EJN fens EUROPEAN JOURNAL OF NEUROSCIENCE

European Journal of Neuroscience, pp. 1–10, 2013 doi:10.1111/ejn.12304

Spectral signatures of viewing a needle approaching one's body when anticipating pain

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Keywords: autonomic nervous system, beamforming, electroencephalography, human, oscillations, pupil dilation response

Abstract

When viewing the needle of a syringe approaching your skin, anticipation of a painful prick may lead to increased arousal. How this anticipation is reflected in neural oscillatory activity and how it relates to activity within the autonomic nervous system is thus far unknown. Recently, we found that viewing needle pricks compared with Q-tip touches increases the pupil dilation response (PDR) and perceived unpleasantness of electrical stimuli. Here, we used high-density electroencephalography to investigate whether anticipatory oscillatory activity predicts the unpleasantness of electrical stimuli and PDR while viewing a needle approaching a hand that is perceived as one's own. We presented video clips of needle pricks and Q-tip touches, and delivered spatiotemporally aligned painful and nonpainful intracutaneous electrical stimuli. The perceived unpleasantness of electrical stimuli and the PDR were enhanced when participants viewed needle pricks compared with Q-tip touches. Source reconstruction using linear beamforming revealed reduced alpha-band activity in the posterior cingulate cortex (PCC) and fusiform gyrus before the onset of electrical stimuli when participants viewed needle pricks compared with Q-tip touches. Moreover, alpha-band activity in the PCC predicted PDR on a single trial level. The anticipatory reduction of alpha-band activity in the PCC may reflect a neural mechanism that serves to protect the body from forthcoming harm by facilitating the preparation of adequate defense responses.

Introduction

A common piece of advice by health professionals when administering an injection is 'to look away'. Support for this advice comes from a recent study that demonstrated that observing a needle pricking a hand that is perceived as one's own enhances the pupil dilation response (PDR) and perceived unpleasantness of pain (Höfle *et al.*, 2012). A particularly interesting finding was that the enhancement of the PDR started a few hundred milliseconds before the onset of electrical stimulation, suggesting that viewing a needle approaching one's body leads to an anticipatory increase of arousal. How the observation of an approaching needle while anticipating pain influences neural processes is, to date, unknown. Moreover, it is unknown whether these processes account for changes in the autonomic nervous system (ANS), as measured by the PDR.

Magneto- and electroencephalographic studies using non-naturalistic cues showed that anticipation of pain is reflected in oscillatory alpha-band (8–12 Hz) activity (Babiloni *et al.*, 2005a, 2006; May *et al.*, 2012). Using electroencephalography (EEG), Babiloni *et al.* (2005a, 2006) observed a reduction of alpha-band activity (ABA) at central scalp contralateral to the site of the expected stimulation during the anticipation of pain. Furthermore, pain anticipation has been found to increase ANS responses (Bitsios *et al.*, 2004; Höfle *et al.*, 2012; Seifert *et al.*, 2012) These findings demonstrate that the anticipation of painful stimuli can lead to both a reduction of ABA and an increase of ANS activity. To date, the interplay between ABA and ANS activity during pain anticipation has not been investigated.

A reduction of ABA has also been found in studies presenting static pictures of body parts in painful and nonpainful situations (Yang *et al.*, 2009; Perry *et al.*, 2010; Whitmarsh & Jensen, 2011). The reduction of ABA was stronger when participants viewed painful compared with nonpainful situations (Yang *et al.*, 2009; Perry *et al.*, 2010; Whitmarsh & Jensen, 2011; but see Mu *et al.*, 2008). Whitmarsh & Jensen (2011) showed that the effects on ABA originate from sensorimotor areas along the central sulcus. Moreover, functional magnetic resonance imaging studies found an involvement of limbic structures (Jackson *et al.*, 2005, 2006; Gu *et al.*, 2010; Lamm *et al.*, 2010). Threatening stimuli presented near the body are known to trigger a defense response, which enables the

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Received 25 May 2013, revised 3 June 2013, accepted 10 June 2013

organism to rapidly react to potentially aversive stimuli (e.g. Graziano & Cooke, 2006). The role of ABA in this context is unknown. Therefore, it is intriguing to study how viewing a needle approaching one's body while at the same time anticipating painful stimulation influences ABA in cortical networks.

In this combined EEG/PDR study, we mimicked a naturalistic situation by displaying a hand on a screen that was pricked by a needle or touched by a Q-tip. Participants placed their hand directly below the displayed hand so that they had the impression of looking at their own hand, i.e. they incorporated the hand. Clips of needle pricks and Q-tip touches were presented together with spatiotemporally aligned painful or nonpainful intracutaneous electrical stimuli for which intensity and unpleasantness ratings were obtained. Linear beamforming was applied to EEG data to examine the neural processes underlying the recently observed anticipatory modulation of the PDR when viewing needle pricks (Höfle *et al.*, 2012). To our knowledge, this is the first study to investigate the relationship between anticipatory neural activity, PDR, and pain perception while viewing painful stimulation inflicted upon incorporated body parts.

Materials and methods

Participants

Nineteen participants took part in the study after voluntarily providing written informed consent. One participant was excluded from the analysis due to extensive muscle artifacts in the EEG recordings. The data of the remaining 18 participants (mean age 25.2 ± 3.5 years; nine women) were subjected to further analysis. All participants had normal or corrected-to-normal vision and reported no history of neurological or psychiatric illness and no acute pain. Participants received monetary compensation for their participation. The study conformed to The Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the *British Medical Journal* (18 July 1964), and was approved by the Ethics Committee of the Medical Association of Hamburg, Germany.

Stimuli

In line with previous studies (e.g. Höfle *et al.*, 2012; Pomper *et al.*, 2013), the intracutaneous electrical model (Bromm & Meier, 1984) was used to induce painful and nonpainful stimuli. This model is especially suited to simulate needle pricks because painful intracutaneous stimuli evoke a stabbing and sharp sensation resembling a short needle prick. Electrical stimuli (16 ms duration) were applied to the tip of the participant's left index finger. Prior to each session, individual sensation and pain thresholds were determined. The sensation threshold was defined as the average intensity at which participants were able to detect a certain stimulus. The pain threshold was defined as the average intensity at which participants reported a given stimulus as painful. The thresholds were determined using five ascending and descending series of electrical stimuli with successive intensity changes of 0.02 mA. During the experiment, painful stimuli were presented at twofold pain threshold (mean, *M*, 0.33 ± 0.09 mA) and nonpainful stimuli at 1.5-fold sensation threshold (*M* = 0.12 ± 0.04 mA).

