Nociceptive SSEPs induced using multisine frequency modulated pulse trains

An exploratory study in inducing multi-frequency SSEPs

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Abstract

Steady state evoked potentials (SSEPs) using multiple frequency inputs are a well-known method to study the underlying dynamics in the visual, auditory and somatosensory system. Multi-frequency SSEPs provide insight into system dynamics such as delay, non-linearities, and frequency response function. Despite extensive research, little is known about the nociceptive system and its dynamics. Previous research showed the possibility to evoke single frequency nociceptive SSEPs using block wave stimulation. In the present study, we explored the feasibility of evoking multi-frequency nociceptive SSEPs using a multisine frequency modulated pulse train. The novel electrical stimulation technique using a frequency pulse train composed of 3, 7 and 13 Hz can stimulate multiple frequencies simultaneously. For the first time, we were able to induce multi-frequency SSEPs indicated by a contralateral maximal signal to noise ratio EEG response for 3 and 7 Hz. The power in 3 and 7 Hz showed significantly higher power compared to all other frequencies up until 40 Hz. The novel stimulation technique offers a unique opportunity to measure multi-frequency SSEPs related to nociceptive processing, allowing better localization of nociceptive signal processing and possible insight into the dynamics of the nociceptive regulation system.

Keywords: **SSEP; EEG; nociception; pulse train; multisine;**

Introduction

Nociception and the sensation of pain are some of the most vital functions to protect the human body from further damage [1, 2]. When nociceptive afferents in the skin are excited, they cause nerve fibers to transduce the nociceptive signal through the spinal cord towards different locations of the brain [3]. However, to date it is still unclear which regions of the brain are involved in nociceptive processing and how the nociceptive signal is processed by the brain in general. To activate the nociceptive pathway, stimuli can be applied, triggering the nociceptive fibers and bringing selective attention to the site of stimulation. Nociceptive stimuli triggering the nociceptive pathway, applied using laser stimulation or electrical stimulation, can be chosen accordingly to stimulate the myelinated, faster Aδ fibers and/or unmyelinated, slow C-fibers, which are thought to play different roles in pain pathologies [3, 4].

Nociceptive responses can be studied in both time and location using imaging techniques such as EEG (high time resolution, low spatial resolution) and fMRI (low time resolution, high spatial resolution) [5]. In recent studies presenting nociceptive stimulation, activations in terms of event-related potentials (ERP) could be seen in the mid-cingulate cortex, primary somatosensory cortex, and insula using EEG [6–8]. ERPs are observed within 500 ms after stimulus onset and therefore require a high time resolution recording. As the applied stimuli are painful, selective attention may be drawn to the site of stimulation. There is an ongoing debate as to what extent the resulting signal obtained from the nociceptive stimulations may reflect the sudden change in selective attention rather than underlying processes occurring in persistent pain [9, 10].

Sustained stimulation may circumvent the sudden change in attention. In studies on processing areas of the visual and somatosensory system, sustained periodic stimulations have been used to induce an easily quantifiable and reproducible electrocortical response, known as the steady-state evoked potential (SSEP) [11–13]. As such, SSEPs can be used for source localization and comparison of activity levels between different stimulation amplitudes and varying conditions over time. Additionally, several clinical applications of SSEPs have been studied, amongst which diagnosing schizophrenia and migraine [14,15]. By using a prolonged stimulation, the activated areas should become less prone to the influence of selective attention straight after stimulation onset. Recently, it was shown that SSEPs can

also be evoked by nociceptive stimulation [16, 17].

Multi-frequency SSEPs, which are currently already used with visual, somatosensory and auditory stimulation, could be used for several aspects of system analysis, ranging from identification of non-linearities to multispectral phase coherence and delays [12, 18, 19]. In a clinical setting, insight into the processing of different frequencies and their gain in power may prove to help diagnose and monitor patients receiving therapy.

Nociceptive multi-frequency SSEPs in response to intra-epidermal stimulation (IES) provide a potential tool to study the nociceptive system in conjunction with the techniques developed throughout decades of visual and auditory SSEP research. IES specifically targets nerve endings in the epidermis, where mostly free nociceptive nerve endings are located. Nociceptive multi-frequency SSEPS could be used both in system analysis and as possible biomarkers. To provoke a multi-frequency SSEP, a novel stimulation technique was developed using a pulse train which was frequency modulated with a sinusoid composed of three frequencies. By applying sinusoidal stimulation instead of block stimulation, several frequencies can be applied simultaneously. This allows for system identification in terms of order, non-linearities upon the presence of harmonics and for possible source localization, presenting insight into pain connectivity and frequency response function.

A discrete multisine frequency modulated pulse train was used for stimulation with the aim to measure the same frequencies as a continuous signal in the EEG response. By analyzing possible second and third-order harmonics and phase shifts, information for potential system identification could be extracted. This study aimed to explore the possibility of evoking multifrequency SSEPs by frequency modulated multisine pulse train stimulation as a potential tool for identification of dynamic properties of the nociceptive regulation system.

Methods

Pilot

Prior to the present study, a pilot study on five healthy male participants, all employees within the TU Delft or UTwente, was performed. In three subjects the presence of 3 Hz was tested, in a single subject 7 Hz and in a single subject 13 Hz. The same procedure and stimulus application as in the present study were used (see 'Procedure' and 'Stimulus application'). No VAS measures were taken.

Stimulation pulse

During the pilot, the same frequency modulation range of the pulse train was used, 20 Hz to 200 Hz (see 'Stimulation pulse and pulsewidth'). Participants were stimulated with a pulse train composed of a carrier wave of 110 Hz and modulation amplitude of 90 Hz. The same technique as shown in fig 3 was used, however a single frequency instead of three frequencies was used.

Present Study

Participants

Ten healthy male participants (aged 23 to 27 years, 9 right-handed) participated in the present study. Written informed consent was obtained from all participants. The study was approved by the local ethics committee.

Procedure

Experiments were performed in a dim, silent, signal-shielding room. Participants were seated upright in a wheelchair facing a blank wall with a single neutral image. Nociceptive stimuli were applied to the back of the right hand during 5 stimulation blocks on different locations of the hand, each consisting of 20 stimulation sequences. Per location a single stimulation block was performed, to prevent habituation of the skin to the nociceptive stimuli. No difference was present in the stimulation pulse trains applied during each block. The location order was chosen such that none of the locations were located directly next to each other to prevent additional desensitization of the skin, resulting in the order presented in fig 1. The timeline for the procedure can be found in fig 2.

