Neuroimage. Author manuscript; available in PMC 2017 February 23.

Published in final edited form as:
Neuroimage. 2017 February 01; 146: 266–274. doi:10.1016/j.neuroimage.2016.11.045.

EEG frequency tagging using ultra-slow periodic heat stimulation of the skin reveals cortical activity specifically related to C fiber thermonociceptors

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Abstract

The recording of event-related brain potentials triggered by a transient heat stimulus is used extensively to study nociception and diagnose lesions or dysfunctions of the nociceptive system in humans. However, these responses are related exclusively to the activation of a specific subclass of nociceptive afferents: quickly-adapting thermonociceptors. In fact, except if the activation of Aδ fibers is avoided or if A fibers are blocked, these responses specifically reflect activity triggered by the activation of Type 2 quickly-adapting A fiber mechano-heat nociceptors (AMH-2). Here, we propose a novel method to isolate, in the human electroencephalogram (EEG), cortical activity related to the sustained periodic activation of heat-sensitive thermonociceptors, using very slow (0.2 Hz) and long-lasting (75 s) sinusoidal heat stimulation of the skin between baseline and 50°C. In a first experiment, we show that when such long-lasting thermal stimuli are applied to the hand dorsum of healthy volunteers, the slow rises and decreases of skin temperature elicit a consistent periodic EEG response at 0.2 Hz and its harmonics, as well as a periodic modulation of the magnitude of theta, alpha and beta band EEG oscillations. In a second experiment, we demonstrate using an A fiber block that these EEG responses are predominantly conveyed by unmyelinated C fiber nociceptors. The proposed approach constitutes a novel mean to study C fiber function in humans, and to explore the cortical processing of tonic heat pain in physiological and pathological conditions.

Keywords

C fiber; Heat; Thermoreceptors; Electroencephalogram; Frequency-domain analysis; Laser stimulation

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Financial disclosures
All authors declare that they have no conflicts of interest.

Europe PMC Funders Author Manuscripts

1 Introduction

The perception of heat-evoked pain results from the activation of heat-sensitive Aδ and C fiber thermonociceptors (Kandel et al., 2000). Based on how they respond to sustained heat, these thermonociceptors can be categorized as either slowly-adapting or quickly-adapting (Meyer and Campbell, 1981; Schepers and Ringkamp, 2010; Treede et al., 1995). Quickly-adapting thermonociceptors respond immediately after the onset of a thermal stimulus and then quickly adapt when the thermal stimulus is maintained over time. In contrast, slowly-adapting thermonociceptors respond gradually following the onset of a thermal stimulus and exhibit little or no adaptation when the thermal stimulus is maintained over time (Meyer and Campbell, 1981; Schepers and Ringkamp, 2010; Treede et al., 1995).

The recording of event-related brain potentials (ERPs) in response to transient heat stimuli (e.g. a brief infrared laser pulse applied onto the skin) is used extensively to study nociception in humans (Garcia-Larrea et al., 2003), and to objectify lesions or dysfunctions of the nociceptive system for the diagnosis of neuropathic pain (Cruccu et al., 2000). However, such ERPs necessarily reflect activity triggered by only a fraction of nociceptive afferents: *quickly-adapting* nociceptors. In fact, except when C fibers are activated in isolation, heat-evoked ERPs are thought to result exclusively from the activation of a very specific category of nociceptive afferents: quickly-adapting Aδ fiber thermonociceptors, also referred to as Type 2 A fiber mechano- and heat-sensitive nociceptors (AMH-2) (Bromm et al., 1984, Meyer and Campbell, 1981; Treede 1995).

Here we propose an alternative method based on EEG “frequency tagging” to isolate cortical activity related to the sustained ultraslow (0.2 Hz) sinusoidal activation of quickly- and slowly-adapting thermonociceptors in humans. In addition to showing that this approach elicits a reliable EEG response which can be readily identified at single-subject level, we also show that the elicited responses essentially reflect activity conveyed by unmyelinated C fibers, which are known to constitute the vast majority of nociceptive afferents. Therefore, the information provided by our approach could be highly complementary to the information provided by the conventional recording of transient heat-evoked ERPs. Furthermore, it could constitute a mean to study the cortical processes involved in the perception of tonic heat pain. To generate these long-lasting periodic stimuli, a temperature-controlled CO_2 laser stimulator was used to produce a very slow 0.2 Hz sinusoidal variation of skin temperature, oscillating between baseline skin temperature and 50°C. Previous studies using microneurography suggest that such slow changes in skin temperature should generate periodic activity in *both* quickly- and slowly-adapting thermonociceptors, in particular, slowly- and quickly-adapting C fiber nociceptors (Meyer and Campbell, 1981; Treede et al., 1995). The general assumption underlying the proposed “frequency-tagging” approach is that the slow periodic activation of heat nociceptors elicits periodic activity within higher-order neurons processing this thermonociceptive input, and that this synchronous neuronal activity should generate peaks in the EEG frequency spectrum at the frequency of stimulation (0.2 Hz) and its harmonics.

2 Methods

2.1 Participants

The study comprised three distinct experiments. Eight healthy volunteers (4 females, aged 24-33 years) took part in Experiment 1. Six healthy volunteers took part in Experiment 2 (2 females, aged 19-27 years). Six other healthy volunteers took part in Experiment 3 (2 females, aged 34-43 years). All participants were right-handed, and had no prior history of neurological or psychiatric disorders. Before taking part in one of the three experiments, participants were familiarized with the experimental setup and exposed to a small number of test stimuli. The study was approved by the local Ethics Committee and conformed to the latest version of the declaration of Helsinki. Written informed consent was obtained from all participants.

2.2 Temperature-controlled CO_2 laser stimulation

In all three experiments, thermal stimuli were generated using a temperature-controlled CO_2 laser stimulator. The power output of this stimulator is regulated online using a feedback control loop based on a continuous measurement of skin temperature at the site of stimulation performed using a radiometer collinear with the laser beam (Laser Stimulation Device, SIFEC, Belgium). A similar feedback-controlled device developed by Meyer et al. (1976) inspired the conception of this laser. Because the radiometer used in the present device is capable of obtaining a reliable estimate of target temperature with a very short integration time, the feedback loop is able to update laser power output at a very fast rate (500 Hz). The heat source is a 25 W radiofrequency-excited CO_2 laser (Synrad 48-2; Synrad, WA). Power control is achieved by pulse width modulation at a 5-kHz clock frequency. The stimuli are delivered through a 6 m optical fiber. By vibrating this fiber at some distance of the source, a quasi-uniform spatial distribution of radiative power within the stimulated area is obtained. At the end of the fiber, optics are used to collimate the beam. The optic used in the present study provided a 12 mm beam diameter at target site.

2.3 Experiment 1

The experiment was conducted in a dim and silent room. Participants were seated in a comfortable chair with the palm of one hand resting on a table. The laser stimulation consisted in a 0.2 Hz sinusoidal variation of skin temperature, oscillating between baseline skin temperature (32.6 ±1.7°C; mean ± sd) and 50°C. The temperature of 50°C was chosen as this temperature is above the thermal activation threshold of both slowly- and quickly-adapting thermonociceptors. The frequency of 0.2 Hz was chosen because microneurography studies performed in human and animals suggest that this should elicit periodic activity in both slowly- and quickly-adapting thermonociceptors (Treede et al., 1995), in particular, slowly-adapting C fiber thermonociceptors (Meyer and Campbell, 1981). The duration of the sustained periodic heat stimulus was 75 s (15 cycles each lasting 5 s). The target of the laser beam on the skin was displaced slightly during the descending part of each stimulation cycle in order to avoid sensitization or habituation of heat-sensitive afferents. A total of 20 stimuli were delivered on each hand, in two separate blocks. Each block lasted ± 40 min.