Visual stimuli comprised 36 naturalistic clips depicting the volar view of a left hand, the index finger of which was either pricked by a needle or touched by a Q-tip. Similar to previous experiments (e.g. Avenanti *et al.*, 2005; Azevedo *et al.*, 2012; Höfle *et al.*, 2012), both items were attached to a syringe (Fig. 1A). In accordance with our previous study (Höfle *et al.*, 2012), an additional clip of a hand alone was presented. Hand-alone trials were not included in the further analyses because they substantially differed from the needle and Q-tip clip trials, prohibiting the interpretation of effects, particularly with respect to PDR and EEG. For the same reason, we had refrained from comparing PDRs to the hand-alone clips with PDR to needle or Q-tip clips in our previous study (Höfle *et al.*, 2012). The presentation of each needle and Q-tip clip started with the first frame of the clip, which was presented for 0.8 s. The following 60 frames were presented at a rate of 60 Hz and the last frame of the clip was sustained on the screen for 1.2 s.

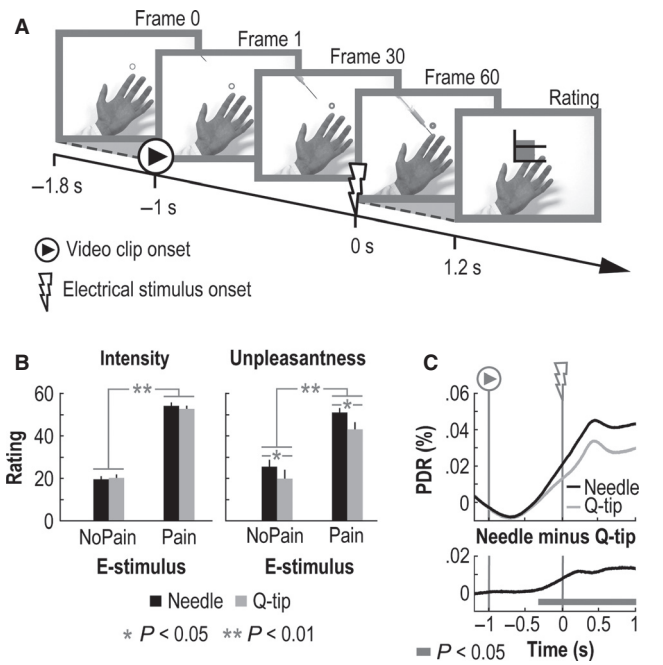

FIG. 1. Experimental setup and influence of visual stimulation on subjective ratings of electrical stimuli and PDR. (A) Illustration of a needle clip with electrical stimulation and rating. Simultaneously with the last frame, in which the needle pricked the skin, participants received a painful or a nonpainful electrical stimulus. The last frame was maintained on the screen for 1.2 s. Afterwards, participants rated the electrical stimulus on a two-dimensional scale (ordinate, intensity; abscissa, unpleasantness). Participants were asked to cross the horizontal line (visual analogue scale > 40) when an electric stimulus was perceived as being painful. The play symbol signifies video clip onset and the flash symbol signifies electrical stimulus onset. (B) Painful electrical stimuli were perceived as more intense (left panel) and unpleasant (right panel) than nonpainful stimuli. Furthermore, electrical stimuli were perceived as more unpleasant when participants viewed needle pricks compared with when they viewed Q-tip touches. The error bars depict the standard error of the mean. (C) PDR was larger when viewing needle pricks compared with viewing Q-tip touches (pooled across trials with painful and nonpainful electrical stimulation) starting at about 0.7 s after clip onset, i.e. −0.3 s before electrical stimulus onset.

Procedure

Participants were seated in front of an infrared eye-tracking system (iView X, SensoMotoric Instruments, Teltow, Germany) with their heads secured. Visual stimuli were spatiotemporally aligned with the intracutaneous electrical stimuli. Specifically, the participant's left hand was placed on a board mounted below a flat screen, so that the position of the hand matched the position of the incorporated hand (i.e. a hand that was perceived as one's own) on the screen (the setup has been illustrated elsewhere; Fig. 1A in Höfle *et al.*, 2012). Participants were instructed to imagine that the hand on the

screen would be their own. Each experimental trial started with the presentation of a clip (Fig. 1A). Simultaneously with the last frame depicting the needle that pricked or the Q-tip that touched the index finger of the incorporated hand, participants received a painful or nonpainful electrical stimulus at the index finger of their own hand. Throughout all clips, participants fixated a gray-shaded circle located above the left index finger. Together with the onset of the video clip, the circle filled from surrounding to center and was filled up when the electrical stimulus was presented 1 s after the clip onset. The filling circle was presented to ensure that the same temporal information about the occurrence of the electrical stimulus was provided in all clips. During each trial, pupil size was monitored from the left eye at a sampling rate of 500 Hz. Following the presentation of the last frame, participants rated the intensity and unpleasantness of the electrical stimulus on a two-dimensional visual analogue scale using a joystick in their right hand. The visual analogue scale, which was superimposed over the finger of the hand on the screen, ranged between 0 and 100 on the vertical intensity axis (0, no sensation; 40, beginning of pain experience, marked by a horizontal line; 100, most intense pain) and 0 and 100 on the horizontal unpleasantness axis (0, not unpleasant at all; 100, extremely unpleasant). The visual analogue scale remained on the screen for 2 s. As the rating procedure was trained beforehand, this time interval was sufficient to respond adequately. Prior to the experimental session, the experimenter instructed participants to rate the perceived intensity and unpleasantness of electrical stimuli, but not how intense or unpleasant the visual stimulation appeared. Each experimental session consisted of 15 blocks comprising 48 trials each; 50% of all needle, Q-tip or hand-alone trials were associated with painful stimulation (i.e. eight out of 16 trials per clip and block). Prior to each block, the eye-tracking system was calibrated and, after the experimental session, participants rated the degree of embodiment of the hand seen on the screen.

