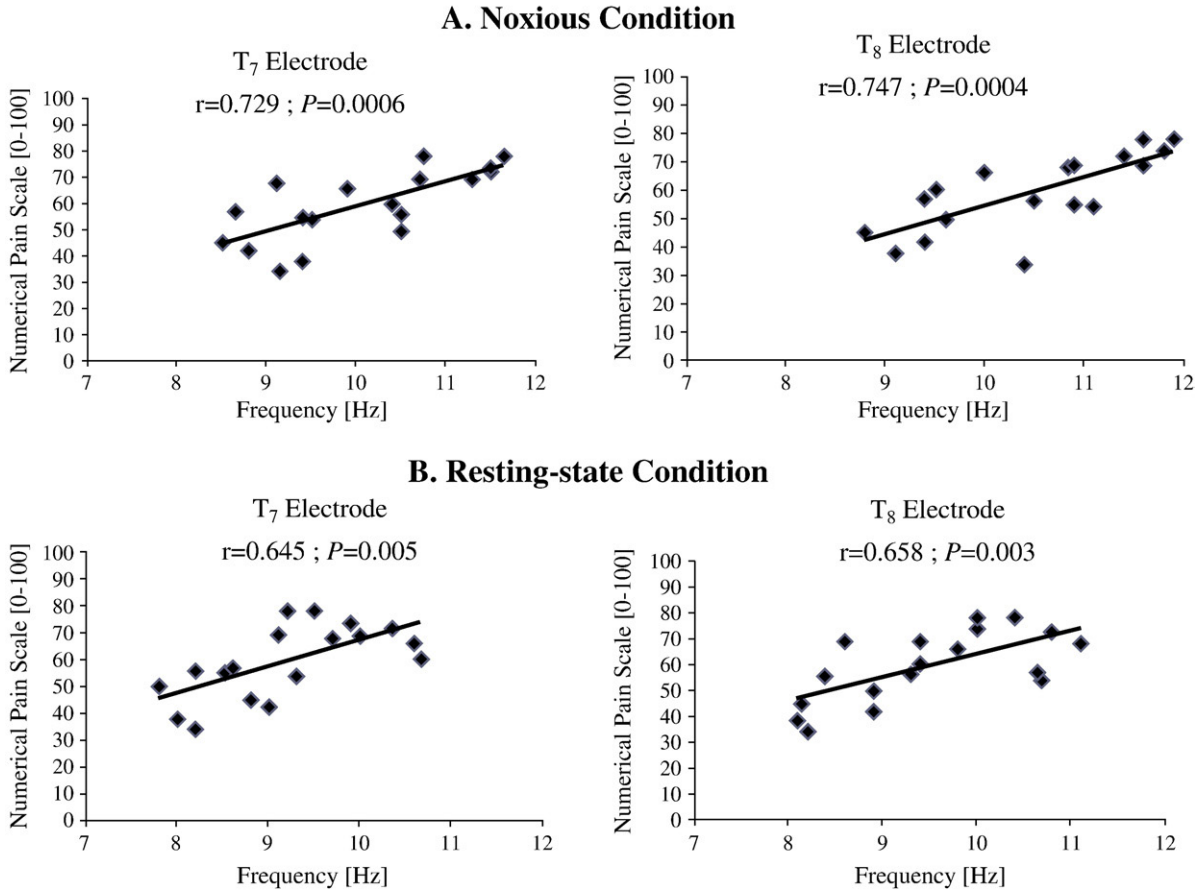

Fig. 1 – Regression analyses incorporating the NPSs of the noxious condition vs. PAFs of the noxious condition (A) and the resting-state condition (B) within the entire group ($N = 18$). T_7: left temporal electrode (ipsilateral to stimulation); T_8: right temporal electrode (contralateral to stimulation). NPS: numerical pain score; PAF: peak alpha frequency.

