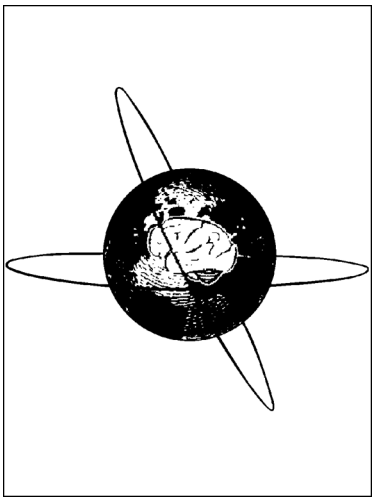

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PII: S1388-2457(20)30397-7
DOI: https://doi.org/10.1016/j.clinph.2020.06.020
Reference: CLINPH 2009298

To appear in: *Clinical Neurophysiology*

Received Date: 10 March 2020
Revised Date: 27 April 2020
Accepted Date: 6 June 2020

Please cite this article as: van den Broeke, EN., de Hemptinne, P., Mercken, M., Torta, DM., Lambert, J., Mouraux, A., Central sensitization of nociceptive pathways demonstrated by robot-controlled pinprick-evoked brain potentials, *Clinical Neurophysiology* (2020), doi: https://doi.org/10.1016/j.clinph.2020.06.020

Central sensitization of nociceptive pathways demonstrated by robot-controlled pinprick-evoked brain potentials

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Keywords:
High-frequency stimulation; central sensitization; robot-controlled pinprick stimulation; pinprick-evoked potentials; stimulus evaluation.

Highlights

- Robot-controlled pinprick stimulation elicit pinprick-evoked brain potentials (PEPs) consisting of a negative and positive peak.
- High-frequency electrical stimulation of the skin (HFS) induces secondary hyperalgesia, which is a manifestation of central sensitization.
- Both PEP peaks are increased after HFS when elicited from the area of secondary hyperalgesia.

ABSTRACT

Objective. The aim of this study was to assess the effect of central sensitization, induced by high frequency electrical stimulation of the skin (HFS), on pinprick-evoked brain potentials (PEPs) using robot-controlled mechanical pinprick stimulation and a stimulus evaluation task.

Methods. In 16 healthy volunteers HFS was applied to the right volar forearm. Robot-controlled pinprick stimuli (64 mN) were applied before and 20 minutes after HFS to the skin surrounding the area onto which HFS was applied. During pinprick stimulation, the EEG was recorded and the quality of perception and perceived intensity of the pinprick stimuli was collected.

Results. After HFS, the skin surrounding the site at which HFS was delivered showed increased mechanical pinprick sensitivity. Both the early-latency negative peak of PEPs and the later-latency peak were significantly increased after HFS.

Conclusions. This study shows increased PEPs after HFS when they are elicited by a robot-controlled mechanical pinprick stimulator and participants are engaged in a stimulus evaluation task during pinprick stimulation.

Significance. This is the first study that shows a significant increase of both PEP peaks, and therefore, it provides a preferred setup for assessing the function of mechanical nociceptive pathways in the context of central sensitization.

1. INTRODUCTION

Cutaneous tissue injury can be accompanied by increased pain sensitivity in the area of tissue damage ("primary hyperalgesia") and in surrounding uninjured skin ("secondary hyperalgesia"). Secondary hyperalgesia is most pronounced for mechanical pinprick stimuli (van den Broeke et al. 2016a; Ali et al. 1996) and is the result of an increased responsiveness of nociceptive neurons in the central nervous system (central sensitization) (Baumann et al. 1991; Simone et al. 1991).

To explore the changes in brain activity related to secondary hyperalgesia we previously recorded pinprick-evoked brain potentials (PEPs) before and after intradermal capsaicin injection (van den Broeke et al., 2015). Intradermal capsaicin injection mimics injury and is a well-established method for inducing secondary hyperalgesia (Magerl et al. 1998). In that study, different pinprick intensities, ranging from 16 to 512 mN, were used to characterize the effect of stimulation intensity on PEPs. We found that when pinprick stimuli were applied to the area of increased pinprick sensitivity around the site of injection (i.e. area of secondary hyperalgesia), a late positive peak of the PEP waveform (between 0.2 s and 0.5 s after stimulus onset) was increased. The magnitude of this increase was dependent on the intensity of pinprick stimulation, with the strongest and only significant increase observed for 64 mN pinprick stimuli.