Embodiment questionnaire

To measure the degree of experienced embodiment of the hand viewed on the screen, a questionnaire was used that addressed factors predictive for the proprioceptive delusion observed in classic studies on the rubber hand illusion (adapted from Longo *et al.*, 2008). The questionnaire comprised 10 items including questions on ownership (e.g. 'It seemed like I was looking directly at my own hand, rather than at a videotaped hand'), location (e.g. 'It seemed like my hand was in the same location as the hand in the clip'), and agency (e.g. 'It seemed like I was in control of the hand on the screen'). All questions were rated on a six-point Likert scale (1, 'strongly disagree'; 6, 'strongly agree'). The original questionnaire (Longo *et al.*, 2008) was translated into German and the wording was slightly modified as a videotaped hand instead of a rubber hand was used in the present study (i.e. the term 'rubber hand' was replaced by 'hand in the clip').

Electroencephalographic recordings

High-density EEG recordings were acquired using a passive electrode system (EASYCAP) with 126 scalp electrodes and two electro-oculogram electrodes below the eyes. The data were recorded with a passband of 0.016–250 Hz and digitised with a sampling rate of 1000 Hz using a BrainAmp amplifier system (Brain Products). EEG data were online recorded against a nose tip reference and offline rereferenced to common average. The data were analysed using Matlab (MathWorks), EEGLAB (http://www.sccn.ucsd.edu/eeglab; Delorme & Makeig, 2004) and FieldTrip (http://www.ru.nl/fcdonders/fieldtrip; Oostenveld *et al.*, 2011). For the offline analysis, data were bandpass filtered between 0.3 and 125 Hz and downsampled to 500 Hz. A narrow band notch filter (49.8–50.2 Hz) was applied to remove line noise. Electrodes with extremely high- and/or low-frequency artifacts throughout the entire recording ($M = 7.2 \pm 3.6$) were linearly interpolated using a model of the amplitude topography at the unit sphere surface based on all nonartifactual electrodes (Perrin *et al.*, 1990). Epochs containing nonstereotyped muscular or technical artifacts were removed. An independent component analysis approach was applied to further reduce artifacts such as eyeblinks, horizontal eye movements, or electrocardiographic activity. Independent components representing artifacts were removed from the EEG data by back-projecting all but these components (for details, see Schneider *et al.*, 2008). Finally, all trials that still exceeded a threshold of 100 μV were rejected automatically. On average, 1.7% (range 0.3–3.1%) of all trials were removed for each participant.

Data analysis

Stimulus ratings

Prior to the statistical analysis, outlier trials were removed from pain ratings. To this end, the mean of intensity and unpleasantness ratings was calculated over nonpainful and painful trials separately, pooled across clips. Trials in which the ratings were below or above 3 standard deviations were excluded from further analyses. Based on this criterion, 0.29% of all trials were excluded (range 0.05–0.69%). The effect of viewing needle and Q-tip clips on stimulus ratings was investigated by subjecting intensity and unpleasantness ratings to separate ANOVAs with the factors visual stimulation (needle prick vs. Q-tip touch) and electrical stimulation (painful vs. nonpainful).

As numerous electrical stimuli (360 painful and 360 nonpainful) were administered, it may be that habituation effects influenced the present findings (Condes-Lara *et al.*, 1981; Babiloni *et al.*, 2006). To examine the possible influence of habituation on the effects in intensity and unpleasantness ratings, additional three-way ANOVAs, including the factor time (first and last 50% of trials within each condition), were conducted.

Pupil dilation responses

The PDR was screened and corrected for outliers in the same way as in our recent study (Höfle *et al.*, 2012). Eye blinks and other artifacts were removed in an interval ranging from 0.2 s before to 0.2 s after blink or artifact onset. Trials were excluded from further analyses if more than 50% of sample points within a trial were artifactual. On average, 1.2% of all trials were excluded following this criterion (range 0–3.1%). For all included trials, periods containing artifacts were linearly interpolated (Siegle *et al.*, 2008). The PDR was normalised as follows: (data−baseline)/baseline. To establish the presence of significant effects in PDRs and to define a time interval for further analyses, point-wise running *t*-tests between the needle prick and the Q-tip touch trials were computed. To account for alpha error accumulation in multiple testing, time intervals were defined as being significantly different if each sample point within a 0.1 s interval reached a threshold of $P = 0.05$. In line with our previous study (Höfle *et al.*, 2012), the correlations of the PDR with stimulus ratings were investigated by calculating Pearson's *r* coefficients between difference values of viewing needle pricks minus viewing Q-tip touches across participants.

Analysis of electroencephalographic data

For several reasons, we restricted the analysis of event-related potentials (ERPs) and oscillatory responses to the interval before the onset of electrical stimuli (i.e. when participants viewed the needle/Q-tip approaching the skin). Firstly, the central goal of our study was to examine the neural correlates of the recently observed modulation of anticipatory arousal and to investigate whether these correlates predict the magnitude of effects on pain perception and PDR. Secondly, given the expected modulation of neural activity prior to the onset of electrical stimuli, the present setup did not allow a proper baseline correction for the analysis of the poststimulus interval (i.e. the interval after electrical stimulation). Thus, any effects found in the poststimulus interval may have already started prior to the actual onset of the electrical stimulation.

Event-related potentials

The EEG data were analysed for needle and Q-tip clips. Data epochs were extracted from −1.8 s before to 1.2 s after electrical stimulus onset and baseline corrected. For the analysis of ERPs a baseline ranging from −1.2 to −1 s was chosen. Trials containing outliers in ratings, PDR, or EEG data, as described above, were not included in the analysis. In total, 3.1% of all trials were removed (range 1.0–5.7%). The same trials were used for the analysis of behavioral data, PDRs, ERPs, and oscillatory responses. For the statistical analysis of ERPs to needle and Q-tip clips, a cluster-based permutation test was applied over all electrodes and a time interval from −1 to 0 s (Maris & Oostenveld, 2007). This test controls the type I error rate in statistical tests involving multiple comparisons by clustering adjacent data points exhibiting the same effect. The dependent samples *t*-tests were thresholded at $P = 0.025$ and the permutation *P*-value of the cluster was set to $P = 0.05$. The time window and region of interest used for the ERP analysis were defined based on the results of the cluster-based permutation test (for significant electrodes see Fig. 2C). Furthermore, for illustration purposes (see Fig. 2A) and in line with previous studies (Murray *et al.*, 2006; Senkowski *et al.*, 2007), ERP traces to needle and Q-tip clips were compared using a point-wise running *t*-test. A significant difference in conditions was defined if at least 0.1 s of contiguous data (i.e. 50 consecutive sample points at a sample rate of 500 Hz) met an alpha criterion of 0.05 (Fig. 2A; Guthrie & Buchwald, 1991; Schneider *et al.*, 2011).