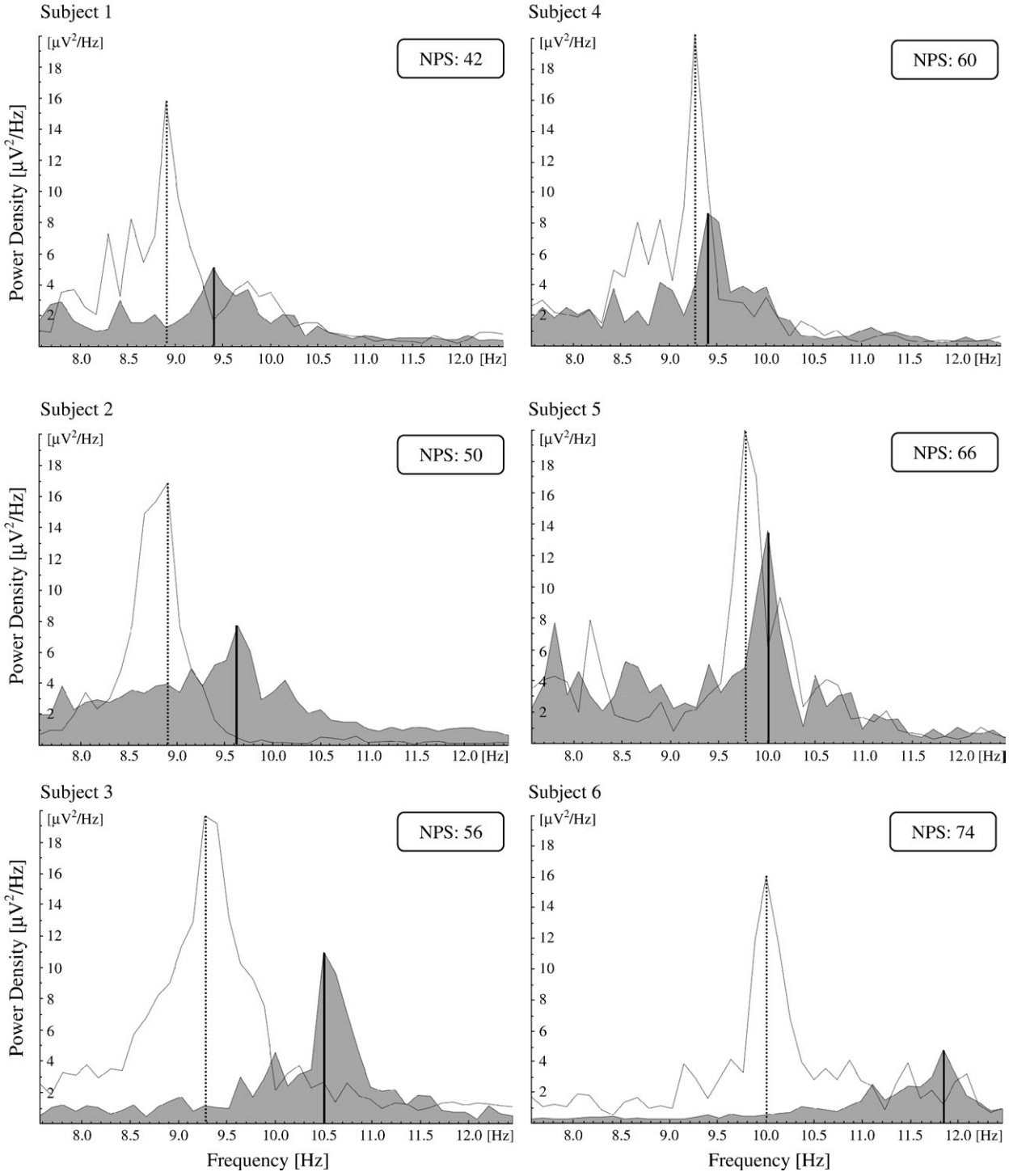

Fig. 2 – Mean NPSs (0–100 scale) to tonic noxious contact-heat stimuli, and PAFs derived from continuous EEG recorded at the temporal scalp contralateral to stimulation (T_8 electrode) in 6 randomly chosen subjects under the noxious condition (solid line in grey fill) and at resting-state (dotted line in white fill). Subjective NPSs correlated with PAFs derived from the noxious condition ($r = 0.747$; $P = 0.0004$; $N = 18$) and the resting-state condition ($r = 0.658$; $P = 0.003$; $N = 18$); as the subjective NPSs of tonic pain increased, so did the PAFs derived from the noxious and resting-state conditions. Increased PAFs may be associated with the observed power loss in lower alpha oscillations and power gain in higher alpha oscillations following the change from resting-state to pain induction within an individual. NPS: numerical pain score; PAF: peak alpha frequency.

Table 2 – Regression analyses of 'pain-60' temperatures vs. numerical pain scores (NPSs) and peak alpha frequencies (PAFs) of the noxious condition. *P-values of PAFs are Bonferroni corrected for multiple comparisons.