2.3.1 EEG recording—The EEG was recorded using 64 Ag-AgCl electrodes placed on the scalp according to the international 10/10 system (Waveguard64 cap, Cephalon A/S, Denmark). Electrode impedances were kept below 10 kΩ. Ocular movements and eye blinks were recorded using two pairs of electrodes, the first placed above and below the right eye, the second placed at the external corner of the left and right eyes. Signals were amplified and digitized using an average reference and a sampling rate of 500 Hz.

2.3.2 EEG signal preprocessing—Analysis of the EEG data was carried out using Letswave 6 (http://nocions.org/letswave) (Mouraux and Iannetti, 2008). The continuous EEG recordings were filtered using a 0.05 Hz high-pass Butterworth filter to remove slow drifts in the recorded signals. Non-overlapping EEG epochs were then obtained by segmenting the recording from 0 to 75 s relative to the onset of the stimulation trains. Each EEG epoch was demeaned using the time-interval ranging from 0 to 75 s. Artifacts due to eye blinks or eye movements were then removed using a validated method based on an Independent Component Analysis (FastICA algorithm) (Hyvarinen and Oja, 2000). Finally, epochs containing artifacts exceeding 500 μv were rejected from further analyses. After this rejection, 17.5±2.7 trials remained when stimulating the left hand and 18.0±3.5 trials remained when stimulating the right hand.

2.3.3 Frequency-domain analysis—For each subject and stimulated hand, artifact-free EEG epochs obtained when stimulating the left and right hand were averaged. The obtained average waveform lasting 75 s was then transformed in the frequency domain using a discrete Fourier transform (FFTW) (Frigo and Johnson, 1998), yielding an amplitude spectrum (μV) ranging from 0 to 250 Hz with a frequency resolution of 0.013 Hz (Bach and Meigen, 1999). Within the obtained frequency spectra, the signal amplitude at 0.2 Hz and at harmonic frequencies (0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 Hz) was measured at each EEG electrode. These measures may be expected to correspond to the sum of the stimulus-evoked periodic EEG response and unrelated residual background noise. Therefore, to obtain valid estimates of the magnitude of the periodic EEG responses, the contribution of this residual noise was removed by subtracting, at each electrode and at each frequency bin, the average amplitude of the signal measured at neighboring frequencies (-0.026 to -0.065 Hz and +0.025 to +0.065 Hz) (Mouraux et al. 2011). In the absence of a periodic EEG response, the noise-subtracted average signal amplitude may be expected to tend towards zero. Hence, to assess the significance of the responses measured at each electrode, a non-parametric Wilcoxon signed-rank test was used to determine whether the magnitude of the noise-subtracted signal amplitude at the base frequency (0.2 Hz) and at harmonic frequencies (0.4 Hz, 0.6 Hz, 0.8 Hz, 1.0 Hz, 1.2 Hz, and 1.4 Hz), averaged across all scalp channels, were significantly greater than zero (SPSS 18, IBM, USA). Significance level was set at $p<0.05$.

To assess the temporal dynamics of the periodic EEG response elicited by the long lasting stimulation trains, an additional set of epochs was computed by segmenting the 75 s waveforms into three successive segments of 25 s. After applying the same analysis procedure, noise-subtracted signal amplitudes obtained in the first segment were compared to the noise-subtracted amplitudes obtained in the third segment using a Wilcoxon matched-pairs test. Significance level was set at $p<0.05$

2.3.4 Hilbert analysis—Previous studies have shown that the perception of sustained pain can be related to sustained changes in the magnitude of ongoing EEG oscillations within different frequency bands (Giehl et al., 2014, Peng et al., 2014, Schulz et al., 2015, Zhang et al., 2016). Therefore, we also examined whether the periodic heat stimulus generated a periodic modulation of the amplitude of ongoing EEG oscillations within the different frequency bands of the EEG frequency spectrum. For this purpose, a band-pass Butterworth filter was applied to the unaveraged EEG epochs to retain the EEG signal within theta (4-8 Hz), alpha (8-12 Hz), beta (12-40 Hz), and gamma (40-100 Hz) frequency bands. A Hilbert transform was then applied to the EEG epochs such as to estimate the envelope of the signal within these frequency bands. Finally, the obtained epochs were averaged across trials, and transformed in the frequency domain using a discrete Fourier transform (FFTW). A Wilcoxon signed-rank test was used to determine whether the magnitude of the noise-subtracted 0.2 Hz amplitude modulation of theta, alpha, beta, and gamma band oscillations, averaged across all scalp channels, was significantly greater than zero. Significance level was set at $p<0.05$

2.3.5 Topographical analysis—Hemispheric lateralization of the obtained EEG responses was assessed using a Wilcoxon matched-pairs test comparing the responses obtained at ipsilateral vs. contralateral central electrodes (C3/C4). Significance level was set at $p<0.05$

2.3.6 Time-domain analysis—In order to assess the lag between the periodic heat stimulation of the skin and the periodic EEG response, each 75-s EEG epoch was segmented in a succession of 15 epochs lasting 5 s and corresponding to each stimulation cycle. These epochs were then averaged in the time domain in order to reveal the time course of the stimulus-induced fluctuations of the EEG signal. In the absence of a lag between the 0.2 Hz oscillations of skin temperature and the 0.2 Hz oscillations of the EEG signals, the peak of EEG activity should coincide with the peak of heat stimulation. A Wilcoxon matched-pairs test was used to determine whether the latency of the peak of the EEG signal was significantly different from the latency of the peak of skin heat stimulation. Significance level was set at $p<0.05$

2.4 Experiment 2: A fiber block

The aim of this second experiment was to determine the respective contribution of myelinated and unmyelinated nociceptors to the periodic EEG responses elicited by the sustained 0.2 Hz sinusoidal variation of skin temperature between baseline and 50°C.

2.4.1 A fiber nerve conduction block—To block selectively the conduction of myelinated fibers, pressure was applied to the left superficial branch of the radial nerve, at the level of the wrist (Magerl et al., 1999; Nahra and Plaghki, 2003; Mouraux et al., 2010). Participants were comfortably seated in a chair with the left forearm immobilized on an armrest with a handle bar in their left hand preventing any pronation or supination of the hand and forearm. The pressure was delivered by means of two 700 g weights hanging at each end of a 1.5 cm rubber band applied on the forearm proximally to the wrist. Development of the nerve conduction blockade was monitored by assessing, every 10

minutes, the ability of the participants to detect five tactile stimuli (calibrated nylon filament exerting a target force of 5.9 mN; Semmens-Weinstein Von Frey Aesthesiometers, evaluator size 3.84, Stoelting Co, USA) and five short-lasting CO_2 laser stimuli (50°C stimuli lasting 100 ms, delivered using the temperature-controlled infrared CO_2 laser stimulator). All test stimuli were delivered to the skin innervation territory of the superficial radial nerve. The block was considered as established when, within the area of hypoesthesia, participants were both no longer able to detect 4/5 tactile stimuli *and* no longer able to detect 4/5 laser heat stimuli with reaction-times (RT) shorter than 650 ms. When stimulating the hand dorsum, a cutoff of 650 ms can be used to distinguish detections triggered by input conveyed by myelinated A fibers (RT < 650 ms) vs. input conveyed by unmyelinated C fibers (RT ≥650 ms) (Churyukanov et al., 2012). During the assessment the view of the hand was prevented.