Oscillatory responses

Time–frequency representations of spectral power were computed for low frequencies (5–30 Hz) by means of a sliding window Fourier transform using a single Hanning taper. The analysis was conducted with a fixed time window ($t = 0.4$ s) and a fixed frequency smoothing ($f = 2.5$ Hz). Total power was computed relative to a baseline interval (−1.6 to −1.2 s before electrical stimulus onset). Average power in the baseline interval was first subtracted from the interval after clip onset and before electrical stimulus onset (prestimulus interval; −1 to 0 s) and the resulting difference was divided by the baseline interval activity as follows: $\mathrm{Pow}(t,f)_{\mathrm{normalised}} = 100 * ((\mathrm{Pow}(t,f)_{\mathrm{prestimulus}} - \mathrm{Pow}(f)_{\mathrm{baseline}})/\mathrm{Pow}(f)_{\mathrm{baseline}})$ (e.g. Pfurtscheller & Aranibar, 1977). For the statistical analysis, a cluster-based permutation test was applied on electrode–time–frequency data (Maris & Oostenveld, 2007; Schneider *et al.*, 2011). The dependent samples *t*-tests were thresholded at $P = 0.005$ and the permutation *P*-value of the cluster was set to $P = 0.05$. For the source reconstruction, a linear beamforming approach was applied (dynamic imaging of coherent sources; Van Veen *et al.*, 1997; Gross *et al.*, 2001). In this approach, source-level power is calculated using an adaptive spatial filter that passes activity from one specific location of interest with unit gain and maximally suppresses activity from surrounding locations. In the present study, one common filter was used, comprising all conditions (i.e. needle and Q-tip) as well as all time intervals (i.e. baseline and prestimulus). As linear beamforming is based on the calculation of the cross-spectral density matrix over trials, this approach is particularly suitable for the analysis of total power in the human electroencephalogram (Schneider *et al.*, 2008, 2011). The leadfield matrix was calculated on a boundary element model for each grid point in the brain with a regular 7 mm grid using a forward model based on closed compartments representing brain tissue (gray and white matter), bone, and skin (Oostenveld *et al.*, 2001). A spatial filter was constructed for each grid point and subsequently applied to estimate the power at that source location.

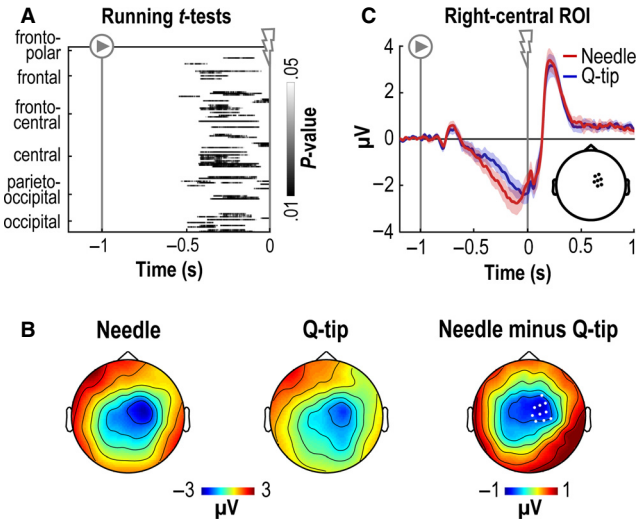

FIG. 2. Viewing needle pricks and Q-tip touches evoked a slow negative potential over right-central electrodes. (A) The result of point-wise *t*-tests for all electrodes revealed a difference between viewing needle and Q-tip clips several hundred milliseconds before electrical stimulus onset. The play symbol signifies clip onset and the flash symbol signifies electrical stimulus onset. (B) Topographic maps of the needle and Q-tip clips, and the difference group-averaged ERPs (−0.3 to −0.2 s). Highlighted in white are significant electrodes revealed by a cluster-based permutation test (dependent samples *t*-tests, $P < 0.025$; permutation *P*-value of the cluster, $P < 0.05$). (C) Grand mean ERPs over the right-central region of interest (ROI) in response to a needle and a Q-tip approaching the hand. The shaded areas depict the standard error of the mean.

In accordance with previous studies on pain anticipation (Babiloni *et al.*, 2005a, 2006) and with the activity patterns observed in the present study, the main focus of the statistical analysis of oscillatory responses was on the examination of ABA (8–12 Hz). The time interval for the source analysis was selected based on the results of the cluster-based permutation test on electrode–time–frequency data (Fig. 3) and was centered at −0.5 s (interval −0.7 to −0.3 s) before electrical stimulus onset; the respective baseline was centered at −1.4 s (interval −1.6 to −1.2 s). Source data were analysed voxel-wise by means of a cluster-based permutation test. The dependent samples *t*-tests for this analysis were thresholded at $P = 0.0001$ and the permutation *P*-value of the cluster was set to $P = 0.05$. Based on the results obtained in the cluster-based analysis of source data (Fig. 5), a region in the posterior cingulate cortex (PCC) and in the right fusiform gyrus (FG) was selected for further analysis.