'Pain-60' temperature vs.	Electrode	R	*P	F (1,16)
NPSs	–	0.065	0.635	0.067
PAFs	C_z	0.04	0.337	0.026
	C_3	0.13	0.322	0.275
	C_4	0.16	0.650	0.420
	T_7	0.003	0.216	0.001
	T_8	0.014	0.541	0.003
	F_{p1}	0.038	0.461	0.032
	F_{p2}	0.21	0.527	0.740

3. Discussion

The main finding of the current study is that peak alpha frequencies may represent an objective marker for subjective perception of tonic heat pain. This is based on the correlation between subjective pain scores and PAF values which were induced by tonic noxious heat stimuli. An additional observation was that inherent attributes of pain responsiveness may be indicated through electroencephalographic data obtained during stimulation-free conditions. This notion stems from the correlation between baseline PAFs recorded during resting-state and pain scores induced by the tonic noxious stimuli in a separate session. The lack of significant correlations between the individual 'pain-60' temperatures and the NPSs as well as PAFs of the noxious condition substantiates that the EEG alterations were probably not due to the applied temperatures and their physiological intensity coding, but rather, due to the subjective experience of tonic pain.

We sought to demonstrate the associations of PAF, an objective neurophysiological parameter, with individual levels of experienced tonic pain, rather than with the physical intensity of the applied tonic stimuli. Thus, rather than employing physically-anchored intensities, we opted for the use of psychophysically-anchored intensities of the tonic noxious stimuli in accordance with the 'pain-60' paradigm (Granot et al., 2006, 2008; Weissman-Fogel et al., 2009). The specific psychophysical 'pain-60' level was chosen so as to preserve a steady pain perception throughout the tonic noxious stimuli, which was considered a prerequisite for utilizing the entire temporal span of the continuous EEG data for subsequent frequency content analyses. In our experience, this precondition is more likely to be met for the psychophysical level achieved using the 'pain-60' intensity; stimuli of lower psychophysical intensities tend to decrease pain scores over the course of stimulation, probably owing to adaptation, whereas stimuli of higher intensities are inclined to increase pain ratings, possibly due to sensitization. As such, the pain scores obtained in the present study remained steady at tolerable but nevertheless significantly painful levels without changing during the prolonged application of the tonic noxious stimuli.

In healthy subjects, studies focusing on the relations between PAF and cognitive performance have demonstrated PAF to be sensitive to various cognitive tasks, increasing with: visuospatial and arithmetic tasks (Osaka, 1984), administration of an auditory working memory task (Osaka et al., 1999), better verbal abilities (Anokhin and Vogel, 1996), higher speed and performance in a number of cognitive tasks (Li et al., 1996) and enhanced attentional demands and/or arousal (Klimesch, 1999). In that regard, as pain perception and arousal are intimately intertwined both functionally and physiologically and reciprocally affect one another (Rimm and Litvak, 1969; Groves and Thompson, 1970; Craig, 2006; Flor and Turk, 2006), it could stand to reason that an arousal effect may have been associated with the findings of the current study. In the clinical arena, Alzheimer's disease patients were reported to demonstrate reduced PAF (Klimesch et al., 1990; Passero et al., 1995), and in the case of chronic fatigue syndrome, decreased PAF was correlated with increased fatigue parameters (Billiot et al., 1997).

In keeping with these non-pain-related PAF reports, the current study suggests that higher PAFs are linearly correlated with increased perception of tonic heat pain. This finding implies that higher PAF characterizes a neural circuitry engaged in more extensive processing, which requires enhanced connectivity between affiliated brain regions (Chen et al., 1998; Posthuma et al., 2001; Mantini et al., 2009). A possible mechanism underlying the observed acceleration of PAF may be an increased activity of cortico-cortical and thalamocortical feedback loops, which may reflect excitatory processes (Klimesch, 1996, 1997). Specifically, this association between the psychophysical and neurophysiological dimensions of tonic pain is in line with the hypothesis that PAF indexes cognitive capacity related to the *state* of brain function (Angelakis et al., 2004), as exemplified by the findings that PAF was affected by emotional states (Kostyunina and Kulikov, 1995; Kostyunina, 1998), as well as the acute administration of various substances (Saletu et al., 1984; Knott, 1988; Tiffin et al., 1995; Lindgren et al., 1999). In this regard, we observed inter-correlations between PAF values across the resting-state, innocuous, and noxious conditions. These associations of PAF values in different states could suggest the possible existence of individual patterns of neuronal responses to varying exogenous stimuli. The correlations between PAF values and NPSs were observed at the bilateral temporal scalp, which is consistent with both pain-related activity in the primary somatosensory cortex and/or the somatosensory association areas located in the parietal operculum and/or insula (Dowman et al., 2008), and with tonic pain related reports in which changes in alpha-band amplitudes were detected at the same location (Chen and Rappelsberger, 1994; Le-Pera et al., 2000; Huber et al., 2006; Dowman et al., 2008).