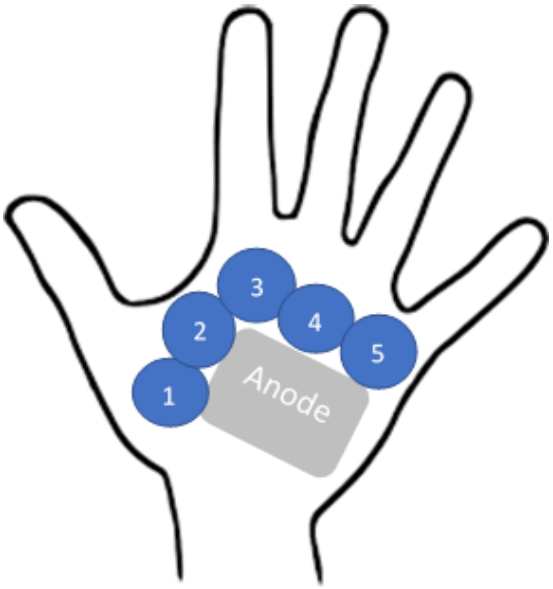


Figure 1: Overview of stimulation block locations. During the first stimulation block location 2 was stimulated, the second stimulation block location 4, then 1, 3 and 5. No locations located next to each other were stimulated directly following stimulation of a neighboring location. All electrode locations were distal from the anode.

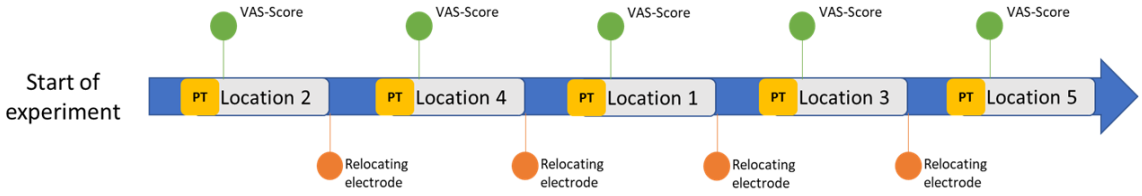


Figure 2: Timeline of the experiment. Each location as seen in fig 1 was stimulated with 20 stimulation sequences. At the start of the 20 stimulation sequences, the perception threshold (PT) was determined. Each sequence lasted 8.5 seconds and after the first sequence, the participant rated the sensation using a VAS-score. After the 20th stimulation sequence, the electrode was moved to the next location.

Stimulation sequences had a length of 8.5 seconds and were interspersed with 10-15 seconds of rest. After each block, the door of the measurement room was opened and small talk to keep the subject attentive was made. The subjects were instructed to keep relaxed and try to avoid blinking during stimulation. The entire preparation and recording lasted about 2 hours per subject. For the full protocol please refer to Appendix B.

Stimuli

Stimulus application

Intra-epidermal stimulation (IES) was used to preferentially stimulate nociceptive Aδ fibers [20]. Nociceptive stimuli were applied to the epidermal nociceptive afferent nerves on the back of the right hand, using a custom made IES electrode composed of five needle cathodes which was placed directly next to a rectangular anode [21]. The electrode was taped onto the skin, gently pressing into the epidermis. After each stimulation block, the electrode was displaced to prevent habituation of the skin. The amplitude was set to twice the perception threshold to ensure the stimulation would be perceived by the subject.

Stimulation pulse and pulsewidth

A frequency modulated pulse train was applied in this experiment. The frequency range was chosen with as highest frequency 200 Hz to allow repolarization of the nerve between different pulses and the lowest frequency 20 Hz to ensure subjects would not notice individual pulses [22]. Three frequencies were evaluated as proposed in earlier research and tested during the pilot experiment [17]. During the present study, the frequency of the generated pulse train was modulated by a multisine composed of 3, 7 and 13 Hz, using a carrier wave of 110 Hz and a modulation amplitude of 30 Hz per frequency as visualised in fig 3. The 13 Hz was shifted with 1/3 π forward and 7 Hz was shifted with 1/3 π backwards. Depending on the amplitude in the time domain the inter pulse interval (ipi) to the next pulse was calculated.

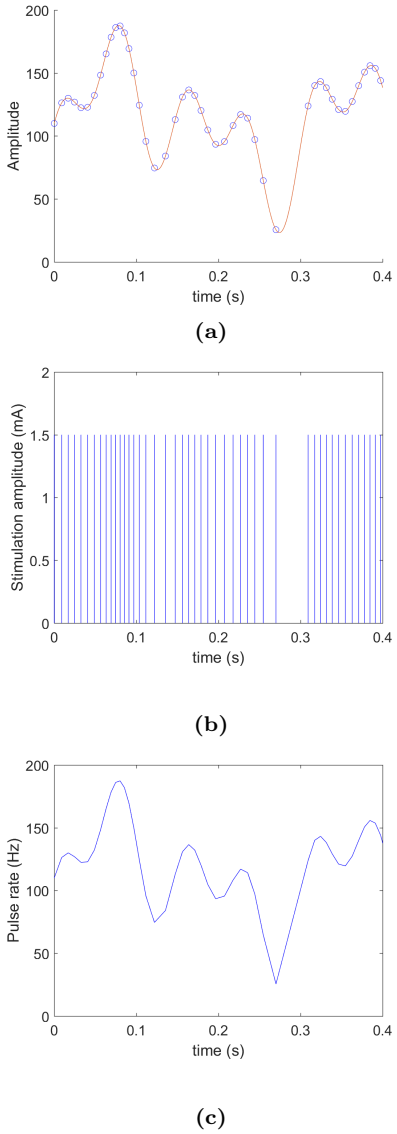


Figure 3: Multisine pulse train as applied in the stimulation. (a) The timedomain representation of the multisine signal using an offset of 110 and an amplitude per frequency of 30. This signal was sampled with a varying inter pulse interval (ipi), depending on the amplitude at each prior sample. (b) The pulse rate as obtained by ipi. The closer together the pulses the higher the amplitude of the original signal. (c) The pulse rate in Hz of each pulse. This figure closely resembles the original signal (a).

Measures

Objective measures

After placing the electrode, the perception threshold of the participant was measured. The perception threshold was determined using a staircase procedure to a 0.5 ms pulse. The participant was asked to press a button until a single pulse was perceived.

Subjective measures

Directly following the first sequence of stimulation of each block, participants were asked to rate their pain perception on a visual analog scale (VAS) ranging from 0 (no sensation at all) to 5 (painful) to 10 (worst pain imaginable). The VAS line mentioned both 0, 5 and 10 and measured 12 cm in length.

EEG measures

The head of the participants was first thoroughly cleaned using 95% disinfectant alcohol to remove grease and styling gel remnants. The EEG signal was recorded using a 128-channel EEG cap (ANT, The Netherlands) and amplifier (TMSi REFA, The Netherlands) according to the international 10/5 system at a sampling rate of 1024 Hz using an average reference. A ground electrode was placed on the right mastoid bone. Electrode impedances were kept below 10kΩ. To decrease impedance further, a tubular net bandage was placed over the EEG cap with a small section cut open for the eyes and mouth. Impedances in each channel were checked after each stimulation block and decreased or channels removed as necessary.

Data analysis

VAS analysis

VAS scores per location were recalculated to values ranging between 0 and 10 based on the distance between the given numbers. To test if a single location was significantly perceived as more painful, a paired two-sided t-test was performed with restraint $p<0.05$.

Amplitude analysis

Stimulation amplitudes were obtained by calibration of the recorded pulse perception amplitudes in the software. To test if a single location had a significantly higher perception threshold, a paired two-sided t-test was performed with restraint $p<0.05$.