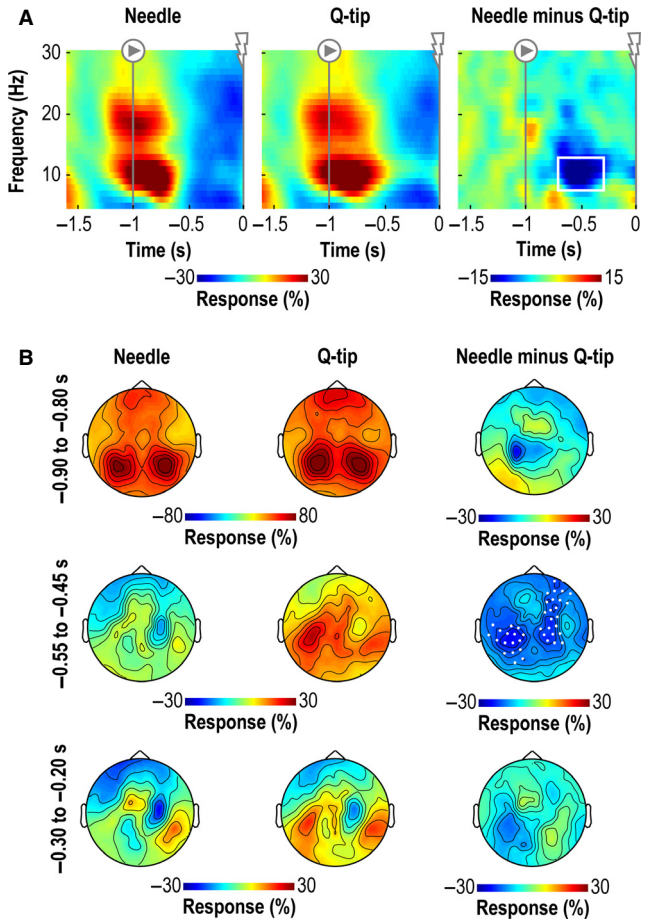

FIG. 3. Observing a needle or a Q-tip approaching the hand causes a reduction of ABA at around −0.5 s prior to the onset of electrical stimulation. (A) Time–frequency representation over right-central areas (the respective electrodes are depicted in Fig. 4) relative to a baseline from −1.6 to −1.2 s. A longer-latency reduction of ABA (around −0.5 s) follows the initial clip onset-related enhancement of ABA. The ABA reduction was more pronounced when viewing needle pricks (left panel) compared with Q-tip touches (middle panel). Outlined in white is the time–frequency window, which was selected for the linear beamforming analysis. The play symbol signifies the onset of the clip and the flash symbol highlights the onset of the electrical stimulus. (B) Topographic maps of anticipatory ABA (10 Hz) prior to the electrical stimulation. Highlighted in white are significant electrodes uncovered by a cluster-based permutation test on electrode–time–frequency data (dependent samples *t*-tests, $P < 0.005$; permutation *P*-value of the cluster, $P < 0.05$).

To investigate whether the ABA effects in the specified regions (i.e. PCC and FG) were related to PDR or stimulus unpleasantness, Pearson's *r* coefficients between difference values of viewing needle pricks minus viewing Q-tip touches were calculated across participants. A further analysis was conducted to investigate whether ABA predicts unpleasantness or PDR across single trials. As baseline normalisation on a trial-by-trial basis might lead to large outliers if a single trial baseline is close to zero, single trials were normalised by the average condition baseline for this analysis. Correlation coefficients were calculated for each participant and subsequently *z*-transformed to account for the fact that Pearson's *r* is not normally distributed: $z = 0.5 * \ln[(1 + r)/(1 - r)]$. The resulting *z*-values were tested against zero by means of a *t*-test. In the case of a significant result, *z*-values were back-transformed to mean *r*-values following the formula $r = (e^{2z} - 1)/(e^{2z} + 1)$, where e represents Euler's number (Corey *et al.*, 1998).

Results

Embodiment questionnaire

The questionnaire inquiring the degree of embodiment of the hand viewed on the screen showed that participants generally had the impression that they were looking at their own hand ($M = 3.52 \pm 0.82$; 13 of 18 participants scored higher than 3). The highest scores were obtained on items that expressed the feeling that the viewed hand was at the location of their own hand and that related to the impression of a causal relationship between the viewed and the experienced event (item 6, 4.17 ± 1.38; item 7, 3.94 ± 1.34; item 8, 4.67 ± 1.33). In addition, participants correctly answered the control question on visual attention ('Which clip was shown in the previous trial?'; asked after 10% of all trials) in 88.9% of all occurrences, demonstrating that participants attended to the clips.

Stimulus ratings

The ANOVA for unpleasantness ratings using the factors electrical stimulation (nonpainful vs. painful) and visual stimulation (needle prick vs. Q-tip touch) revealed a significant main effect of electrical stimulation ($F_{1,17} = 58.65$, $P < 0.001$). Painful electrical stimuli were perceived as more unpleasant than nonpainful stimuli (Fig. 1B). Furthermore, a significant main effect of visual stimulation ($F_{1,17} = 8.60$, $P < 0.01$) revealed that painful and nonpainful electrical stimuli were perceived as more unpleasant when participants saw a needle prick ($M = 38.09$) compared with a Q-tip touch ($M = 31.32$). No other significant effects were found. The ANOVA for intensity ratings revealed a significant main effect of electrical stimulation ($F_{1,17} = 418.67$, $P < 0.001$). Ratings were higher for painful compared with nonpainful stimuli (Fig. 1B). Moreover, a significant interaction of the factors electrical stimulation × visual stimulation was observed ($F_{1,17} = 4.82$, $P = 0.042$). Follow-up ANOVAs, which were conducted separately for painful and nonpainful stimuli, did not reveal any significant simple main effects for the factor visual stimulation (painful stimuli: needle, $M = 53.97$, Q-tip, $M = 52.52$, $F_{1,17} = 4.39$, $P = 0.052$; nonpainful stimuli: needle, $M = 19.41$, Q-tip, $M = 20.05$, $F_{1,17} = 1.27$, $P = 0.276$). To further investigate whether the effects on pain ratings were influenced by habituation to electrical stimuli, ratings were subjected to three-way ANOVAs comprising the factors electrical stimulation, visual stimulation and time (first and last 50% of trials). This analysis did not reveal significant effects in relation to the factor time, suggesting that habituation effects did not substantially contribute to the present findings.

Pupil dilation responses

PDR traces for needle and Q-tip clips (pooled across nonpainful and painful trials) are depicted in Fig. 1C. The dilation started at about 0.4 s after clip onset. PDR traces to needle and Q-tip clips already differed before electrical stimulus onset. A running *t*-test between both PDR traces revealed significant differences between the clips starting from about −0.3 s before electrical stimulus onset until the end of the trial. For the correlation analysis, we selected the time interval based on our previous study (Höfle *et al.*, 2012) from −0.2 s before to 0.6 s after electrical stimulus onset. Data points were averaged within the interval to obtain a single value for further analyses. The correlation analysis conducted on the average effect (needle minus Q-tip) across participants revealed a significant positive relationship between PDR and perceived unpleasantness

(r_{17} = 0.48, P = 0.046). This finding directly replicated the results of our previous study (Höfle *et al.*, 2012), where a positive correlation of r_{24} = 0.49 was found for this analysis.