In two follow-up studies we then recorded PEPs before and after transcutaneous high frequency electrical stimulation of the skin (HFS) (van den Broeke et al. 2017; 2016b). Such as capsaicin, HFS also induces a clear increase in mechanical pinprick sensitivity of the skin surrounding the site at which HFS is applied (Klein et al. 2004). Similarly to the results with capsaicin, we observed in both studies an increase in the magnitude of a late positive peak of

PEPs when elicited by 64 mN pinprick stimuli delivered to the area of increased pinprick sensitivity. The increase in amplitude was maximal at central-posterior scalp regions (van den Broeke et al., 2016b; 2017).

In these previous studies the signal-to-noise ratio of PEPs was relatively low, and no clear negative peak could be observed before the late positive peak. This could be due to the fact that the pinprick stimuli were delivered to the skin manually, which may affect the reproducibility of the pinprick stimulation. To overcome this, we ran a third follow-up study using a robot-controlled mechanical pinprick stimulator to elicit PEPs before and after HFS at the volar forearm (van den Broeke et al., 2019d). In that study, in addition to a late positive peak which was maximal approximately 320 ms after stimulation onset, we observed an earlier negative peak, which was maximal approximately 120 ms after stimulation onset. Both peaks were prominent at the scalp vertex. Although this negative peak was, on average, increased after HFS, this increase was not significant. A similar negative peak had also been reported by Iannetti et al. (2013). In that study, the magnitude of this negative peak was significantly increased after intradermal injection of capsaicin. One important difference between our study that did not show a significant increase of PEPs after HFS (van den Broeke et al. 2019d) and studies that showed a significant increase after HFS or capsaicin (van den Broeke et al. 2015; 2016b; 2017; Iannetti et al. 2013) was task relevance of the pinprick stimuli. In van den Broeke et al. (2019d), participants were asked to provide an average rating of the pinprick stimuli at the end of the stimulation block, while in the other studies, participants had to provide a rating of the perceived intensity and/or had to indicate the quality of perception after individual pinprick stimuli. Engaging participants in a task requiring to evaluate the perceived intensity and/or quality of the sensation elicited by individual pinprick stimuli increases the task relevance of the stimuli.

The aim of the present study was to assess the effect of HFS on the negative and positive peaks of PEPs elicited by a robot-controlled mechanical pinprick stimulator in conditions where pinprick stimuli are made task relevant by asking participants to evaluate their quality of perception and perceived intensity.

2. METHODS AND MATERIALS

2.1 Participants

Sixteen healthy volunteers took part in the experiment. In two subjects, technical problems led to a loss of stimulation triggers or loss of EEG data in one of the two recording sessions. Data from these two subjects was discarded. Therefore, the data presented here consists of the remaining fourteen subjects (9 men and 5 women; aged 18 – 27 years; 21.9 ± 2.3 years [mean ± sd]). "The experiment was conducted according to the declaration of Helsinki (except preregistration of the trial). Approval for the experiment was obtained from the local Ethical Committee (Commission d'Éthique Biomédicale Hospitalo-Facultaire) of the Université catholique de Louvain (UCL) (B403201316436). All participants signed an informed consent form and received financial compensation for their participation" (van den Broeke et al. 2019d).

2.2 Experimental design

The design of the experiment is summarized in Figure 1. HFS of the right volar forearm was used to induce central sensitization, verified by an increased pinprick sensitivity of the surrounding skin (Klein et al. 2014; van den Broeke and Mouraux 2014; van den Broeke et al. 2016a; van den Broeke et al. 2019c; Vo and Drummond 2013; Xia et al. 2016). Robot controlled mechanical pinprick stimuli (64 mN) were applied (approximately 20 minutes) before and 20

minutes after applying HFS ("test area"). During pinprick stimulation, the quality of perception and perceived intensity elicited by the pinprick stimuli were collected. Moreover, the EEG was continuously recorded. Because we have shown in previous studies (van den Broeke et al. 2017; van den Broeke et al. 2019c; van den Broeke et al. 2019d) that both the perceived intensity and PEPs elicited by pinprick stimuli delivered to the arm contralateral to the one that received HFS do not change after HFS, we did not collect the perceived intensity and PEPs from the left (contralateral control) arm.