Artifact

Earlier pilots in UTwente had experienced a big influence of artifact directly at stimulus onset. To ensure the response to the stimuli measured was not influenced by stimulation artifact, the raw EEG response of the first and last block of stimulation was studied. The epochs were first high-pass filtered using a 100 Hz FIR filter, after which the response in each channel to each individual pulse in the pulse train was averaged. The amplitude over time for all channels was calculated for 20000 single pulses as well as the topographical location for the maximum amplitude and the amplitudes just after stimulus onset (t=4 ms).

EEG analysis

Preprocessing steps included filtering the data with a 1 Hz high-pass and a 40 Hz low-pass filter and epoching the data from -0.5 to 8.5 seconds to the start of stimulation. The range of filters was chosen to prevent signal leakage (1 Hz) and exclude noise (>40 Hz). Additionally, epochs containing movement and EMG artifacts were rejected and EOG components such as eye-blink artifacts were removed using ICA decomposition.

Temporal dynamics

In many EEG analyses, the ERP is of interest. In this study, the aim is to study the SSEP, occurring after the initial ERP. To prevent the influence from the ERPs, the initial time interval including ERP activity should be excluded. With a time window from [-0.5s to 2s], an average across all trials per channel in the time domain was conducted on the pre-processed data to identify ERP latency.

Scalp topographies

From the time domain signals, all trials per subject were divided into 2s epochs from 0.5s after stimulation quadrupling the number of epochs, where the onset was chosen based on the temporal dynamics. Signal to noise ratio (SNR) was calculated after transforming all 2s epochs to the Fourier domain and using the power defined as:

$$E_x(k) = \left| \frac{1}{p} \sum_{p=1}^{P} X^{[p]}(k) \right|^2 \tag{1}$$

where P is the number of epochs and k the frequency.
The sample variance was calculated using:

$$N_x(k) = \frac{1}{p} var(X^{[p]}(k)) \tag{2}$$

The SNR was then computed as:

$$SNR_X = \frac{\sum_{k=0}^{N-1} E_x(k)}{\sum_{k=0}^{N-1} N_X(k)} \tag{3}$$

with N being the number of frequencies, varying for the number of frequencies evaluated.

Using this SNR, topographies were plotted for every single frequency, the base frequencies(3, 7 and 13 Hz) and second- and third-order combinations of 3, 7 and 13 Hz, see formula 6.
Apart from per subject analyses, group analyses were performed by averaging the SNR, magnitude, and variance as calculated on a per subject basis. To prevent data deficiency, removed bad channels were spherically interpolated on a per subject basis in the time domain before calculating the Fourier transform.

Power spectra

The power spectra was calculated using the same 2s epochs. Two approaches were used to respectively compute the sample variance and the auto spectral density for the average of all epochs:

- The sample variance was computed by first performing a Fourier transform on all epochs and then applying formula (2).
- The auto spectral density was computed by first averaging across all epochs per subject, after which the Fourier transform was taken from this time-domain averaged epoch.

The auto spectral density was then computed as:

$$E_x(k) = |X(k)|^2 \tag{4}$$

with $X(k)$ the Fourier transform per frequency k.

Both the stimulated frequencies as well as their second and third order harmonics as presented in formula 5 were evaluated:

$$f^n = \pm n_1 3 \pm n_2 7 \pm n_3 13 \tag{5}$$

with $n_x \geq 0$.

Second order harmonics f^2 are defined by $n_1 + n_2 + n_3 = 2$ and third order harmonics f^3 are defined by $n_1 + n_2 + n_3 = 3$.
Using a 95% confidence interval a frequency is perceived as significant when the power of that frequency value exceeds ±1.96 SD or when the SNR value exceeds 2.

Phase Shift

To evaluate the possible use for system identification, the relative phase shift for each frequency was calculated on the non-interpolated data with 2s epochs. Using the Fourier transform of all epochs the phase was computed as:

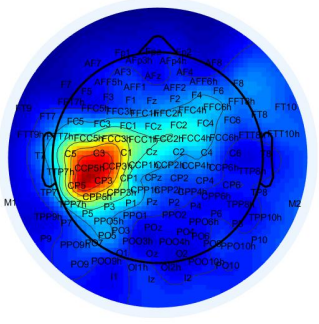
$$\Phi_{ssep}(k) = \angle\left(\sum_{p=1}^{P} \frac{X^{[p]}}{P}\right) + 2k\pi \tag{6}$$

with $0 < \Phi_{ssep} \leq 2\pi$

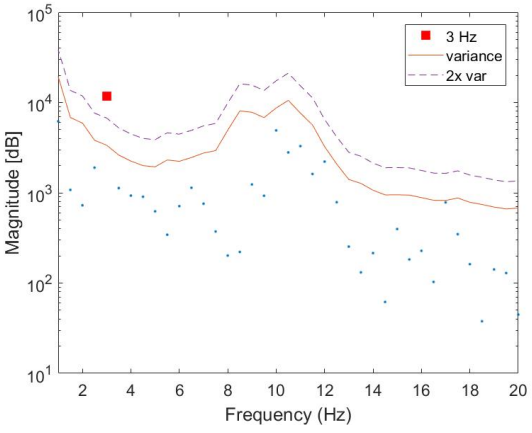
The resulting Φ_{ssep} was averaged across subjects showing significant 3, 7 or 13 Hz activity and the standard deviation was computed. The phase difference was calculated by subtracting the initial phase shift given to 3, 7 and 13 Hz at stimulus onset $\pm 2\pi$ to ensure $0 < \Phi_{diff} \leq 2\pi$. The corresponding delay was calculated using:

$$\tau = (\Phi_{diff} \pm k\pi)/2\pi \cdot T \tag{7}$$

where k is any integer for $\tau > 0$ and T is the period of the evaluated frequency.



(a)



(b)

Figure 4: Pilot results of single frequency 3 Hz SNR topography and normalized amplitude spectrum. (a) The average SNR of the pilot subjects stimulated with 3Hz is shown. A clear peak can be seen around channel CCP3h, indicating by the bright red. (b) The power in channel C3 is plotted using a significance level of 1.96 SD. All frequencies are plotted using dots and are considered significant when the power is higher than the dashed line. The power in 3Hz is labeled by the red square and significant ($p<0.05$).

Results

Pilot study: single frequency

To test the feasibility of a sinusoidal frequency-modulated pulse train to induce SSEPs, first a single frequency (3Hz) modulated signal was tested. For each subject, a scalp topography and averaged filtered normalized amplitude plot was computed. In all three subjects, a contralateral response was seen for a 3Hz response (fig 4). In the normalized amplitude plot for C3 respectively, a significant peak ($p<0.05$) is visible, confirming the presence of 3Hz activity.

After validation of 3 Hz activity, additional pilot tests with 7 and 13 Hz as a single frequency were performed (see appendix A fig 11). Both for 7 and 13 Hz activity was seen in channels C3 and CCP3h. Upon plotting the power spectra, both frequencies were significantly present ($p<0.05$). Following the fact that all three individual frequencies induced by a sinusoidal frequency modulated pulse train could be retrieved as a continuous signal in the EEG response, it was assumed feasible to test the multi-sine frequency-modulated pulse train. The three different frequencies showed the highest power in the channels C1, C3 and CCP3h, which are therefore the channels of interest for the present study. The following sections describe the present study.