Event-related potentials

A cluster-based analysis on mean ERP values computed over all electrodes and a time interval from −1 to 0 s revealed significant differences between viewing needle pricks and Q-tip touches from about −0.4 to −0.1 s (illustrated by means of a running *t*-test in Fig. 2A) and at right-central electrodes, i.e. contralateral to the forthcoming electrical stimulation (Fig. 2B). The mean ERP traces for these electrodes showed a slow negative potential within the time interval of interest, which was more pronounced when viewing needle clips compared with Q-tip touches (Fig. 2C). In the following, we will refer to this slow negative potential as stimulus-preceding negativity (SPN; e.g. Brunia & van Boxtel, 2001). Mean ERP amplitudes (−0.4 to −0.1 s) at right-central electrodes were selected for the further correlation analyses.

Oscillatory responses in the alpha band

Time–frequency representations (5–30 Hz) of total oscillatory responses at right-central electrodes showed an initial increase in the alpha band peaking at about 0.1–0.2 s after clip onset (Figs 3A and 4). The alpha power increase was maximal at occipital sites (Fig. 3B, first row). Following the increase, a reduction of ABA was found, which was strongest at right-central electrodes (Fig. 3B, last row). The cluster-based permutation test over electrode–time–frequency points revealed two significant clusters at medio-central and posterior electrodes from about −0.7 to −0.2 s. The reduction in ABA was stronger when viewing needle pricks compared with Q-tip touches (Figs 3B and 4). The pattern in ABA was not due to phase-locked responses to the onset of the video clip (see Supporting Information and Fig. S1 for a comparison of total and induced activity). In the next step of the analysis, the ABA modulations (10 Hz, −0.7 to −0.2 s) were examined in source space. The linear beamforming analysis revealed an ABA increase in occipital areas, which was stronger for Q-tip trials compared with needle-prick trials (Fig. 5, left and middle panels). In Q-tip trials the ABA increase extended to parietal areas. Moreover, a slight reduction of ABA was found in needle-prick trials contralateral to the forthcoming stimulation site, including the cingulate cortex, as well as parietal and frontal areas. The cluster-based permutation test revealed significant differences between conditions for two clusters in the right PCC (i.e. contralateral to the forthcoming electrical stimulation) and right FG (Fig. 5, right panel). In both clusters the ABA was lower when participants viewed needle pricks compared with Q-tip touches. The mean activity within each of these clusters was computed for further correlation analyses. As previous studies on viewing painful stimulation have found modulations in the sensorimotor cortex (e.g. Whitmarsh & Jensen, 2011), we explored whether this area also showed an effect on ABA in the present study. To this end, we created virtual channels for the sensorimotor cortex and the significant source clusters in the PCC and FG (see Supporting Information and Fig. S2 for details). The correlation analysis between the ABA effect (i.e. needle minus Q-tip) in the PCC and FG and the effect on PDR, SPN, and pain ratings did not reveal any significant correlations across participants. However, there was a trend towards significance for the correlation between ABA in the PCC and the PDR (r_{17} = −0.44, P = 0.071). Next, the relationships between ABA, PDR, SPN, and pain ratings were investigated at the single trial level (see Materials and methods). This analysis revealed a positive relationship between ABA in the PCC and ABA in the FG (t_{17} = 11.77, P < 0.0001; average correlation coefficient over subjects: r_{17} = 0.31). Furthermore, a small but significant negative relationship was found between ABA in the PCC and the PDR (t_{17} = −3.36, P = 0.0037; average correlation coefficient over subjects: r_{17} = −0.07). No other significant relationships were observed.

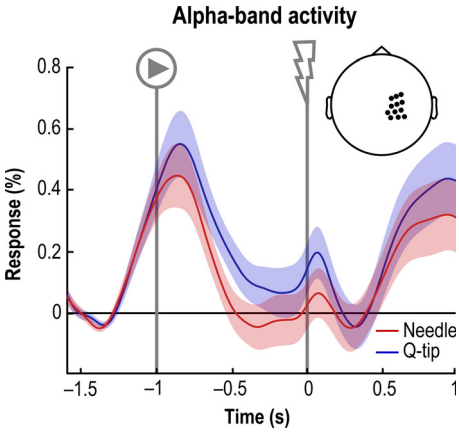

FIG. 4. Traces of ABA (10 Hz) averaged over the right-central electrode cluster for the whole trial relative to baseline (−1.6 to −1.2 s). The traces for needle and Q-tip clips differed from about −0.7 to −0.3 s before stimulus onset. The shaded areas depict the standard error of the mean. The play symbol signifies the onset of the clip and the flash symbol highlights the onset of the electrical stimulus.

Discussion

This study examined the impact of viewing a needle pricking a hand that is perceived as one's own on anticipatory oscillatory activity, PDR, and subjective stimulus ratings to painful and nonpainful electrical stimuli. Replicating the results of our previous study (Höfle *et al.*, 2012), we observed that electrical stimuli were perceived as more unpleasant when viewing a needle prick compared with a Q-tip touch and that this effect was paralleled by enhanced PDRs. The key novel finding of our study is a reduction of ABA in the PCC and FG when viewing a needle compared with a Q-tip approaching the incorporated hand. Moreover, we observed a negative relationship between PDRs and alpha-band responses in the PCC.

Following the onset of the video clips, we found an increase in ABA, which was followed by a reduction of ABA. This reduction, which started at about −0.7 s prior to the electrical stimulation, was stronger when participants viewed a needle compared with when they watched a Q-tip approaching the incorporated hand. Reduction of ABA has previously been ascribed to activation of the respective sensory system (Hari & Salmelin, 1997; Pfurtscheller & Lopes da Silva, 1999; Ploner *et al.*, 2006; Klimesch *et al.*, 2007; Jensen & Mazaheri, 2010). Along the same lines, previous studies related ABA reduction to attention and stimulus anticipation (Babiloni *et al.*, 2005a, 2006; Thut *et al.*, 2006; Siegel *et al.*, 2008). For instance, in a bimodal attention task, reduced alpha power was found over the sensory cortex of the attended modality (Foxe *et al.*, 1998). Furthermore, the ABA reduction is spatially specific, being located contralateral to the attended site (Worden *et al.*, 2000; Van Ede *et al.*, 2011; Bauer *et al.*, 2012). In the present study, reduction of ABA was found at central electrodes contralateral to the forthcoming electrical stimulation site (Fig. 3B, last row), possibly reflecting increased attention to the incorporated hand.