2.3 High frequency electrical stimulation (HFS)

HFS consisted of 12 trains of 42 Hz electrical biphasic pulses lasting 1 s each (van den Broeke et al., 2019c). The time interval between the onsets of each train was 10 s. "The biphasic pulses consisted of 2-ms square-wave pulse followed, after 0.1-ms delay, by a 4-ms compensation pulse of opposite polarity having half the intensity of the first pulse. The entire HFS protocol lasted 2 min" (van den Broeke et al. 2019c). HFS was delivered to the right volar forearm, 10 cm distal to the cubital fossa (Fig. 1). "The intensity of stimulation was individually adjusted to 20x the absolute detection threshold to a single pulse (0.28 ± 0.11 mA; mean ± sd). Detection thresholds were estimated using the method of limits. The electrical pulses were triggered by a National Instruments digital-analog interface (NI6343, National Instruments, Austin, TX), produced by a constant current electrical stimulator (Digitimer DS5, Welwyn Garden City, UK), and delivered to the skin using a custom electrode (Fig. 1) designed and built at the Centre for Sensory-Motor Interaction (Aalborg University, Denmark). "The cathode consists of 16 blunt stainless-steel pins with a diameter of 0.2 mm protruding 1 mm from the base. The 16 pins are placed in a circle with a diameter of 10 mm. The anode consists of a surrounding stainless-

steel ring having an inner diameter of 22 mm and an outer diameter of 40 mm" (van den Broeke et al. 2019c).

2.4 Robot-controlled mechanical pinprick stimulation

A custom-built calibrated robot-controlled pinprick stimulator was used to deliver reproducible mechanical pinprick stimuli (van den Broeke et al. 2019d). "The robot consists of three linear computer-controlled stages. The first two stages control the horizontal (X/Y) position of the pinprick probe. The third stage, onto which the pinprick probe is mounted, controls the vertical (Z) position of the probe. Each stage can be displaced at a speed of 25 mm/s, with a resolution of 0.1 mm. The pinprick probe consists of a stainless-steel flat tip probe (diameter: 0.35 mm, flat geometry) on top of which rests a calibrated cylindrical weight. The probe and weight are mounted inside an aluminium tube, held by the robot. When applied perpendicular to the skin, the probe and weight slide freely inside the tube, thereby applying a constant normal force which is entirely determined by the total mass of the probe and weight. A high-resistance switch generates a trigger in the EEG that marks stimulation onset. When the probe touches the skin, this switch is triggered by the reduced impedance between the probe and an electrode placed on the skin at the wrist. A thin layer of conductive gel was applied onto the skin to lower the impedance between the contacting probe and the skin (van den Broeke et al. 2016b; 2017). Before the start of each block, the X/Y/Z position of the pinprick stimulator was adjusted to position the probe approximately 5 mm above the skin, at the centre of the test area" (van den Broeke et al. 2019d).

During the pinprick stimulation, participants were sitting in front of a table with their right arm inside the custom-built robotic pinprick stimulator. A 48 x 57.5 cm panel with an opening for

the arm was placed in front of the pinprick robot to prevent view of the stimulated arm. Moreover, a sound-attenuated headphone and flexible earplugs were used to mask any sound generated by the pinprick robot during movements.

In each block (before and after HFS) a total of thirty pinprick stimuli were administered. “The pinprick robot stimulated at random positions within the test area and never the same spot twice by first displacing the probe to the corresponding position in the X/Y plane, and then performing a downward 16 mm movement (Z-axis). After 1.2 s, the pinprick probe was raised by performing an upward 16 mm movement. Both movements were performed at a constant speed of 33.33 mm/s. The test area was defined at the beginning of the experiment by drawing a circle of 4 cm diameter that exactly matches the size of the HFS electrode at the ventral forearm (Fig. 1)” (van den Broeke et al. 2019d).

“A computer screen in front of the participant was used to display a fixation cross. Each trial started with the presentation of this fixation cross on the computer screen, which remained during the whole trial. After the fixation cross appeared, the pinprick stimulus was delivered onto the skin after a randomized interval between 10 to 14 seconds. Eight seconds after delivery of the pinprick stimulus the fixation cross disappeared, indicating the end of the trial. During each trial, participants were instructed to fixate their gaze at the dot” (van den Broeke et al. 2019d).