Accumulating imaging-based evidence associate patterns of signal coherence across brain regions in resting-state with functional–anatomical networks involved in somatosensory and cognitive processes, memory, motor function and executive functioning (Biswal et al., 1995; De Luca et al., 2005, 2006; Damoiseaux et al., 2006; Shmuel and Leopold, 2008). These patterns were suggested to be a useful probe for functional alterations in the brain reflecting changes in cognitive states, disease, or pharmacological interventions (De Luca et al., 2005, 2006; Damoiseaux et al., 2006). The present study adds to these

emerging findings by demonstrating for the first time the relationship between PAFs of the resting-state condition and the NPSs of the tonic noxious stimulation. This finding suggests that the central processing of persistent noxious events within individuals can be indicated through the intrinsic neural functioning of their pain network at rest, possibly enabling the prediction of subjective tonic pain responsiveness without the use of external stimuli. An affiliated view proposes that 'spontaneous' alpha oscillations do not necessarily reflect 'passive states' or 'idling' of the brain, but rather signals with quasi-deterministic properties relating to diverse brain functions, including sensory and memory processes (Başar et al., 1997). Likewise, PAF was suggested to index cognitive capacity related to the *trait* of brain function (Angelakis et al., 2004), as represented by the mentioned positive associations of PAF with both memory abilities and processing speed in healthy individuals, and by the chronically suppressed cognitive capacity of individuals with traumatic brain-injury induced decreased PAF (Angelakis et al., 2004).

The associations we observed between NPSs and PAFs were across the entire alpha spectrum. This may stem from the fact that the elements reflected by alpha sub-bands are affiliated, and so their associations with individual responses to exogenous stimuli may be comparable, specifically in the arena of pain research (Chen and Rappelsberger, 1994; Chang et al., 2001a; Chang et al., 2002a,b; Dowman et al., 2008). In keeping, previous studies investigated the attributes of the entire alpha span so as to characterize these oscillations collectively (Li et al., 1996; Başar et al., 2000, 2001; Suldo et al., 2001; Angelakis et al., 2004; Mu et al., 2008).

The applicability of the resting-state PAF measure to the pain-related clinical and research arenas is supported by the intra-individual stability of PAF at rest, which has repeatedly been demonstrated to show high reproducibility over periods of up to four years (Gasser et al., 1985; Dustman et al., 1999; Kondacs and Szabo, 1999; Poulos et al., 2002; Maltez et al., 2004; Vuga et al., 2006). Moreover, of the various EEG-derived frequency content parameters, the peak and mean alpha frequencies at rest were the most stable features measured at an interval of 3–4 months (Salinsky et al., 1991). In a large twins study with 688 participants, PAF was found to be highly heritable with 71–83% of the total variance reported to be genetic (Posthuma et al., 2001). These high heritability levels of PAF may be indicative of a stable inherent trait, which is influenced little by developmental plasticity or individual experiences and is instead explained largely by genetic factors. This trait may signify the evolutionary importance of PAF in the faster neural processing of both cognitive and physical stimuli.

Future research should concentrate on several limitations within the current study. First, as subjects were healthy young adults, further investigation is needed to expand the current findings to clinical pain populations and older adults. Second, we examined pain perception of tonic pain using thermal modality. Whether analogous findings as to tonic pain perception and its association with PAF will be observed using additional modalities calls for additional exploration. Finally, while the present study focuses on the alpha-band frequencies due to their aforementioned features, additional bands are to be assessed for correspondence with subjective experiences of tonic pain.

In conclusion, current findings extend prior research regarding the characteristics of PAF as a direct, objective and experimentally stable measure of cognitive as well as sensory processes by reporting that increased PAF values derived from EEG recordings of resting-state and noxious conditions were correlated with higher NPSs. These results may have important implications for objectively and straightforwardly assessing pain responsiveness in pain research and clinical interventions.

4. Experimental procedures

4.1. *Subjects*

Participants were 18 healthy right-handed volunteers, including 9 females and 9 males. Since differences between individuals in alpha frequency can be attributed to age (Klimesch, 1999), an age-wise homogenous group (mean ± SD: 26 ± 2.1) was recruited for the study. Volunteers were enrolled after meeting the following criteria: (i) absence of pain; (ii) no current or previous history of a relevant neurological or psychiatric disease; and (iii) no current regimen of any medications known to affect EEG recording. The Institutional Review Board of Rambam Health Care Campus approved the study protocol in accordance with the Helsinki Declaration, and written consent was obtained from each subject prior to the initiation of each experiment.