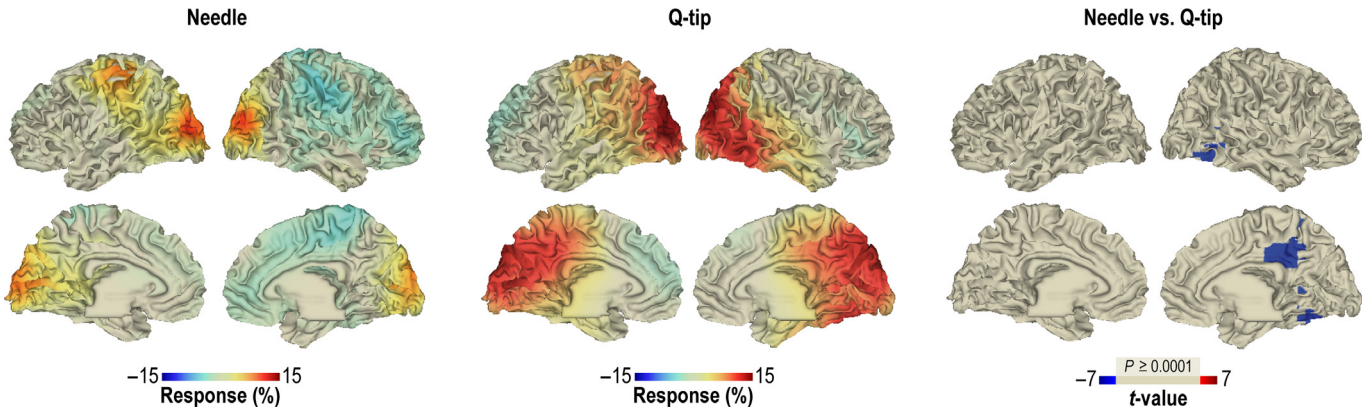

FIG. 5. Linear beamforming of ABA (10 Hz, −0.7 to −0.3 s before electrical stimulus onset) for needle (left panel) and Q-tip (middle panel) clips, and the difference response (right panel). ABA increase was source localised in visual areas, extending to temporal and parietal areas, especially in Q-tip trials. A slight reduction of ABA was specifically found in needle-prick trials contralateral to the forthcoming stimulation site in medial, parietal and frontal areas. The statistical analysis between needle and Q-tip clips revealed reduced ABA when viewing needle pricks compared with Q-tip touches in the right PCC and FG. Displayed are voxels with *t*-values corresponding to $P < 0.0001$. The depicted *t*-values resulted from dependent samples *t*-tests calculated at each voxel as part of the cluster-based permutation test (see Materials and methods).

The reduction of ABA was stronger when participants viewed a needle compared with a Q-tip approaching the incorporated hand. This effect was observed up to −0.2 s before electrical stimulus onset. As a Hanning window with a length of 0.4 s was used for the time–frequency analysis, anticipatory activity directly preceding the electrical stimulus (i.e. beginning at −0.2 s) already involved poststimulus responses. Thus, temporal smearing during the time–frequency transformation might have masked possible ABA effects immediately prior to the electrical stimulus onset.

In general, the observation of stronger ABA reduction when viewing needle pricks compared with Q-tip touches is in line with previous magneto- and encephalographic studies in which participants viewed static pictures depicting limbs in painful and nonpainful situations in extrapersonal space (Perry *et al.*, 2010; Whitmarsh & Jensen, 2011). In these studies, the reduction of ABA was stronger when participants viewed painful compared with nonpainful situations. Interestingly, the effect of viewing painful situations in extrapersonal space was found in the sensorimotor cortex (Whitmarsh & Jensen, 2011). The present study differs from the above-mentioned studies in some important aspects. Firstly, we presented nonpainful and painful electrical stimuli that were spatiotemporally aligned with the visual input and, thus, participants were anticipating actual pain while viewing the approaching needle and the Q-tip. Pain anticipation has previously been shown to involve activity in sensorimotor regions but also in the insula, anterior cingulate cortex and PCC (Porro *et al.*, 2002, 2003; Wager *et al.*, 2004; Koyama *et al.*, 2005; Brown *et al.*, 2008; Atlas *et al.*, 2010; Drabant *et al.*, 2011; Worthen *et al.*, 2011; Seifert *et al.*, 2012). Secondly, we used dynamic visual stimuli instead of static pictures, which possibly enhanced the threatening aspect of the needle (Ehrsson *et al.*, 2007). Activity within the PCC has been repeatedly associated with processing of threat-related stimuli (for a recent meta-analysis see Hayes & Northoff, 2012). Finally, the focus of our analysis was on the interval before the needle or the Q-tip hit the hand. These differences in experimental protocols may have accounted for the different effects of visual stimulation on ABA in the present compared with some previous studies (Perry *et al.*, 2010; Whitmarsh & Jensen, 2011).

The effect of viewing a needle prick on anticipatory ABA was robustly localised to the PCC. The PCC has frequently been related to the default mode network and to different cognitive processes such as memory, attention, and change detection (for reviews see Vogt, 2005; Pearson *et al.*, 2011). The PCC is also involved in visual aversive conditioning (Maddock & Buonocore, 1997), pain anticipation (Porro *et al.*, 2003; Brown *et al.*, 2008; Seifert *et al.*, 2012), and the initial detection of threat (Mobbs *et al.*, 2009, 2010). Furthermore, larger PCC activity has been observed during the anticipation of aversive compared with neutral pictures (Grupe *et al.*, 2013). Based on its anatomical connections, comprising amongst others the anterior cingulate cortex and cingulate motor regions (Vogt *et al.*, 2006), the PCC has been supposed to play a role in orienting the body to motivationally salient stimuli (McCoy & Platt, 2005; Vogt, 2005). Salient sensory stimuli, especially threatening stimuli, presented near the body have been shown to evoke defensive responses (for reviews see Graziano & Cooke, 2006; Legrain *et al.*, 2011). Thus, in the present study, the effects on ABA and PDR may reflect the preparation of adequate defensive behavior when viewing a needle approaching the body.