Once the block was established, EEG responses to the sustained 0.2 Hz sinusoidal heat stimulation of the area of hypoesthesia were recorded in each participant. The thermal stimulation profile was identical to the stimulation profile used in Experiment 1. A total of 15 trials were recorded. At the end of this EEG recording, persistence of the A fiber block was verified using the testing procedure used to assess development of the block. The A fiber block was then released. To allow a within-subject assessment of the effect of the A fiber block, EEG responses to the sustained 0.2 Hz periodic heat stimulation were also obtained from the contralateral hand of each participant. In half of the participants, these responses were collected before collecting the EEG responses from the blocked hand, i.e. while waiting for the block to establish itself. In the other half, EEG responses from the contralateral hand were collected immediately after collecting the EEG responses from the blocked hand. Finally, to assess the effect of the block on the percept elicited by sustained periodic heat stimulation, participants were asked to rate the intensity of the percept elicited by the first, seventh and last trial of each of the two EEG sessions using a numerical rating scale between 0 (minimum reported intensity) and 10 (maximum reported intensity). Each EEG session lasted 20-30 minutes.

The EEG was recorded using 32 Ag-AgCl electrodes placed on the scalp according to the international 10/10 system (Waveguard64 cap, Cephalon A/S, Denmark). Signal preprocessing and analysis were identical to those performed in Experiment 1. After artifact rejection, 11.5±2.59 trials remained when stimulating the blocked hand and 11.67±3.27 trials remained when stimulating the control hand.

Based on the scalp topographies of the responses recorded in Experiment 1, a Wilcoxon signed-rank test was used to determine whether, at electrode Cz, the magnitude of the noise-subtracted signal amplitude at 0.2 Hz in the frequency domain analysis was significantly greater than zero. The same test was used to determine whether, at the contralateral central electrode C3 or C4, the magnitude of the noise-subtracted amplitude of the 0.2 Hz modulation of theta, alpha, and beta band oscillations was significantly greater than zero.

In addition, Wilcoxon matched-pairs tests were used to compare the intensity of perception, the magnitude of the 0.2 Hz periodic EEG response at electrodes Cz, and the magnitude of the 0.2 Hz modulation of theta, alpha, and beta band oscillations at the contralateral central electrode C3 or C4, during stimulation of the blocked and control hands. Furthermore, to

assess whether the A fiber block had an effect on the time course of the elicited EEG response, a cross-correlation analysis was performed on the EEG signals averaged across stimulation cycles. A Wilcoxon signed-rank test was used to assess whether the lag at maximum correlation was significantly different from 0. Significance level was set at $p<0.05$.

2.5 Experiment 3: time course of heat perception

The aim of this third experiment was to assess the time course of the heat percept elicited by the 0.2 Hz sinusoidal variation of skin temperature used in Experiments 1 and 2. The thermal stimulation profile was identical to the stimulation profiles used in the other experiments except for the fact that the stimulation duration was 25 s (five cycles). All stimuli were delivered to the left hand dorsum. During stimulation, the participant was asked to evaluate continuously the intensity of perception by manipulating a 10 cm vertical slider with their right hand. The lower end of the slider was defined as “not perceived” and the upper end of the slider was defined as “maximum intensity”. Participants were asked to move the slider button continuously according to the perceived intensity. The position of the slider as well as the temperature of the skin measured by the laser stimulator was digitized at 1000 Hz using an analog/digital converter (USB 1208HS-40A, Measurement Computing, MA). The displacements of the slider used to monitor the changes in intensity of perception were normalized for each participant to obtain ratings of intensity of perception extending between 0 (minimum reported intensity) and 1 (maximum reported intensity). To evaluate the temporal dynamics of the intensity of perception elicited by the 25 s of stimulation, the normalized ratings were averaged across trials. A Wilcoxon signed rank test were then used to compare the maximum intensity of perception elicited by the first cycle of skin heating with the maximum intensity of perception elicited by the last cycle of skin heating. Significance level was set at $p<0.05$. Moreover, to assess the lag between perception and skin heating, a cross-correlation analysis was performed between intensity rating and skin temperature waveforms. A Wilcoxon signed-rank test was used to assess whether the lag at maximum correlation was significantly different from 0. Significance level was set at $p<0.05$.

3 Results

3.1 Experiment 1

3.1.1 Frequency domain analysis—The sustained periodic sinusoidal oscillation of skin temperature between baseline skin temperature and 50°C elicited a marked increase of EEG power at the frequency corresponding to the frequency of skin temperature oscillation (0.2 Hz) and its harmonics (Fig.1A). The scalp topography of this periodic response was maximal at the scalp vertex, symmetrically distributed over the two hemispheres, and clearly identifiable in each participant.

After subtraction of the surrounding frequency bins to account for residual background noise, the magnitude of the periodic EEG response, averaged across all scalp channels, was significantly greater than zero at 0.2 Hz ($p=0.008$), 0.4 Hz ($p=0.008$), 0.8 Hz ($p =0.008$), 1.0 Hz ($p =0.008$), and 1.2 Hz ($p=0.02$) (Fig. 1B).

The magnitude of the 0.2 Hz EEG response averaged across all scalp channels remained constant over time. Indeed, the magnitude of the response recorded during the first 25 s of stimulation (0.17± 0.16μV) and during the last 25 s stimulation (0.15±0.12 μV) were not significantly different (p=0.74).

3.1.2 Hilbert analysis—A clear peak at 0.2 Hz was noted in the theta, alpha and beta frequency bands (Fig. 2A). No peak was observed in the gamma frequency band. After subtraction of the surrounding frequency bins to account for residual background noise, the magnitude of the 0.2 Hz periodic modulation of ongoing EEG oscillations was significantly greater than zero in the theta (p=0.02), alpha (p=0.008) and beta (p=0.008) bands, but not in the gamma band (p=0.19). The group-level average scalp topographies of these responses were maximal over the central-parietal regions contralateral to the stimulated hand (Fig. 2B). This hemispheric lateralization was confirmed by the results of the Wilcoxon matched-pairs test showing that the response was significantly greater at the contralateral vs. ipsilateral central electrode (C3/C4) in the alpha (p=0.02) and beta (p=0.008) frequency bands, but not in the theta band (p=0.19) or gamma band (p=0.38).

3.1.3 Time-domain analysis—There was a clear lag between the 0.2 Hz oscillation of skin temperature and the 0.2 Hz variation of the EEG signal (Fig. 3). On average, the EEG signal peaked 1.0±0.3 s after the peak of heat stimulation. This lag was significant (p=0.008).

3.2 Experiment 2: A fiber block

3.2.1 Frequency domain analysis—The sustained periodic sinusoidal oscillation of skin temperature between baseline skin temperature and 50°C elicited a marked increase of EEG power at 0.2 Hz and harmonics both when the stimulus was applied to the blocked hand and when it was applied to the control condition (Fig. 4A). After subtraction of the surrounding frequency bins to account for residual background noise, the magnitude of this periodic EEG response, measured at electrode Cz, was significantly greater than zero both in the block condition and in the control condition at 0.2 Hz (Wilcoxon signed-rank test: block condition p=0.03, control condition, p=0.03). There was no significant difference between the amplitudes of the responses obtained from the two hands (Wilcoxon matched-pairs test, all p=1).

3.2.2 Hilbert analysis—Such as in Experiment 1, a clear peak at 0.2 Hz and harmonics was noted for theta, alpha and beta frequency bands (Fig. 4B). After subtraction of the surrounding frequency bins to account for residual background noise, the Wilcoxon signed-rank tests showed that the magnitude of the 0.2 Hz modulation of theta, alpha and beta band oscillations measured at the contralateral central electrode C3 or C4 was significantly greater than zero when stimulating the control hand in the alpha band (p=0.03) and marginally greater than zero in the beta band (p=0.06) and in the theta band (p=0.06). In the block hand, the magnitude of the 0.2 Hz modulation was not greater than zero in the alpha band (p=0. 22), marginally different from 0 in the beta band (p=0.06) and not in the theta band (p=0.31). There was no significant difference in the magnitude of the responses elicited by stimulation

of the blocked vs. control hands in the alpha, beta and theta band (Wilcoxon matched-pairs test: all p>0.15).