4.2. *Stimulator*

A thermal contact-heat stimulator equipped with a round thermode was used to deliver tonic innocuous and noxious stimuli (PATHWAY sensory evaluation system, Medoc Ltd., Ramat-Yishai, Israel) by contacting a cutaneous area of 572.5 mm^2 (27 mm in diameter). All stimuli were administered to the left, non-dominant volar forearm. The thermode comprises a heating thermofoil (Minco Products, Inc., Minneapolis, MN, USA), which is covered with a 25 μm layer of thermo-conductive plastic (Kapton®, thermal conductivity of 0.1 to 0.35 W/mK at 23 °C). Two thermocouples are embedded 10 μm within this conductive coating, which contacts the skin directly, thus providing an estimate of the skin temperature at the thermode surface. The thermofoil permits an estimated heating rate of up to 70 °C/s, and the Peltier device incorporated in the thermode allows an approximated cooling rate of 40 °C/s. These maximal values were used in our study at the initiation and ending of each tonic stimulus, respectively.

4.3. *Continuous EEG recording*

Continuous EEG was recorded from 32 Ag/AgCl electrodes using an electrode cap (Easy Cap Q40, FMS Falk Minow Services, Herrsching, Germany) according to the 10%-system (an extended montage of the standard 10–20% system). All electrodes were referenced to a midline electrode positioned

at the chin. Extracranial activity was continuously recorded using a Quick Amp EEG system (Brain Products GmbH, Munich, Germany) within a 0.15 Hz and 100 Hz bandpass filter, and was digitized at a sampling rate of 500 Hz. Throughout the experiments, ongoing real-time recordings were monitored, and the impedance at each electrode position was kept below 5 kΩ. A notch filter was set to 50 Hz in order to reduce electrical interference. An artifact rejection program controlled eye-movement artifacts, with the maximal allowed voltage-step set to 50 μV. EEG data were stored on disk and were analyzed offline (Recorder and Analyzer Software, Brain Products GmbH, Munich, Germany). Stimulus onset and offset were marked by electrical transistor–transistor logic (TTL) pulses, which were delivered from the stimulator to the Quick Amp at the initiation and ending of each stimulus, respectively.

4.4. *Experimental protocol*

Subjects were seated in a comfortable armchair in a quiet room with an ambient temperature of ~22 °C. Experiments were performed during the same morning hours to exclude an impact of circadian factors on the EEG.

4.4.1. *Subjective numerical pain scores (NPSs)*

Subjects were asked to verbally rate the level of perceived pain on a 0–100 numerical pain scale, in which 0 was defined as "no pain sensation" and 100 as "the worst imaginable pain." Four NPSs were obtained in total throughout the experiment, of which three were obtained during the application of the five-minute-long tonic stimuli, both innocuous and noxious, namely one after each third (100 s). A fourth overall NPS was collected up to 10 s after the tonic stimuli, both innocuous and noxious, in response to the question: "Which NPS would best reflect the overall pain you have experienced during the stimulus?"

Importantly, these four NPSs were collected during and after the application of both innocuous and noxious stimuli in order to minimize the differences in the attentional loads and arousal levels between the two conditions, and accordingly, to enhance the specificity of the results to individual processing of tonic pain.

4.4.2. *EEG recording procedure*

Continuous 32-electrode EEG recording was performed during relaxed, but alert, wakefulness. Subjects were instructed to keep their eyes closed during EEG recording, as alpha peaks can occur at the frequency that is most depressed by opening of the eyes (Klimesch, 1999; Posthuma et al., 2001). The five-minute-long EEG recordings were conducted under three separate conditions: during the aforementioned two thermal stimulation intensities (innocuous 32 °C and noxious 'pain-60' temperature) as well as throughout a baseline resting-state condition, during which no experimental stimulation was applied. Continuous EEG was always recorded first during the resting-state condition, followed by EEG recordings during the thermal stimuli, which were counter-balanced in order. Breaks of at least 20 min were maintained between the three recording conditions.