2.5 Intensity and quality of perception elicited by the pinprick stimulation

“Participants were asked to indicate after each pinprick stimulus, when the fixation cross disappeared, whether the stimulus was perceived as pinprick, touch or not detected. Moreover, within each block of thirty stimuli, participants were asked to rate the intensity of the percept elicited by ten randomly selected stimuli using a numerical rating scale (NRS)

ranging from 0 (no perception) to 100 (maximal pain), with 50 representing the transition from non-painful to painful domains of sensation" (van den Broeke et al. 2017).

To confirm the successful induction of the increase in perceived pinprick intensity after HFS a paired sample t-test on the NRS scores before versus after HFS was performed. A paired sample t-test was chosen because the difference scores (after HFS minus before HFS) were approximately normally distributed as indicated by the D'Agostino & Pearson omnibus normality test (GraphPad Prism v5, GraphPad Software, California, USA), and there were no significant outliers. The level of significance was set at $p<0.05$ for a one-sided test. The statistical analysis was conducted using SPSS 18 (SPSS Inc., Chicago, IL, USA).

2.6 EEG recording

"The EEG was recorded using 64 actively shielded Ag-AgCl electrodes that were mounted in an elastic cap and arranged according to the international 10-20 system (Waveguard64 cap, Advanced Neuro Technologies, The Netherlands). The EEG signals were amplified and digitized using a sampling rate of 1000 Hz and an average reference (64-HS Advanced Neuro Technologies, The Netherlands). Eye movements were recorded using two surface electrodes placed at the upper-left and lower-right sides of the left eye. Electrode impedances were kept below 20 kΩ" (van den Broeke et al. 2019d).

2.7 EEG analysis

"The EEG signals were analysed offline using Letswave 6.0 (www.nocions.org/letswave). After applying a 0.3-30 Hz band pass zero-phase Butterworth filter to the continuous EEG recordings, the signals were segmented into epochs extending from -500 to +1500 ms relative to stimulus onset" (van den Broeke et al. 2017). An Independent Component Analysis was used to attenuate the contribution of eye movements or eye blinks artefacts (ICA, Jung et al.,

2000)). Independent Components (ICs) were identified as capturing electro-oculographic activity based on their time course typical of eye blink artefacts and their anterior scalp topography. After removal of these ICs, the denoised epochs were then baseline-corrected (reference interval: -500 to 0 ms). Finally, epochs with amplitude values exceeding ±75 μV were rejected as these were likely to be contaminated by artefacts. Separate averaged waveforms were computed for each participant and time point (before and after HFS).

A previous study found that the early-latency negative peak of PEPs is maximal at the scalp vertex (Iannetti et al. 2013). In that study and in our own previous study (van den Broeke et al. 2019d) this negative peak is maximal between 0.1 and 0.2 s following stimulation onset. Based on these studies we identified this negative peak between 0.1-0.2 s at electrode Cz, in each average waveform of each participant. Similarly, in our previous studies we found that the later positive peak is maximal between 0.2 and 0.5 s following stimulation onset (van den Broeke et al. 2015; 2017) and is maximal at central-posterior electrodes (van den Broeke et al. 2016b; 2017). Therefore, we identified this positive peak between 0.2-0.5 s at electrode Cz, CPz and Pz, in each average waveform of each participant. To test whether the increases in magnitude of these peaks following HFS were statistically significant, we performed paired sample t-tests on the individual maximal peak values recorded before versus after HFS. The paired sample t-test was chosen for the same reasons as mentioned for the analysis of the perceived intensity ratings. The critical p-value was set at $p<0.05$ for a one-sided test when assessing the HFS-induced increase of the negative peak at electrode Cz. The critical p-value was set at $p<0.016$ for a one-sided test when assessing the HFS-induced increase of the positive peak at electrodes Cz, CPz and Pz (Bonferroni correction for the number of electrodes). The statistical analysis was conducted using SPSS 18.