In agreement with our previous study (Höfle *et al.*, 2012), we observed a positive correlation between the effects in the PDR and perceived unpleasantness across participants. Interestingly, we found a difference in timing between the effect in the PCC and PDR. The effect in the PCC started at about −0.7 s, whereas it started at about −0.2 s in the PDR. This observation might be due to the more sluggish response of the PDR, which takes several hundred milliseconds to differentiate between stimulus content. For instance, in our previous study, we found that the pupil starts differentiating between painful and nonpainful electrical stimulation at about 0.4 s after electrical stimulus onset (Höfle *et al.*, 2012). Another interesting observation of the present study was the negative correlation between the PDR and ABA in the PCC. Enhanced reduction of ABA, which presumably reflects stronger activation, was associated with larger PDRs. This finding is in line with functional magnetic resonance imaging studies that showed a positive relationship between PCC activity and ANS arousal during pain anticipation (Porro *et al.*, 2003; Maihöfner *et al.*, 2011; Seifert *et al.*, 2012). As the PCC does not have direct autonomic connections (Vogt, 2005; Vogt *et al.*, 2006), it may be that subcortical structures are involved in mediating the observed relationship between responses of the central nervous system and ANS (Carrive, 1993; Brandão *et al.*, 2003;

Graziano & Cooke, 2006; Samuels & Szabadi, 2008; Cohen & Castro-Alamancos, 2010). A subcortical structure involved in mediating the observed effects could be the locus coeruleus (Zhang *et al.*, 1997; Berridge & Waterhouse, 2003; Samuels & Szabadi, 2008; Carter *et al.*, 2010). Animal studies have shown that phasic locus coeruleus responses are evoked by salient (e.g. threatening) stimuli of different modalities (Berridge & Waterhouse, 2003; Samuels & Szabadi, 2008; Sara, 2009). Furthermore, phasic locus coeruleus activation is known to evoke a PDR (Koss, 1986; Einhäuser *et al.*, 2008; Samuels & Szabadi, 2008) and to facilitate cortical stimulus processing (McCormick, 1992; Berridge & Waterhouse, 2003; Samuels & Szabadi, 2008; Sara, 2009). Moreover, the cingulate cortex (including the PCC) receives projections from midline and intralaminar thalamic nuclei, which in turn have prominent innervations by norepinephrinergic axons primarily originating from the locus coeruleus (Vogt *et al.*, 2008). The role of subcortical structures in the present findings could be investigated in future studies using, for instance, functional magnetic resonance imaging.

In addition to the significant cluster within the PCC, we found significant effects on anticipatory ABA within the FG. The FG has previously been related to the processing of faces (e.g. Vuilleumier *et al.*, 2001) and other body-related stimuli (Peelen & Downing, 2005). Furthermore, this area has been shown to be involved in the recognition of biological motion (Grossman & Blake, 2002), attention (Martínez *et al.*, 1999; Tallon-Baudry *et al.*, 2005; Davidesco *et al.*, 2013), and processing of emotional cues and threat (Hadjikhani & de Gelder, 2003; Kret *et al.*, 2011). In the present study, we observed a positive relationship between anticipatory ABA in the FG and PCC, suggesting interplay between these areas. Moreover, as the FG and PCC participate and interact in object recognition, as well as in sensorimotor transformations for visually guided actions (Goodale & Milner, 1992; Vogt *et al.*, 2006), they might mutually facilitate the preparation of defensive responses when viewing a needle approaching the body.

Finally, we observed an effect of viewing a needle prick on anticipatory slow-wave ERPs, resembling an SPN (Brunia, 1988). The SPN has been related to the contingent negative variation (Walter *et al.*, 1964; Tecce, 1972; Hultin *et al.*, 1996; Hamano *et al.*, 1997), and to pain anticipation (Babiloni *et al.*, 2005b; Brown *et al.*, 2008). The sources of the SPN prior to the onset of a simple finger movement comprise, in addition to primary motor areas, the anterior cingulate cortex and inferior parietal cortex as well as occipital and prefrontal areas (Gómez *et al.*, 2003). Thus, the stronger anticipatory negative drift over the central scalp for needle compared with Q-tip clips in the present study may reflect enhanced preparation for the processing of the subsequently presented electrical stimulus.

An aspect that was not addressed by the present study is the effect of viewing a needle prick on the neural responses to electrical stimulation. The clips in our study were presented immediately before the onset of the electrical stimuli, triggering anticipatory processes that probably overlap with the responses to the electrical stimulus. Therefore, it is not possible to disentangle whether any poststimulus effects would actually be linked to the processing of the electrical stimuli or are due to anticipatory processes that start prior to the electrical stimulation. Future studies may include unimodal visual trials, in which the clips are presented without subsequent electrical stimulation. Neural activity to these stimuli could be subtracted from the activity to bimodal visual-pain stimuli (Busse & Woldorff, 2003; Senkowski *et al.*, 2011). However, the inclusion of unimodal visual stimuli would have substantially changed the stimulation protocol of our original study (Höfle *et al.*, 2012). For this reason, we did not include unimodal visual stimuli in the present study and restricted the analysis of electrophysiological data to the interval prior to electrical stimulation.

Conclusion

Our study showed that viewing a needle pricking a hand that is perceived as one's own enhances the unpleasantness of spatiotemporally aligned painful and nonpainful electrical stimuli. Moreover, our study demonstrated that viewing a needle compared with viewing a Q-tip approaching the body enhances PDRs and reduces anticipatory alpha-band responses in the PCC and FG. Thus, our study uncovered a spectral signature that was associated with the previously reported effect of viewing a needle prick on the PDR (Höfle *et al.*, 2012). Viewing a needle approaching the body modulates neural activity in the PCC and FG probably to orient the body to the forthcoming stimulation and to prepare adequate defense responses to protect the integrity of one's body.

Supporting Information

Additional supporting information can be found in the online version of this article:

Data S1: Supporting analyses of induced activity and of virtual channels in source space.

Fig. S1. Time-frequency representations of total power and induced power.

Fig. S2. Time-frequency representations of virtual channels in source space comprising PCC, FG, and right sensorimotor hand area.

Acknowledgements

This study was supported by grants from the German Research Foundation (DFG) (SE 1859/1-2 to D.S.; SFB TRR 58 B04 to A.K.E.) and the European Union (ERC-2010-StG_20091209 to D.S.; ERC-2010-AdG-269716 to A.K.E.). We thank C. Beckmerhagen and R. Zimmermann for help with the preparation of the experimental setup, C. Reißmann and K. Deazle for support in data recording, and J. Lorenz for helpful discussion. There are no conflicts of interest that may arise as a result of the research presented in this article.

Abbreviations

ABA, alpha-band activity; ANS, autonomic nervous system; EEG, electroencephalography; ERP, event-related potential; FG, fusiform gyrus; M, mean; PCC, posterior cingulate cortex; PDR, pupil dilation response; SPN, stimulus-preceding negativity.

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