Present Study

Perception amplitude

The perception threshold was measured at the start of each stimulation block. The perception amplitudes are shown in fig 5. No significant difference between locations was found ($p>0.05$). Linear regression analysis did not show a significant correlation between increased amplitude and SNR ($p>0.05$).

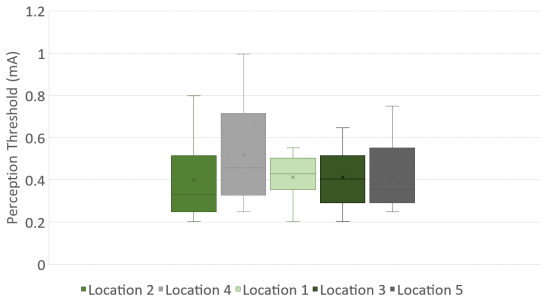


Figure 5: Perception amplitude median and standard deviation for different stimulation locations in order of stimulation. A large spread in perception threshold ratings was seen, none differed significantly. For different locations, the spread of perception amplitudes varied.

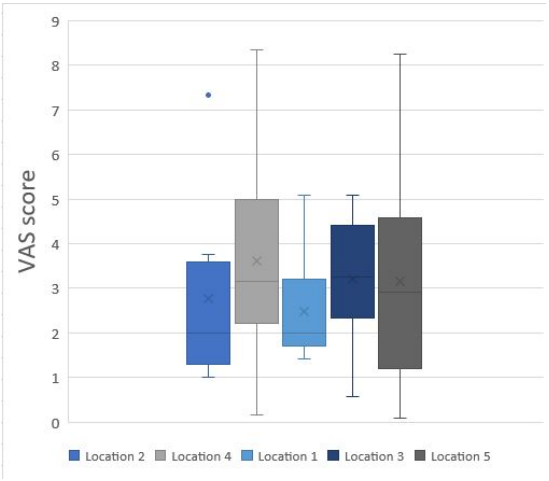


Figure 6: VAS score per location of the hand in order of stimulation. The locations used per block were arranged in the order 2, 4, 1, 3 and 5, as seen in fig 1. Locations 3, 4 and 5 were located near the knuckles. A large spread in VAS ratings was seen, none differed significantly.

Pain rating

After the first pulse train of each stimulation block, participants rated their sensation to the single pulse train. Nine subjects were included as one participant forgot to rate the sensation. None of the locations were perceived as painful, but the three locations near the knuckles were perceived stronger (fig 6). Location 4 seems to be the most noticeable, although not statistically significant($p=0.052$). None of the locations differed significantly ($p>0.05$). A significant linear regression correlation was found between the pain rating and the average SNR across channels C1, C3, and CCP3h ($p<0.05$). A trend ($p=.08$) was seen between a higher perception threshold and a higher pain rating assessed using linear regression.

Artifact

Using an average across 20000 pulse onsets, no indication of substantial influence of stimulus artifact was found. Apart from subjects 1002 and 1006, the maximum SNR peaks were seen to the side of the head, likely evoked by EMG activity. The channels showing high activity in the previously mentioned subjects were either excluded or given the very small amplitude discarded as influential as values were lower than 0.1 μV. For channel C3, no amplitude over 0.28 μV was found in any of the subjects. For a complete overview of artifact amplitudes per subject and respective scalp topographies, please refer to Appendix A fig 12.

Temporal dynamics

The time-domain response was averaged using a 2.5-second window across all trials per channel per

subject to evaluate the time window of ERPs generated by the nociceptive stimulation. In all channels, a clear ERP was visible considering a fast negative peak N1 followed by a positive peak P3. As can be seen in fig 7, the ERP signal in the channels of interest (C1, C3, CCP3h) lasts from approximately 0 to 0.5 seconds from stimulus onset based on the biphasic negative-positive behavior of ERPs. The ERPs for individual channels can be found in Appendix fig 13.

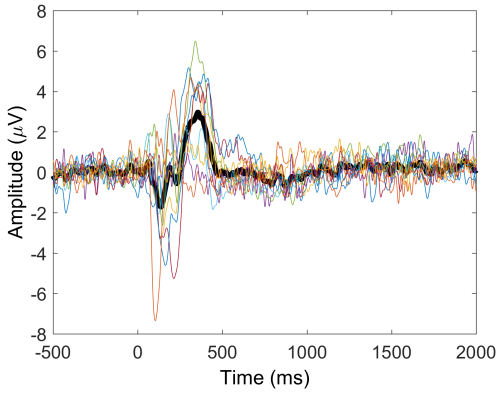


Figure 7: Average time domain response for the average of channels C1, C3 and CCP3h to establish ERP time window. In black the average across all subjects is shown, whilst coloured lines show the results per subject. In all subjects the P3 ends before 500 ms.

Group level analyses

Scalp topographies

Group level topographies were computed for both individual frequencies (3, 7 and 13 Hz) as well as for base, second and third-order combinations of these three frequencies. Based on the frequency response location as seen in the pilot experiments, contralateral activity to the side of stimulation was expected around channels C1, C3, and CCP3h. The group data shows a high SNR for all individual frequencies at the parietal cortex contralateral to the side of stimulation (fig 8). The peak for 13 Hz is located a bit more posterior to the peak seen for 3 and 7 Hz. Second-order harmonics show increased SNR in the frontal cortex contralateral to stimulation. Third-order harmonics SNR peaks are localized in the same location as the individual frequencies 3 and 7 Hz, although the peak shows a bigger spread across channels.

Group level power

Group level power spectra were computed for channels C1, C3, and CCP3h. In channels C1 and CCP3h, 3 and 7 Hz are significantly present ($p<0.05$). In channel C3 only 7 Hz activity is seen ($p<0.05$). On a group level, none of the higher-order harmonics or 13 Hz were significantly present ($p>0.05$). The power of second and third-order harmonics varied per channel, whilst the ratio in power for 3, 7 and 13 Hz remained constant per channel.

Per subject analyses

Scalp topographies

The scalp topographies of nociceptive SSEPs show large variations between subjects. Per subject presence of 3, 7 and 13 Hz activity was analyzed. In fig 10 an overview of the scalp topographies per subject for each stimulated frequency is visualized. For all subjects, activity was seen in the hemisphere contralateral to stimulation. In most subjects, the increased SNR is seen around channel C3 but frequency location results vary per frequency and subject. Out of all subjects, 6 subjects show an increased SNR for 3 Hz, 6 subjects show an increased SNR for 7 Hz and 2 subjects show an increase in signal to noise ratio for 13 Hz in one or more of the three channels of interest.