3.2.3 Cross-correlation *analysis*—The cross-correlation analysis showed that, on average, the time course of the periodic 0.2 Hz EEG responses recorded in the block vs. control conditions were highly similar (Fig. 5A). The lag at maximum correlation (r=0.75±0.14) between the time courses of the EEG responses recorded in the block vs. control conditions was - 0.003±0.13 (Fig. 5B). This lag was not significantly different from zero (Wilcoxon signed-rank test: p=0.84).

The intensity of perception in the block condition 6.2±2.0 and in the control condition 6.3±2.2 were not significantly different (Wilcoxon matched-pairs test: p=1).

3.3 Experiment 3: time course of heat perception

The intensity of the heat percept generated by the 0.2 Hz sinusoidal variation of skin temperature remained stable during the entire 25 seconds of stimulation (Fig. 6). The maximum intensity of the percept elicited by the first stimulation cycle and the last stimulation cycle were not significantly different (Wilcoxon matched-pairs test: p=0.09). Such as for the EEG response observed in Experiment 1, there was a clear lag between the 0.2 Hz oscillation of skin temperature and the intensity of perception ratings (Fig. 6). On average, the maximum intensity of perception was reported 1.5 s after the peak of heat stimulation. The cross-correlation analysis showed that the lag at maximum correlation between rating and skin temperature waveforms (1.5±0.3 s) was significantly greater than zero (p=0.031).

4 Discussion

Slowly and periodically increasing and decreasing skin temperature during 75 seconds at a rate of 0.2 Hz elicited, in all participants, a robust periodic EEG response at the frequency of stimulation and its harmonics. The high signal-to-noise ratio of the obtained EEG response is explained by the fact that it is concentrated within very narrow frequency bands (0.2 Hz and its harmonics). Importantly, this response was entirely unaffected by blocking the conduction of myelinated A fibers, indicating that ultra-slow periodic heat stimulation of the skin can be used to reliably “frequency tag” cortical activity related predominantly to the sustained activation of C fiber thermonociceptors.

The scalp topography of the 0.2 Hz EEG response was maximal over frontal-central electrodes, and symmetrically distributed over the two hemispheres. This observation is in line with the results of our previous studies, in which EEG responses to the rapid succession of brief laser pulses (Colon et al., 2014; Mouraux et al., 2011), or to the rapid repetition of intra-epidermal electrical stimuli (Colon et al., 2012) also displayed a symmetric scalp distribution maximal over frontal-central electrodes. Although, one can only speculate on the cortical generators of these responses due to the spatial uncertainty inherent to scalp EEG, we showed in a previous study that this scalp topography could be explained by activity originating from midline brain structures such as the anterior cingulate cortex (ACC) and/or deep lateral brain structures such as the left and right operculo-insular cortices (Mouraux et

al., 2011). This is also compatible with the results of functional neuroimaging studies having shown that the application of sustained heat using a contact thermode elicits a significant increase of regional cerebral blood flow within the anterior cingulate, as well as the left and right operculo-insular cortices (Peyron et al., 2000).

Sustained ultra-slow periodic activation of thermonociceptors also elicited a periodic modulation of EEG power in the theta, alpha and beta frequencies. Interestingly, these responses showed a significant hemispheric lateralization, being maximal over the central and parietal areas contralateral to the stimulated hand. These results are compatible with the results of previous EEG studies having shown that tonic pain delivered to the hand can induce a sustained and asymmetric reduction of alpha-band power maximal over contralateral central areas, and hypothesized to originate at least in part from the contralateral sensorimotor cortex (Peng et al., 2014, Zhang et al., 2016). Whereas other studies have shown that tonic pain can also induce an enhancement of higher-frequency gamma-band oscillations (Peng et al., 2014, Schulz et al., 2015), no significant periodic modulation of gamma-band oscillations was observed in the present study.

The lack of any effect of the A fiber nerve conduction blockade on both the EEG responses and the heat percept elicited by the sustained ultra-slow periodic modulation of skin temperature demonstrates that these responses are essentially conveyed by heat-sensitive C fiber nociceptors. This could be explained by differences in the response properties of slowly-adapting Aδ and C fiber thermonociceptors. Slowly-adapting Aδ thermonociceptors (AMH-1) respond to sustained thermal stimulation such as heating the skin to 53°C during 30 s (Schepers and Ringkamp, 2010; Treede et al., 1995; Treede et al., 1998). However, discharge within these fibers is markedly delayed relative to the onset of heat stimulation (average response latency ± 5 s), and gradually increases over time to reach maximum firing rates towards the end of the 30 s stimuli. Therefore, at 0.2 Hz, the periodic changes in skin temperature might still be too transient to elicit consistent periodic activity within these afferents. Such as AMH-1, slowly-adapting C fibers respond gradually to sustained heat, and exhibit little or no adaptation when the heat stimulus is maintained over time (Meyer and Campbell, 1981; Schepers and Ringkamp, 2010; Wooten et al., 2014). However, the response latency of slowly-adapting C fibers appears to be, on average, shorter than the response latency of AMH-1. For example, Meyer and Campbell (1981) found that the maximum response of slowly-adapting C fibers occurred approximately 1 s after the onset of the heat stimulus, and adapted slowly to reach a stable level after 10 s (Meyer and Campbell, 1981). For this reason, it is likely that varying skin temperature at a 0.2 Hz frequency generates strong periodically-modulated afferent activity in slowly adapting C fibers, but not in slowly-adapting Aδ fibers.

Further supporting the view that the EEG responses elicited by a 0.2 Hz modulation of skin temperature are conveyed predominantly by C fibers is the finding that the A fiber conduction blockade did not exert any effect on the time course of the elicited EEG responses. Specifically, the absence of any lag between the EEG responses elicited by stimulation of the blocked and control hands indicates that the afferent fibers generating the responses had similar conduction velocities in both conditions. Moreover, in Experiment 1, both the peak of the EEG response and the peak of heat perception were delayed by

approximately 1-1.5 seconds relative to the peak of skin heating. This lag is compatible with the slow conduction velocity of C fibers (0.5-2 m/s) but not with the higher conduction velocity of Aδ fibers (12-30 m/s) (Almeida et al., 2004). Taken together, the results of the three experiments suggest that both the EEG activity and the perception elicited by the 0.2 Hz sinusoidal heating of the skin were predominantly mediated by slowly-adapting and/or quickly-adapting C fibers thermonociceptors(s).

The proposed EEG “frequency tagging” approach (Regan, 1989) to isolate cortical activity related to the sustained periodic activation of thermonociceptors could constitute a mean to study the cortical processes involved in the perception of tonic heat pain, with potential clinical applications. Because transient heat stimuli activate almost exclusively quickly-adapting thermonociceptors whereas long-lasting heat stimuli generate sustained activity within slowly-adapting thermonociceptors, the processes involved in the perception of tonic heat could differ from those involved in the perception of transient heat. Moreover, slowly-adapting thermonociceptors have been related to the development of heat hyperalgesia, and could thus play a prominent role in the development of chronic pathological pain. For example, Wooten et al. (2014) found that, following a mild burn injury, slowly-adapting C fiber thermonociceptors sensitize more than quickly-adapting C fiber thermonociceptors. Furthermore, considering that the innervation density of C fibers is far greater than that of Aδ fibers, C fiber thermonociceptors are probably the main mediators of the primary hyperalgesia induced by tissue injury or inflammation (Campbell and Meyer, 1983; Lamotte et al., 1983). This periodic EEG approach could thus be used to explore the cortical processing of tonic heat pain and the involvement of C fibers in various pathological pain conditions.