3. RESULTS

3.1 Perceived pinprick intensity

In agreement with previous studies, HFS induced an increase in pinprick sensitivity of the skin surrounding the site at which HFS was applied (Figure 2). This was confirmed by a paired-t-test between the individual NRS scores elicited by the pinprick stimuli before versus after HFS (t (13) = 3.778, p=.0012, Cohens *dz* = 1.010).

3.2 Quality of perception

Before applying HFS, pinprick stimuli were qualified as 'pinprick' in approximately 50% of the trials, and as 'touch' in the remaining 50% of the trials (Fig. 2C and Supplementary Figure S1). After HFS, the number of stimuli qualified as 'pinprick' increased to 85% of the trials.

3.3 Pinprick-evoked brain potentials (PEPs)

The group-level average waveforms of the PEPs elicited by the 64 mN stimulation before and after HFS at electrodes Cz, CPz and Pz are shown in Figure 3. Individual waveforms are shown in Supplementary Figure S2.

The magnitude of the negative peak of PEPs was increased after HFS and this increase was maximal at electrode Cz (Fig. 4). A paired t-test on the individual peak values before versus after HFS revealed a significant increase of the magnitude of the negative peak after HFS (t (13) = 3.956, p=.0008, Cohens *dz* = 1.058, Fig. 5).

The magnitude of the positive peak of PEPs was also increased after HFS and this increase was maximal at electrode CPz (Fig. 4). Paired t-tests revealed a significant increase in the magnitude of the positive peak after HFS at electrode CPz (t (13) = 4.169, p = .0006, Cohens *dz*

= 1.115, Fig 5), but not at Cz (t (13) = 1.693, p=.0572, Cohens *dz* = .4527) and Pz (t (13) = 2.159, p=.0251, Cohens *dz* = .5773).

4. DISCUSSION

The present study shows that robot-controlled mechanical pinprick stimuli elicit both an early-latency negative peak maximal at the scalp vertex (Cz) and a later-latency positive peak maximal at the more posterior electrode (CPz). Both peaks were significantly enhanced after HFS.

Whereas we did not observe a significant increase in PEPs in our previous study (van den Broeke et al., 2019d) we do here. In both studies we used robot-controlled pinprick stimuli to elicit PEPs before and after HFS. However, in the present study (as well as the other studies in which an enhancement of PEPs was observed after HFS or capsaicin treatment of the skin; Iannetti et al. 2013; van den Broeke et al. 2015; 2016b; 2017), we engaged our participants in a task that required evaluating the quality of perception and intensity of the pinprick stimuli. Hence, the pinprick stimuli in the present study were likely more task relevant than the pinprick stimuli delivered in the previous study where no significant HFS-related enhancement of PEPs was observed. Our results therefore strongly suggest that the effect of HFS on the magnitude of PEPs is dependent on task relevance. The finding that the increase of PEPs after HFS is dependent on subject engagement further shows that care must be taken to control for cognitive factors when clinical assessments are made.

The mean (±SD) latencies of our PEPs at baseline, at electrode Cz, were 136 ± 25 ms for the negative peak and 259 ± 40 ms for the positive peak. The mean (±SD) latencies of PEPs (at Cz, across all conditions) in the study of Iannetti et al. (2013), where they had to correct latencies

for a trigger delay, were 111 ± 8 ms for the negative peak and 245 ± 17 ms for the positive peak. In that study they observed a significant increase in the negative peak but not in the later-latency positive peak. However, the authors restricted their analyses to the waveforms recorded at electrode Cz, whereas we also tested the more posterior electrodes CPz and Pz. The latencies of the PEPs elicited by mechanical pinprick stimulation of the skin are compatible with the peripheral conduction velocities of both A-fiber low-threshold mechanoreceptors (LTMs) and high-threshold mechanoreceptors (HTMs). Indeed, Nagi et al. (2019), using microneurography in healthy participants recorded similar peripheral conduction velocities between A-fiber HTMs and A-fiber LTMs. Interestingly, they also showed that intraneural electrical stimulation of single A-fiber HTMs elicits a painful perception qualified as sharp or pinprick (Nagi et al. 2019). The fact that our pinprick stimuli elicited a percept qualified as "pricking" in the majority of cases suggests that our pinprick stimulus activates A-fiber HTMs. It has yet to be investigated if these HTMs contribute to mechanical secondary hyperalgesia.