Power spectra

Autospectral density and SNR per frequency were calculated to show if 3, 7 and/or 13 Hz activity was present on an individual basis. All subjects were evaluated at channels C1, C3 and CCP3h. The SNR values per frequency are plotted in fig 9 to evaluate the number of participants showing a significantly increased SNR. In 6 of the subjects 3Hz was significantly present ($p<0.05$), in 6 of the subjects 7 Hz ($p<0.05$) and in 2 of the subjects 13 Hz ($p<0.05$). For a full overview of the magnitude spectra including noise levels per subject, refer to Appendix A fig 14.

Phase shift

For each subject, the phase of the EEG response (Φ_{SSEP}) was determined in channels C1, C3 and CCP3h for 3, 7 and 13 Hz. Each frequency showed a different phase. Using the phase at stimulus onset, Φ_{Diff} was calculated. Only the subjects showing significant power in the evaluated frequency were included in the phase shift measurements as shown in Table 1. The Φ_{Diff} for 3, 7 and 13 Hz all differed significantly from each other ($p<0.05$). The delay τ between input and output signal is around 0.18s. For an overview of all subjects see Appendix A fig 15.

Table 1: Phase difference and delay output signal

	3Hz	**7Hz**	**13Hz**
Phase	3.36	1.21	4.54
Std	*0.57*	*1.04*	*0.19*
Delay	0.18 s $\pm \frac{k}{6}$	0.03 s $\pm \frac{k}{14}$	0.06 s $\pm \frac{k}{26}$

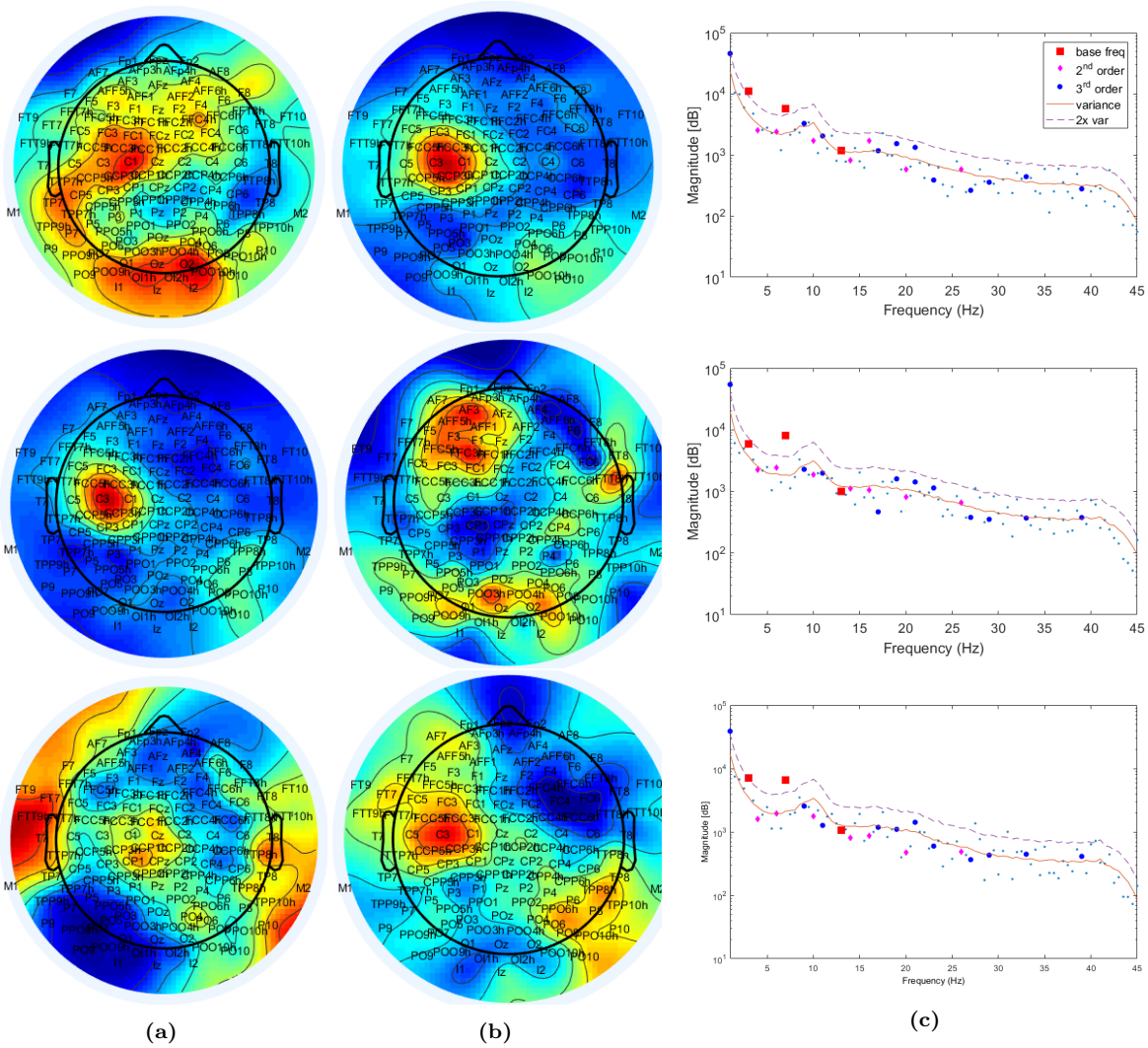


Figure 8: Topographies and magnitudes averaged across all subjects. (a) The SNR topographical plots for single frequencies from top to bottom 3, 7 and 13 Hz. Each show mid-parietal activity contralateral to stimulation. (b) The SNR topographical plots for respectively base-, second- and third-order harmonics. Base and third-order frequencies show mid-parietal activity contralateral to stimultion. Second-order acitivity is located more frontal. (c) The power spectra including 95% confidence interval for channels C1, C3 and CCP3h from top to bottom is shown. In all channels only 3 and 7 Hz are significantly present.

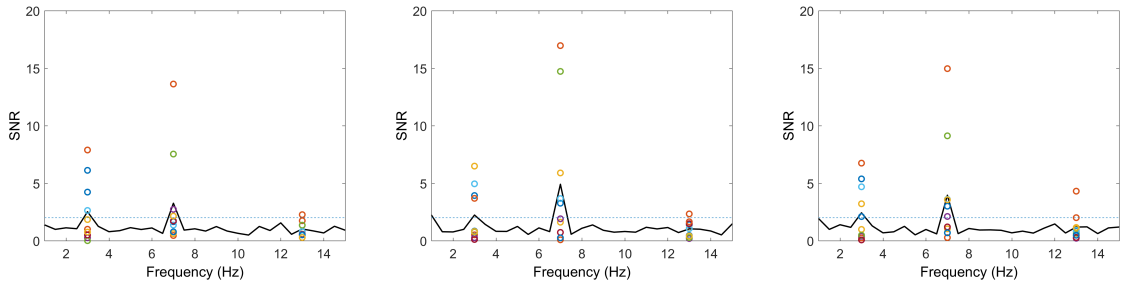


Figure 9: SNR values per frequency for channels C1 (a), C3 (b) and CCP3h (c). To analyse if the group data was heavily influenced by a single outlier, the SNR for all subjects for the 3, 7 and 13 Hz is plotted using coloured markers. The average SNR is plotted in black. Using a 95% confidence interval, all values above 2 SNR are considered significant. Although 7 Hz does show a high peak, 6 subjects show 3 and 7 Hz activity ($p < 0.05$) and 2 subjects show 13 Hz activity ($p < 0.05$), confirming that the group results were not caused by a single outlier.