The present study is not our first attempt at recording steady-state EEG responses to periodic heat stimulation of the skin. In two previous studies, we recorded EEG responses to periodic heat stimulation using a much higher frequency of stimulation (6-7 Hz) (Colon et al., 2014; Mouraux et al., 2011). In these studies, brief laser or electrical pulses were applied onto the skin. Consequently, these periodic EEG responses were predominantly related to the activation of quickly-adapting AMH-2. The signal-to-noise ratio of the elicited responses in these studies was also very low. In contrast, the EEG responses obtained in the present study were characterized by a very high signal-to-noise ratio, and by the fact that the responses were clearly identifiable in each participant. This difference could be explained by the fact that, as compared to short-lasting heat stimulation cycles, long-lasting heat stimulation cycles activate a greater number of heat-sensitive afferents, in particular, slowly-adapting C fiber thermonociceptors. Furthermore, the magnitude of steady-state EEG responses is determined not only by the strength of the afferent input but also by the phase synchronization of the afferent input when it reaches the cortex as well as the consistency of its phase over the repeated cycles. The time course of the afferent input generated by heating the skin is dependent on the initial transduction of heat into neural impulses by heat-sensitive nociceptive afferents. Variability in the time course of activation of individual afferents could thus smear the periodicity of the afferent input generated by the stimulus. Furthermore, cycle-by-cycle variations in heat transfer to the skin as well as variations in the conduction velocity of individual fibers may constitute important additional sources of within-trial and inter-trial variability. These different sources of temporal jitter can be

expected to have much more impact on the phase consistency of afferent input elicited by high-frequencies of stimulation, and could thus also explain why robust steady-state EEG responses are obtained when very low frequencies of stimulation are used, but not when high frequencies of stimulation are used.

5 Conclusion

In conclusion, we show that very slow periodic heat stimulation of the skin elicits a reliable and robust periodic EEG response having a symmetric scalp topography maximal over frontal-central electrodes. In addition, we show that this periodic heat stimulus also induces a periodic modulation of the magnitude of ongoing EEG oscillations in theta, alpha and beta frequency bands. Interestingly, these responses were lateralized, and maximal over the central and parietal regions contralateral to the stimulated hand. The lack of any effect of an A fiber block on the amplitude and latency of these EEG responses indicates that they predominantly reflect activity conveyed by unmyelinated C fibers. This EEG “frequency tagging” approach could be used in future studies to explore the cortical processes underlying the perception of tonic heat in physiological and pathological pain conditions.

Acknowledgments

AM is supported by an ERC Starting Grant awarded to André Mouraux (PROBING-PAIN, 336130). EC is supported by the Belgian American Educational Foundation (B.A.E.F.) and the Wallonie-Bruxelles International - World Excellence Fellowship (W.B.I., Belgium). GL is supported by the Belgian Fund for Scientific Research (F.R.S.- FNRS, Belgium). These funds did not exert any editorial direction or censorship on any part of this manuscript.

References

Almeida TF, Roizenblatt S, Tufik S. Afferent pain pathways: a neuroanatomical review. Brain Res. 2004; 1000:40–56. [PubMed: 15053950]

Bach M, Meigen T. Do's and dont’s in Fourier analysis of steady-state potentials. Doc Ophthalmol. 1999; 99:69–82. [PubMed: 10947010]

Bromm B, Jahnke MT, Treede RD. Responses of human cutaneous afferents to CO2 laser stimuli causing pain. Exp Brain Res. 1984; 55(1):158–166. [PubMed: 6086371]

Campbell JN, Meyer RA. Sensitization of unmyelinated nociceptive afferents in monkey varies with skin type. J Neurophysiol. 1983; 49:98–110. [PubMed: 6298377]

Churyukanov M, Plaghki L, Legrain V, Mouraux A. Thermal detection thresholds of ad and C-fibre afferents activated by brief CO_2 laser pulses applied onto the human hairy skin. PLoS ONE. 2012; 7(4):e35817. [PubMed: 22558230]

Colon E, Nozaradan S, Legrain V, Mouraux A. Steady-state evoked potentials to tag specific components of nociceptive cortical processing. Neuroimage. 2012; 60:571–581. [PubMed: 22197788]

Colon E, Legrain V, Mouraux A. EEG frequency-tagging to dissociate the cortical responses to nociceptive and non-nociceptive stimuli. J Cog Neurosci. 2014; 26:2262–2274.

Cruccu G, Sommer C, Anand P, Attal N, Baron R, Garcia-Larrea L, Haanpaa M, Jensen TS, Serra J, Treede R-D. EFNS guidelines on neuropathic pain assessment: revised 2000. Eur J Neurol. 2000; 17:1010–1018.

Frigo, M., Johnson, SG. FFTW: an adaptative software architecture for the FFT. Paper presented at the International Conference of Acoustics, Speech, and Signal Processing; Seattle, WA: 1998.

Garcia-Larrea L, Frot M, Valeriani M. Brain generators of laser-evoked potentials: from dipoles to functional significance. Clin Neurophysiol. 2003; 33:279–292.

Giehl J, Meyer-Brandis G, Kunz M, Lautenbacher S. Responses to tonic heat pain in the ongoing EEG under conditions of controlled attention. Somatosensory and Motor Research. 2014; 31(1):40–48. [PubMed: 24320554]

Hyvarinen A, Oja E. Independent component analysis: algorithms and applications. Neural Networks. 2000; 13:411–430. [PubMed: 10946390]

Kandel, ER., Schwartz, JH., Jessell, TM. Principles of neural science. 4th ed. McGraw-Hill, Health Professions Division; New York: 2000.

Lamotte RH, Thalhammer JG, Robinson CJ. Peripheral neural correlates of magnitude of cutaneous pain and hyperalgesia: a comparison of neural events in monkey with sensory judgement in human. J Neurophysiol. 1983; 50:1–26. [PubMed: 6875640]

Magerl W, Ali Z, Ellrich J, Meyer RA, Treede RD. C- and A delta-fiber components of heat-evoked cerebral potentials in healthy human subjects. Pain. 1999; 82:127–137. [PubMed: 10467918]

Mouraux A, Iannetti GD. Across-trial averaging of event-related EEG responses and beyond. Mag Reson Imaging. 2008; 26:1041–1054.

Mouraux A, Iannetti GD, Plaghki L. Low intensity intra-epidermal electrical stimulation can activate Ad-nociceptors selectively. Pain. 2010; 150:199–207. [PubMed: 20510515]

Mouraux A, Iannetti GD, Colon E, Nozaradan S, Legrain V, Plaghki L. Nociceptive steady-state evoked potentials elicited by rapid periodic thermal stimulation of cutaneous nociceptors. J Neurosci. 2011; 31:6079–6087. [PubMed: 21508233]

Meyer RA, Walker RE, Mountcastle VB. A laser stimulator for the study of cutaneous thermal and pain sensations. IEEE T BIO-MED ENG. 1976; 23:54–60.

Meyer RA, Campbell JN. Evidence for two distinct classes of unmyelinated nociceptive afferents in monkey. Brain Res. 1981; 224:149–152. [PubMed: 7284829]

Nahra H, Plaghki L. The effects of A-fiber pressure block on perception and neurophysiological correlates of brief non-painful and painful CO2 laser stimuli in humans. Eur J Pain. 2003; 7:189–199. [PubMed: 12600801]

Peng W, HU L, Zhang Z. Changes of spontaneous oscillatory activity to tonic heat pain. PLos ONE. 2014; 9(3):e91052. [PubMed: 24603703]

Peyron R, Laurent B, Garcia-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiolo Clin. 2000; 30(5):263–288.