4.4.3. *Training session*

A training session without EEG recording was conducted 30 min prior to the beginning of the experiment in order to familiarize the subjects with the sensation of the experimental contact-heat stimuli and in order to accustom them to report NPSs using the numerical pain scale. In this session, subjects were exposed to three contact-heat stimuli (43 °C, 45 °C, and 47 °C) of 10 s each, starting from the time that the stimulus intensity had reached the destination temperature. As instructed beforehand, subjects were asked to rate the level of pain intensity on the numerical pain scale 3 s before the end of each stimulus.

4.5. *Continuous EEG analysis*

The EEG recordings at the electrodes positioned at C_z (central scalp), C_3 (left central scalp, ipsilateral to stimulation), C_4 (right central scalp, contralateral to stimulation), T_7 (left temporal scalp, ipsilateral to stimulation), T_8 (right temporal scalp, contralateral to stimulation), F_{p1} (left frontal scalp, ipsilateral to stimulation), and F_{p2} (right frontal scalp, contralateral to stimulation) were referenced to the midline chin electrode and incorporated in subsequent analyses (detailed hereunder). We focused on the aforementioned central, temporal and frontal electrodes due to ample evidence pointing at their particularly substantial relevance in research of tonic experimental pain (Backonja et al., 1991; Chen and Rappelsberger, 1994; Chen et al., 1998; Chang et al., 2001a,b, 2002a,b; Le-Pera et al., 2000; Huber et al., 2006; Dowman et al., 2008). Each EEG recording of 5 min was segmented into 300 segments of 1 s each. All segments contaminated with electro-oculographic or muscle artifacts were eliminated. Segments with speech artifacts, which were induced by the NPSs of subjects collected during specific points throughout the tonic stimulation, were removed as well. Encompassing the entire temporal span of the five-minute long tonic EEG recordings, 60 artifact-free, one-second segments were selected for further analysis on each subject, with 20 segments randomly selected from each third of the recorded EEG. As changes in alpha activity may be visible on single epochs, selection of segments was randomized in order to avoid systemic bias. This procedure was repeated for all three recording conditions. After downsampling to 256 Hz, power spectral densities were computed by averaging the Fast Fourier Transformation (FFT) power spectra of the one-second data windows of all 60 artifact-free trials of each condition. Spectral windows were shifted in 0.125-second overlapping intervals. The peak amplitudes of the alpha-band power–density curves were calculated, as well as their corresponding frequencies. The present study aimed at investigating whether PAF values induced by tonic noxious thermal stimuli could serve as an objective cortical measure associated with subjective perception of tonic heat pain. Accordingly, analyses focus only on the PAF measure. The analyzed alpha-band frequencies were defined as 7.5–12.0 Hz.

4.6. *Statistical analysis*

All statistical analyses were conducted using JMP Version 8 (SAS Institute, Cary, NC, USA). One-way repeated measures

analysis of variance (RM-ANOVA) was performed in order to identify significant differences between the three NPSs collected throughout the application of tonic stimuli, and a two-way RM-ANOVA tested significant differences between the PAF values in the three corresponding epochs within each recording condition, which were subsequently averaged for analysis; these RM-ANOVAs incorporated Segment (either third of the recorded data)×Recording electrode location (C_z, C_3, C_4, T_7, T_8, F_{p1}, or F_{p2}). Changes in PAF values were evaluated using a two-way RM-ANOVA incorporating Condition (resting-state, innocuous stimulation, or noxious stimulation)×Recording electrode location (C_z, C_3, C_4, T_7, T_8, F_{p1}, or F_{p2}). PAF values were compared under each condition at each electrode location when there was a significant Condition×Recording electrode location interaction. All ANOVAs were performed using the Tukey–Kramer test to correct for multiple comparisons. Relationships between continuous variables (NPSs of the noxious condition, PAF values, and 'pain-60' temperatures) were assessed using linear regressions and Pearson correlations, with *P*-values corrected for multiple comparisons using the Bonferroni method. Specifically, two correlations examined the study question of whether PAF values induced by tonic noxious thermal stimuli could serve as an objective cortical measure associated with the subjective perception of tonic heat pain: (1) between PAF values obtained during the application of tonic noxious stimulation and the induced NPSs; and (2) between baseline PAF values attained during resting-state, i.e. throughout a stimulation-free condition, and NPSs induced by the tonic noxious stimulation in a separate session. A paired two-tailed *t*-test was used to compare the grand-average of the pain ratings collected during the 'pain-60' intensity and the fourth overall NPS collected after the stimulus ending. The present study did not aim at addressing differences in the examined parameters as a function of gender. Means and standard deviations (SD) were computed for the various parameters of the study data. Statistical significance was set at $P<0.05$.

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