Discussion

The goal of the present study was to investigate the possibility of provoking nociceptive multi-frequency SSEPs using a frequency modulated pulse train and the feasability to use it as a tool for studying the

dynamics of the nociceptive regulation system. Several measures were used to assess the potential of this method.

Scalp topographies

Both in the present study and the pilot, increased SNR activity for 3, 7 and 13 Hz was located mid-parietal on the side contralateral to stimulation. Despite the presence of a significant topographical location, it was not feasible to perform analysis on an ICA component level. This is related to the fact that the nociceptive SSEPs have a low SNR compared to SSEPs in the somatosensory and visual system [9]. More exact localization of the signal was therefore not possible. In a study on nociceptive SSEPs using block wave stimulation, a more frontal response was seen [17]. In that study, both left and right-hand stimulation were performed after which a t-test revealed no difference in the EEG response depending on the stimulation side. The EEG response was then averaged for both sides. The study at present used 100 epochs whilst the aforementioned study presented 40 epochs used for calculations. The difference in epochs may explain the increased frontal activity seen in previous studies. Somatosensory studies showed similar topographical plots for 3, 6 and 12 Hz [9]. The evoked nociceptive SSEPs are located mid-parietal to stimulation location, but given their relative low SNR, the signal is thought to emerge from deeper brain tissue. When the signal originates from deeper brain tissue, a lower SNR and higher spread among the cortex is expected.

Power spectra

From the presented data, 3 Hz and 7 Hz could be clearly distinguished on a per subject and grand average basis in at least 6 of the participants. A 13 Hz signal however, was not significantly present, neither were higher-order harmonics. Earlier research hypothesized that different neuronal populations are expected to resonate at preferred frequencies [17]. The absence or less preferred resonating may lead to a lack of 13 Hz activity. Higher-order harmonics such as the second- and third-order that were evaluated in the present study, were expected to have increased power compared to first-order frequencies when present [23]. Assuming stimulation was performed directly onto the nerve, little harmonics are to be expected as there are no non-electrical physiological components involved in the pathway directly activating the nerves. The results, therefore, indicate a linear system, however, more tests have to be executed to know for sure. On a per subject level significant harmonics are present, but upon calculating the group power spectra, these harmonics average out. It is therefore possible that these peaks are either generated at random or large deviations between subjects exist. Additional data per subject to increase the SNR could be used to obtain more information.

Phase Shift

A significant phase difference for 3, 7 and 13 Hz was found. However, 7 Hz showed a large variation between different channels and participants. As the number of subjects evaluated is low (n=6), the phase may be influenced by different artifacts, circularity and participant compliance [24]. However, it does show the potential of applying the multisine frequency-modulated pulse train to look at the gain and phase shift of the underlying system. In a future study, it may be feasible to assign a random phase to each frequency to ensure the suggested linear characteristics by the power spectra. For example the visual system is known to be a non-linear system so additional tests in the nociceptive system taking harmonics into account could be performed to ensure the linearity of the system and evaluate the dynamics.

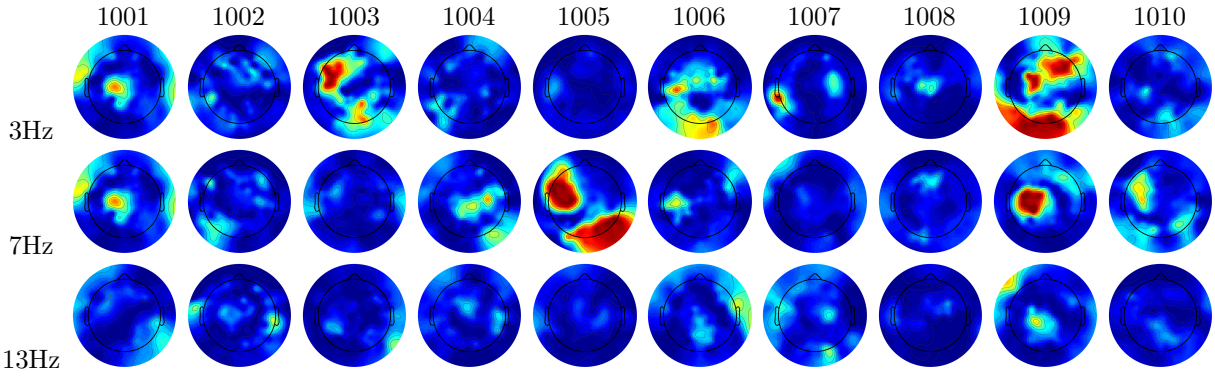


Figure 10: SNR scalp topographies per subject showing differences between frequencies within and between subjects. Per column the results per subject are shown. On the first row the SNR for 3 Hz is plotted, on the second row for 7 Hz and the third row the SNR for 13 Hz. The same scale was used across all plots, where SNR peaks are indicated by yellow or red.

Pain rating and Perception Threshold

The VAS score and SNR were linearly correlated. This implies that a higher pain rating led to an increased SNR. By ensuring the signal is perceived as painful, a better SNR may be obtained. In the present study, stimulation in none of the locations was perceived as painful. Location 4 showed a trend for being perceived as the most noticeable. A certain level of accommodation is expected in sustained nerve stimulation, and as the order of stimulation was not randomized between different subjects it is difficult to interpret these results. Overall the knuckle region was perceived as more noticeable, with more small bones and tendons present near the surface of the top of the hand. In future studies, a location with as little bones and tendons near the skin surface may be more feasible to test an increased number of stimulation blocks.
The perception threshold did not vary significantly per location, however, a trend was seen between PT and pain rating. No correlation between PT and SNR was found. From a clinical perspective, the stimulation intensity may be correlated with the amplitude seen in the SSEP signal [25, 26]. As the present study was evaluated using a small dataset (N=10) and participants proclaimed they were sometimes unsure whether they felt the needles protruding or the pulse, a bigger dataset should be obtained to test this possible relation. In the current study, Aδ fibers were targeted. Different electrodes or different electrode setups may be more suited to preferentially select certain nociceptive fibers mimicking different types of chronic pain [27]. Additionally other setups may increase the VAS scores and possibly SNR.

Pulse train

During the pilot, all subjects showed a clear SSEP response in the SNR topographies as well as in the power spectra for every single frequency. In the present study, although the frequency modulated pulse train showed activity for 3 and 7 Hz, the results were more variable. In the present study, a modulation amplitude per frequency of 30Hz was used, while in the proof of concept an amplitude of 90Hz was used to allow a range from 20 to 200 Hz. Additional steps to increase the response should be considered, either in the form of re-evaluating the range of the modulated signal to either below 20 Hz or above 200 Hz, or by including only 2 instead of 3 different frequencies in the multisine.