Regan, D. Human brain electrophysiology. Evoked potentials and evoked magnetics fields in science and medicine. New York, NY: Elsevier; 1989.

Schepers RJ, Ringkamp M. Thermoreceptors and thermosensitive afferents. Neurosci Biobehav R. 2010; 34:177–184.

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. Cerebral Cortex. 2015:1–8. [PubMed: 23926113]

Treede RD. Peripheral acute pain mechanisms. Ann Med. 1995; 27(2):213–216. [PubMed: 7632416]

Treede RD, Meyer RA, Raja SN, Campbell JN. Evidence for two different heat transduction mechanisms in nociceptive primary afferents innervating monkey skin. J Physiol. 1995; 483:747–758. [PubMed: 7776255]

Treede RD, Meyer RA, Campbell JN. Myelinated mechanically insensitive afferents from Monkey hairy skin: heat-response properties. J Neurophysiol. 1998; 80:1082–1093. [PubMed: 9744923]

Wooten M, Weng HJ, Hartke TV, Borzan J, Klein AH, Turnquist B, Dong X, Meyer RA, Ringkamp M. Three functionally distinct classes of C-fiber nociceptors in primate. Nat Commun. 2014; 5:4122. [PubMed: 24947823]

Zhang CH, Sohrabpour A, Lu Y, He B. Spectral and spatial change of brain rhythmic activity in response to the sustained thermal pain stimulation. Human Brain mapping. 2016; 37:2976–2991. [PubMed: 27167709]

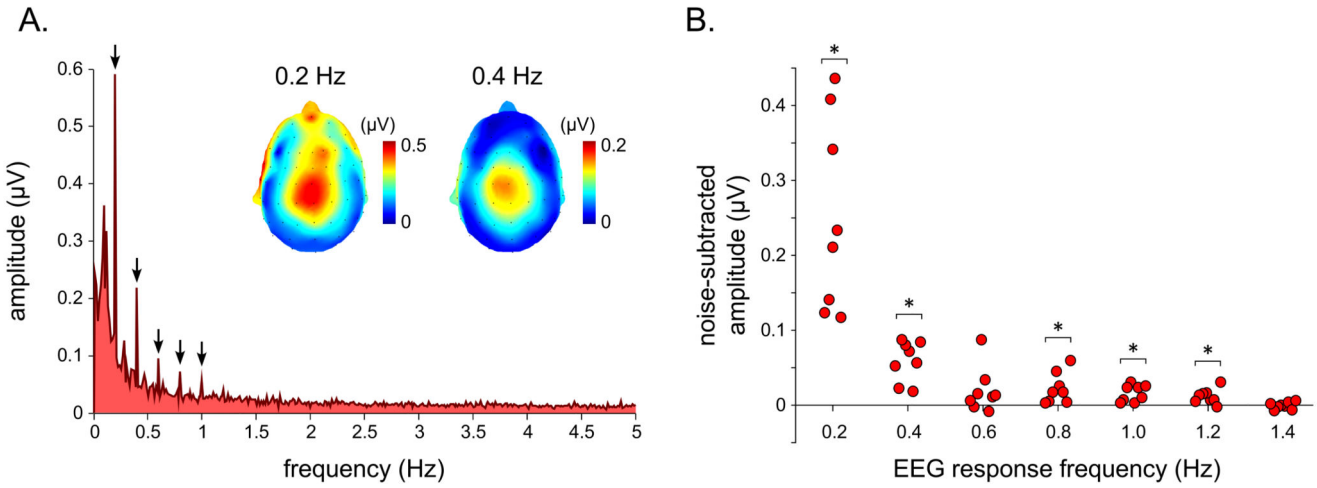

Figure 1.

Frequency-domain analysis of the periodic EEG response elicited by 0.2 Hz periodic heating of the skin to 50°C during 75 s (Experiment 1). *A*. Group-level average frequency spectrum of the signal measured at the scalp vertex (electrode Cz vs. an average reference). Note the clear peak in the EEG spectra at 0.2 Hz and its harmonics (vertical arrows). Note that the periodic EEG response is maximal at the scalp vertex, and symmetrically distributed over the two hemispheres, both at 0.2 Hz and 0.4 Hz. *B.* Group-level average noise-subtracted amplitude of the EEG response elicited at the base frequency (0.2 Hz) and harmonic frequencies (0.4 Hz, 0.6 Hz, 0.8 Hz, 1.0 Hz, 1.2 Hz and 1.4 Hz), averaged across all scalp channels, for each participant. In the absence of a periodic EEG response, noise-subtracted amplitudes should not be significantly different from zero. * p <.05 (Wilcoxon signed ranked test against zero).

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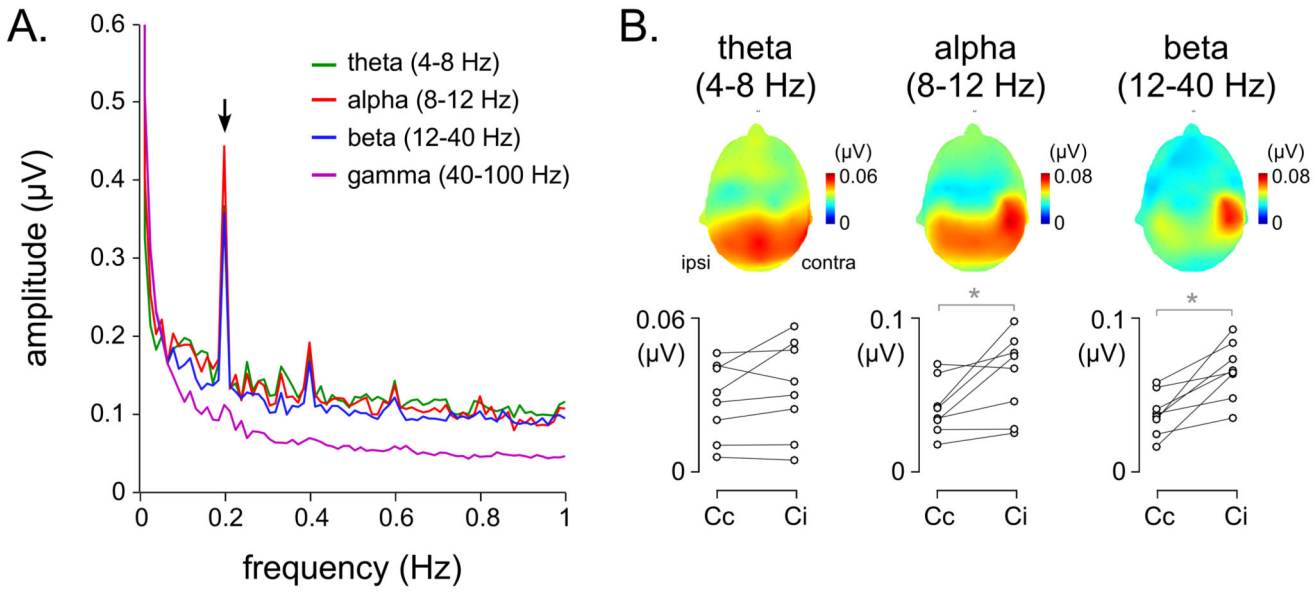

Figure 2.