No clear early-latency negative peak was observed in our previous study (van den Broeke et al. 2017) in which the pinprick stimuli were applied manually to the skin. However, we did observe an early-latency negative peak in a follow-up study (van den Broeke et al., 2019) in which we used the robot to deliver the pinprick stimuli. This suggests that the use of a robot to deliver the pinprick stimuli increases the likelihood of observing the early-latency negative peak. A robot-controlled mechanical pinprick stimulator may increase the reproducibility of the pinprick stimulation compared to manual application and, thereby, reduce across-trial variations in the latencies of the elicited EEG responses. Moreover, the use of a robot makes the pinprick stimulation operator independent and, hence, less prone to biases. Notably, this also increases the probability of replicability of the current findings. Therefore, we recommend the use of a robot-controlled mechanical pinprick stimulator and a stimulus

evaluation task to record PEPs in the context of central sensitization. Future studies should determine the optimal velocity of stimulation to elicit the maximal PEPs and/or HFS-related differences in PEP magnitude, and assess test-retest reliability.

To conclude, the present study shows that robot-controlled mechanical pinprick stimulation of the skin elicits PEPs consisting of an early-latency negative peak followed by a later positive peak, and that both peaks are increased when the stimuli are delivered to the area of HFS-induced increased pinprick sensitivity when participants are engaged in a stimulus evaluation task during pinprick stimulation.

Acknowledgements

EvdB is supported by the Fonds de Recherche Cliniques (FRC) of the Université catholique de Louvain (UCL), Brussels, Belgium.

Conflict of interest

None of the authors have potential conflicts of interest to be disclosed.

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Figure legends

Figure 1. Experimental set-up. (A) High frequency electrical stimulation of the skin (HFS) was applied to the right volar forearm. Robot-controlled pinprick stimuli (64 mN) were applied to the skin surrounding the area onto which HFS was applied. (B) Geometric characteristics of the HFS electrode. (C) The effect of HFS on the perception and EEG responses elicited by pinprick stimuli was assessed at two different time-points: before HFS and 20 minutes after applying HFS.

Figure 2. Effect of HFS (high frequency electrical stimulation of the skin) on intensity and quality of perception elicited by the 64 mN pinprick stimulation. (A) Group-level average and standard deviation of the intensity of perception before and twenty minutes after applying HFS (numerical rating scale, NRS). **(**B) Individual difference in perceived pinprick sensitivity (after minus before HFS). Each dot represents a subject. (C) Group-level average proportion of trials reported as 'pinprick', 'touch' or 'not detected' before and after HFS.

Figure 3. Group-level average waveforms of PEPs (pinprick-evoked brain potentials) recorded before and after applying HFS (high frequency electrical stimulation of the skin) at electrodes Cz, CPz and Pz (64 mN pinprick stimulation intensity).

Figure 4. (A) Group-level average difference waveforms [after minus before HFS (high frequency electrical stimulation of the skin)] recorded at electrodes Cz, CPz and Pz. (B) *Left.*

Scalp distribution of the negative peak at the maximal voltage difference at electrode Cz (0.15 s). The blue colour indicates the *increase* in magnitude of the negative peak. *Right.* Scalp distribution of the positive peak at the maximal voltage difference at electrode CPz (0.31 s). The red colour indicates the *increase* in magnitude of the later positive peak.

Figure 5. (A) Mean and standard deviation of the magnitude of the negative peak of PEPs (pinprick-evoked brain potentials) measured at electrode Cz in the time window 0.1s to 0.2 s, before and after HFS (high frequency electrical stimulation of the skin). (B) Individual difference in peak amplitudes (after minus before HFS) at electrode Cz. Each dot represents a subject. (C) Mean and standard deviation of the magnitude of the late positive peak of PEPs, measured at electrode CPz in the time window 0.2 s to 0.5 s, before and after HFS. (D) Individual difference in peak amplitude (after minus before HFS) at electrode CPz. Each dot represents a subject.

Supplementary material

Supplementary Figure S1. Proportion of trials reported as 'pinprick', 'touch' or 'not detected' before and after HFS (high frequency electrical stimulation of the skin), in each individual participant.

Supplementary Figure S2. Individual waveforms of PEPs (pinprick-evoked brain potentials) recorded before and after applying HFS (high frequency electrical stimulation of the skin) at electrodes Cz and CPz.