Artifact

The location of the artifact did not coincide with the SNR activity peaks locations. Additionally the level of artifact in the present recording is very low, indicating the results presented are not influenced by stimulus artifact. As the channels showing some artifact activity were not of interest to the present study, the effect is limited, but overall in the recording, a few points should be taken into account. Many studies use EOG electrodes to selectively filter out epochs with many EOG artifacts which were manually filtered in this study [28]. During the recordings of the pilot, there were some problems with the ground electrode placement on the wrist being unstable. To circumvent this stability problem, the ground electrode was placed next to the ear in the present study. However, this site is known to produce more EMG activity and therefore more artifact during a recording. Lastly, some troubles with the EEG caps were present during the recordings. Depending on the bending direction of the wires the impedances of some channels would fluctuate or suddenly increase to $>10\text{k}\Omega$ over time.

Conclusion

In 6 out of 10 subjects, 3 and 7 Hz activity was significantly present and in 2 out of 10 subjects, significant 13 Hz activity was seen. There was no correlation found between subjects showing 3 Hz activity, subjects showing 7 Hz activity, or subjects showing 13 Hz activity. Some of the subjects showed activity in all three frequencies whilst others only showed a single frequency. Slight variations in the topographical location were seen, but as the topographical plot was not adjusted to custom fMRI data, slight changes are expected. Although the response was not seen in all subjects, the frequency-modulated pulse train was able to transmit the frequency content through the nociceptive pathway. The artifact was not present in the channels of interest, creating a reliable power, phase, and SNR estimation. A different phase shift for each frequency was seen.

In conclusion, the present study shows that the novel multisine frequency-modulated pulse train can induce nociceptive multi-frequency SSEPs reflecting cortical processes distinct from ERPs, providing possible evidence that the nociceptive pathway does not show harmonics, acting as a first-order system. This novel method may be used to directly study the dynamics of the nociceptive system and provides a potential tool to study the phase and gain of the system.

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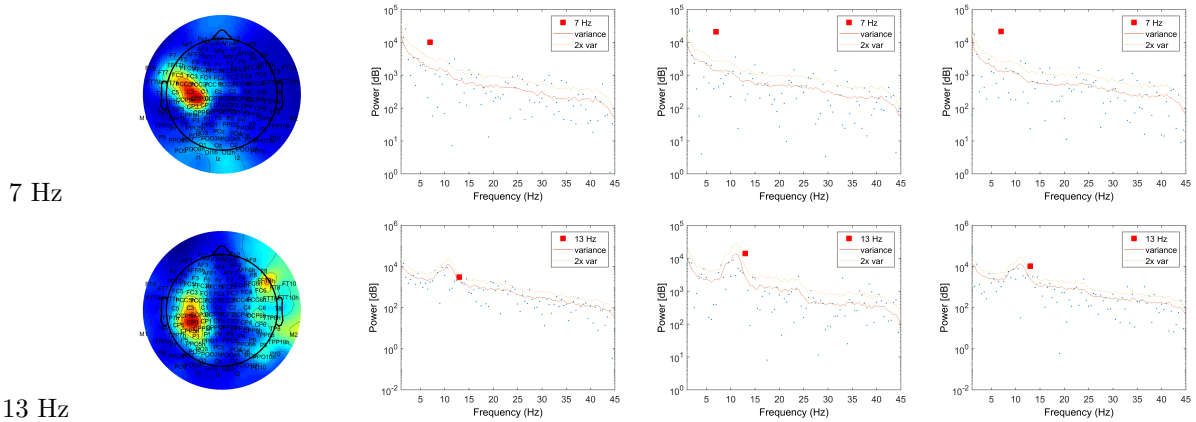
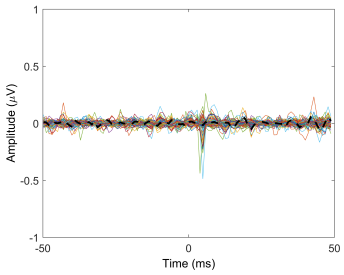
A Additional figures

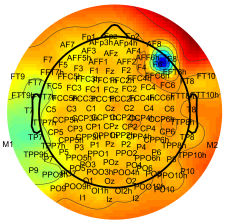
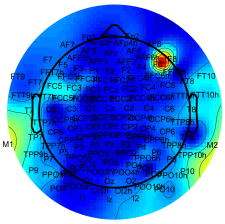
On the following pages, additional figures are presented that were deemed as too much to also include in the present study. A description below each figure is given.



(a) (b) (c) (d)

Figure 11: Topographies and Power spectra for single subjects as performed as a pilot for the inducement of 7 and 13 Hz signals using frequency modulated pulse trains. The topographies show the SNR in respectively 7 and 13 Hz (a), the power in channels C1 (b), C3 (c) and CCP3h (d) using a 95% confidence interal of 1.96 SD.



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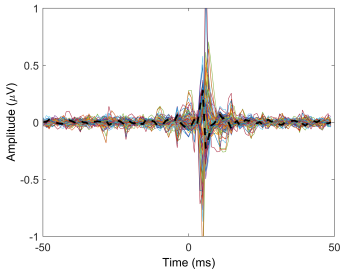

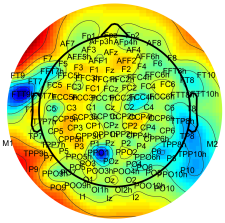
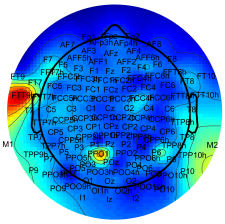

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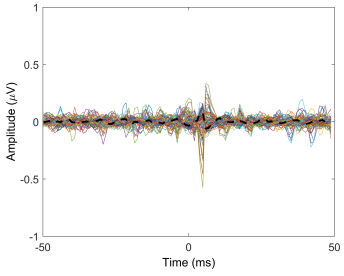

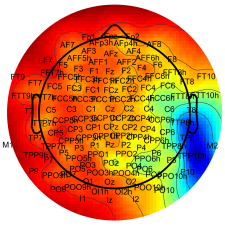
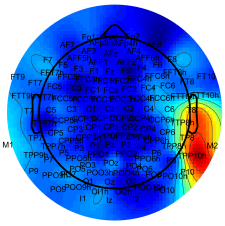

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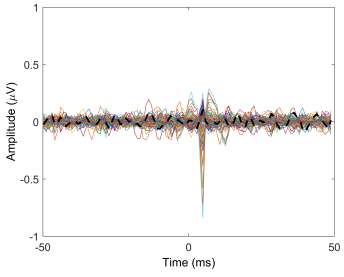

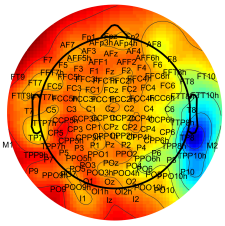
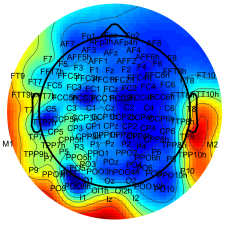