Hilbert analysis of the periodic modulation of ongoing EEG oscillations elicited by 0.2 Hz periodic heating of the skin to 50°C during 75 s (Experiment 1). *A.* Group-level average frequency spectrum of the EEG signal within theta, alpha, beta and gamma frequency bands. Note the significant periodic modulation of EEG power in the theta, alpha and beta frequency bands, and the lack of modulation in the gamma frequency band. *B.* Group-level average scalp topography of the periodic 0.2 Hz modulation of theta-, alpha- and beta-band oscillations, and individual amplitudes of modulation measured at the central electrodes contralateral (Cc) vs. ipsilateral (Ci) to the stimulated hand (* p <.05; Wilcoxon matched pairs test). Note that alpha- and beta-band responses are maximal over the parietal area contralateral to the stimulated hand.

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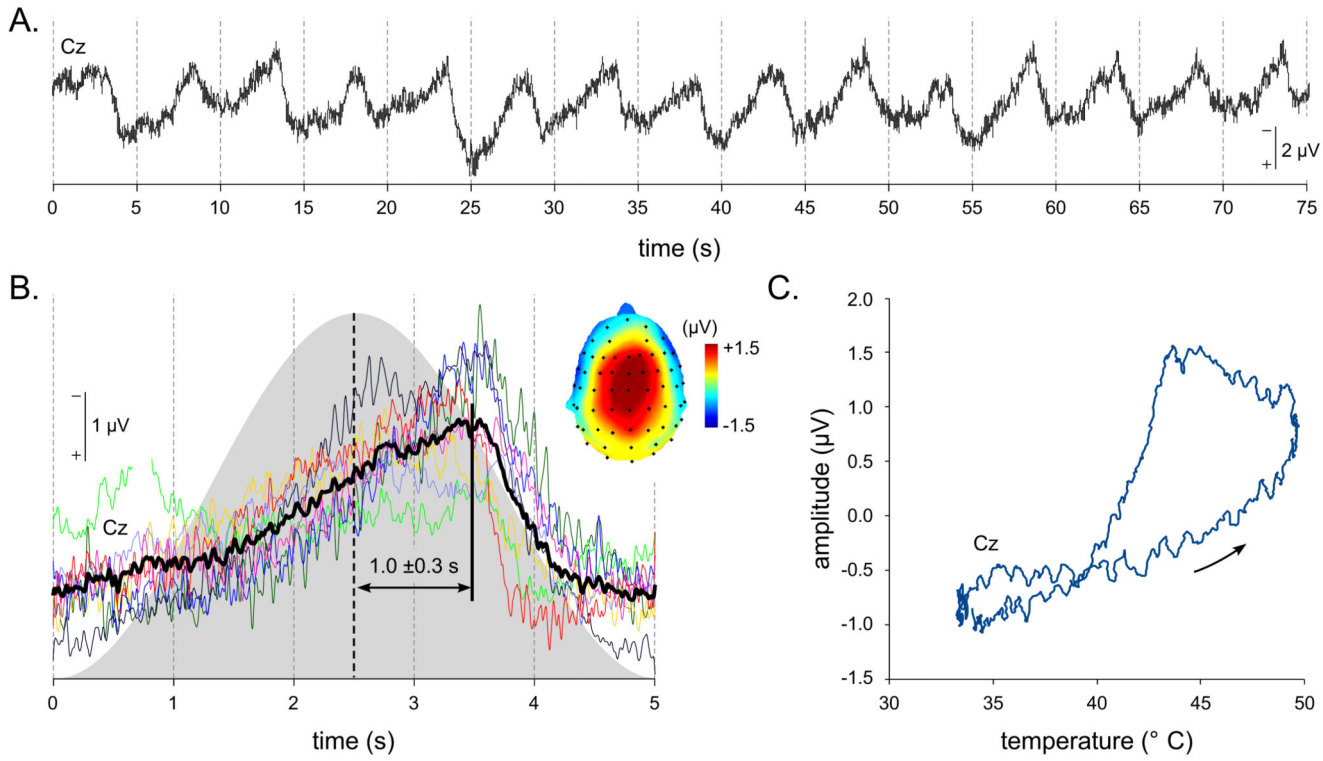

Figure 3.

Time-domain analysis of the periodic EEG response elicited by 0.2 Hz periodic heating of the skin to 50°C during 75 s (Experiment 1). *A.* Group-level average time course of the EEG signal measured during stimulation at electrode Cz vs. an average reference. Note the periodic 0.2 Hz variation of the EEG signal. *B.* Individual (colored waveforms) and group-level average (black waveforms) EEG signal measured at electrode Cz, obtained after averaging signals across stimulation cycles (5 seconds). Note that, as compared to the time course of heat stimulation (illustrated in light grey), the EEG response is delayed. On average, the peak of the periodic EEG response occurred 1.0 ±0.3 s after the peak of heat stimulation. The scalp map shows the topographical distribution of the EEG signal peak. *D.* Lissajous plot of the average time course of skin temperature vs. the group-level average EEG signal at electrode Cz.

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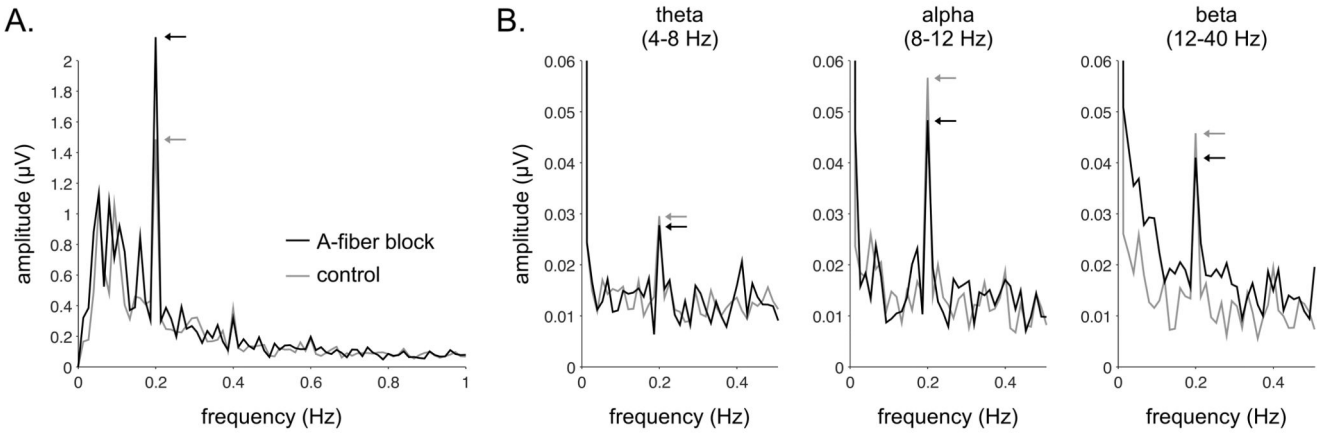

Figure 4.

Effect of an A fiber conduction block on the magnitude of the periodic EEG responses elicited by 0.2 Hz periodic heating of the skin to 50°C during 75 s (Experiment 2). *A.* Group-level average frequency spectrum of the EEG signal measured at electrode Cz vs. an average reference). *B.* Group-level average frequency spectrum of the envelope of the EEG signals measured at the contralateral central electrode (C3 or C4) and bandpass-filtered within theta, alpha, and beta frequency bands. Note the lack of effect of the A-fiber block on the magnitude of the elicited EEG responses.

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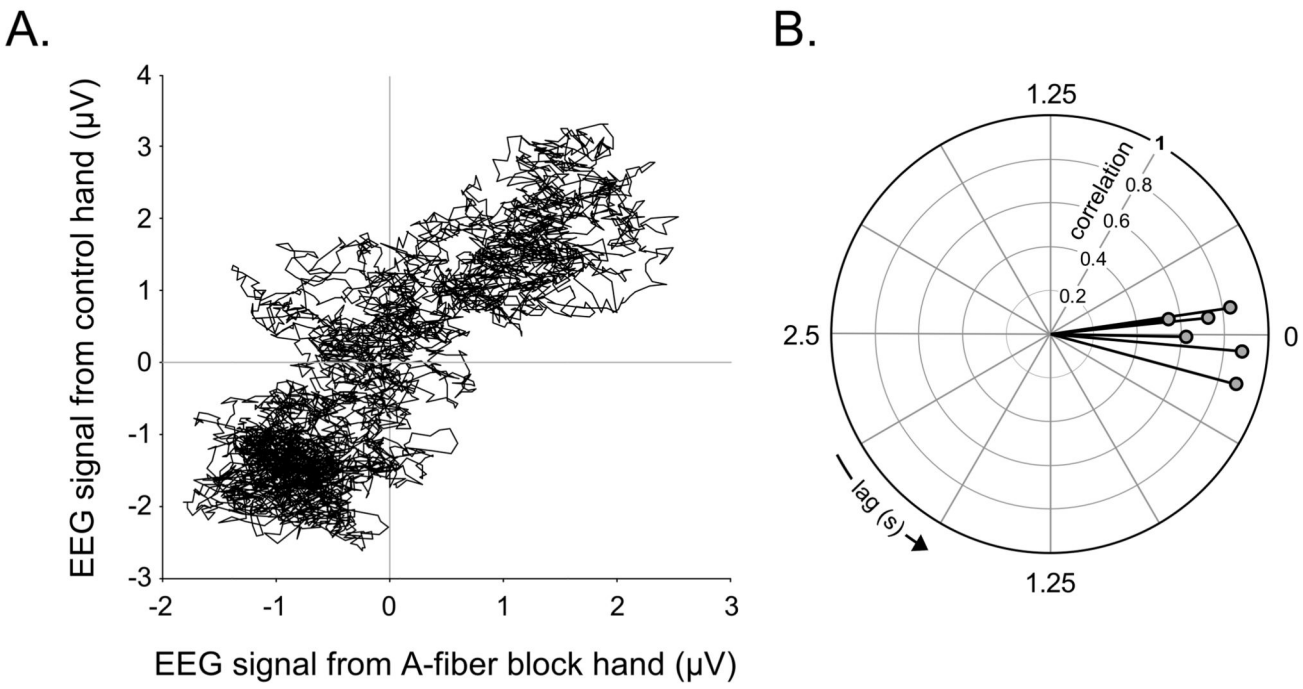

Figure 5.

Effect of an A fiber conduction block on the latency of the periodic EEG responses elicited by 0.2 Hz periodic heating of the skin to 50°C during 75 s (Experiment 2). *A.* Lissajous plot of the EEG signal measured during stimulation of the blocked hand (x-axis) vs. the contralateral hand (y-axis) in one representative subject. *B.* Maximum correlation and lag at maximum correlation between the EEG signals measured during stimulation of the blocked hand vs. stimulation of the control hand in each participant. Note that in all participants, the phase angle was close to zero, indicating the absence of a systematic lag between the two signals.

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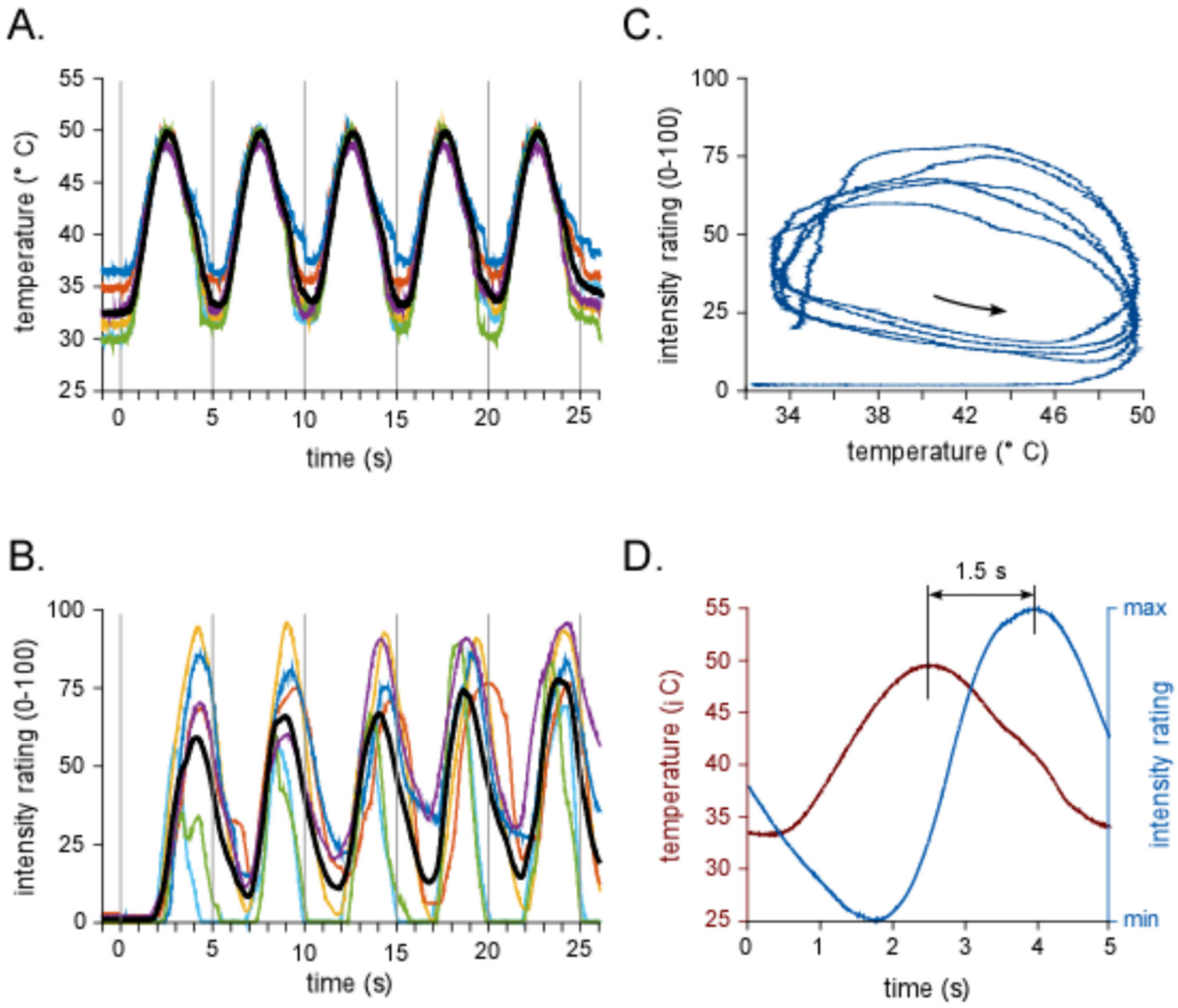

Figure 6.

Time course of the heat percept elicited by 0.2 Hz sinusoidal variation of skin temperature between baseline and 50°C during 25 seconds (Experiment 3). *A.* Time course of the changes in skin temperature on the hand during stimulation. *B.* Time course of intensity ratings during stimulation. The group-level averages are shown in black, and the individual waveforms for each participant are shown in color. *C.* Lissajous plot of the average variation in target skin temperature vs. the average variation in intensity ratings. *D.* Skin temperature and intensity ratings averaged across stimulation cycles (5 seconds). Note that the intensity ratings are significantly delayed relative to the heat stimulus. For each cycle, maximum intensity ratings were reported 1.5 ±0.5 s after the peak of heat stimulation.