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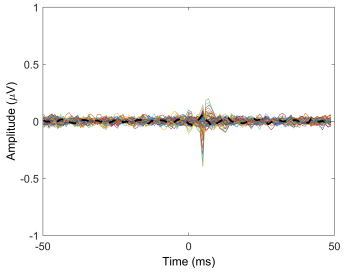

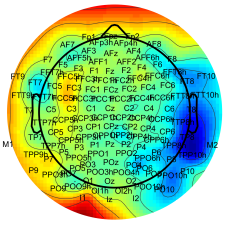
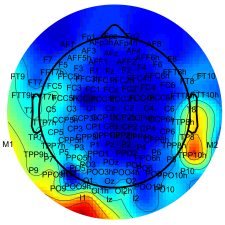

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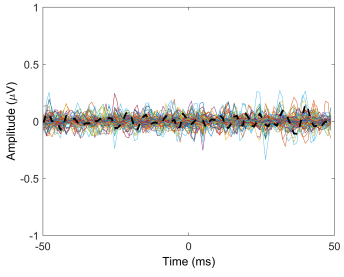

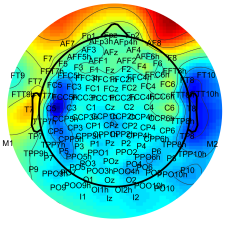
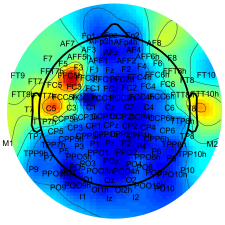

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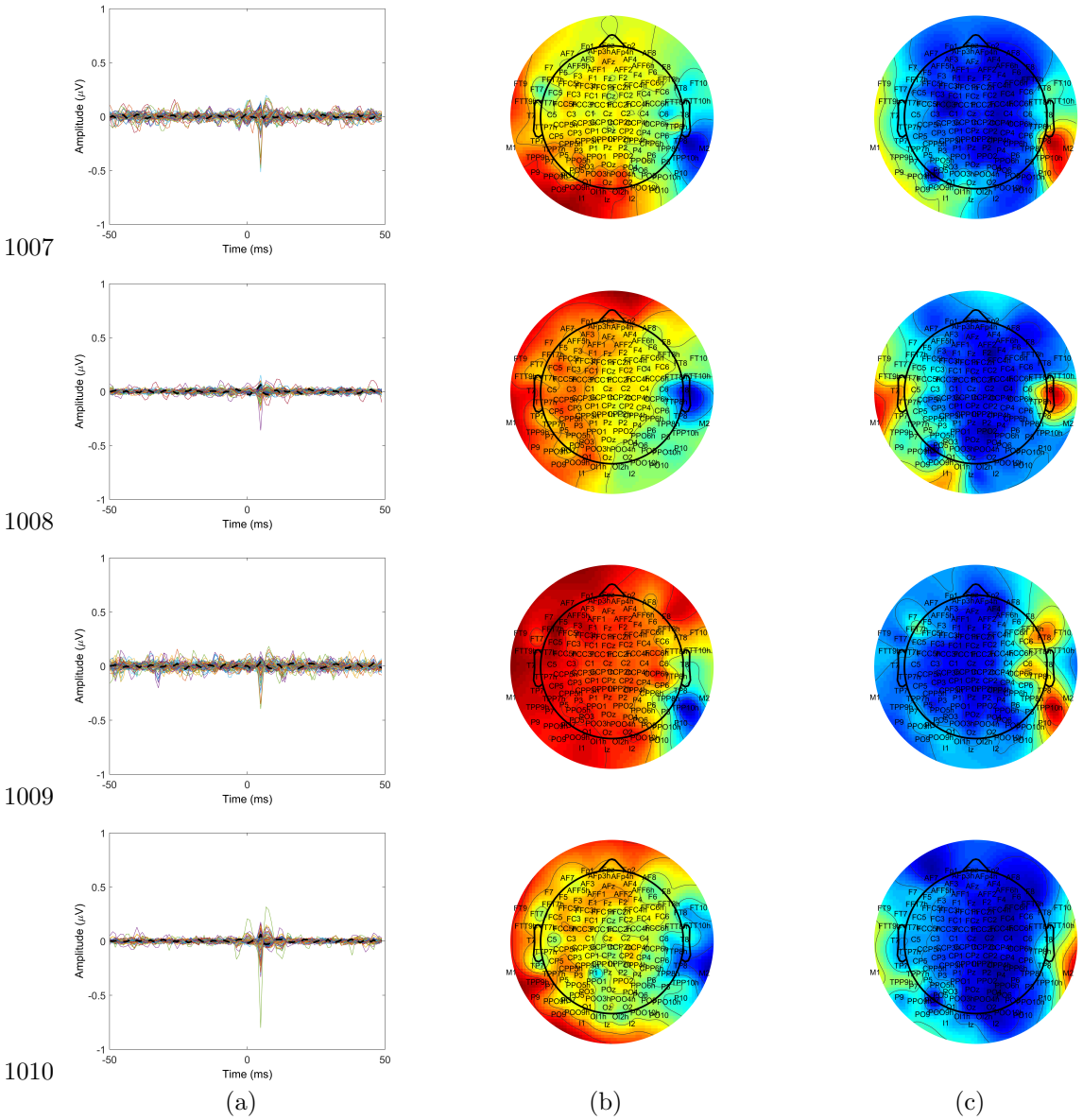

Figure 12: Artifact amplitudes and scalp distributions for all subjects. The average amplitudes of all pulses for each channel are plotted in (a). The black dashed line shows the response for channel C3. The amplitude per channel is plotted using a scalp distribution amplitude at t=4ms in (b), just after the stimulus is applied (t=0ms). The maximum amplitude for each channel within the timewindow of [-50ms 50ms] using a scalp distribution is shown in (c). The topographical plot in (c) is composed of different timepoints for each channel.

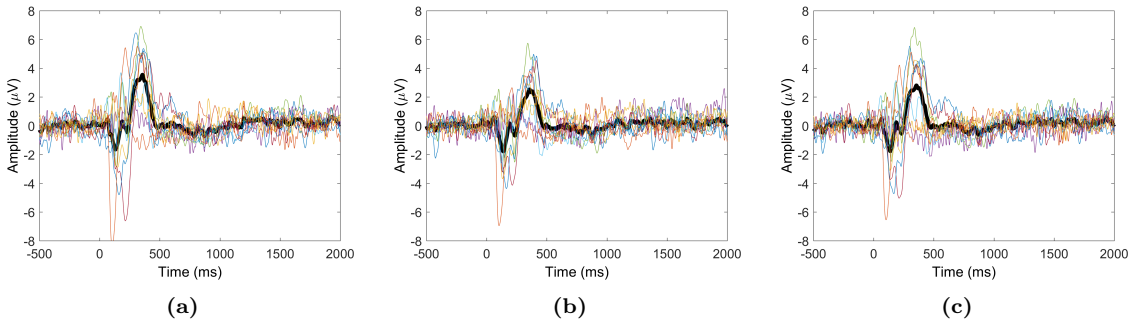

Figure 13: Time domain average per channel to asses the ERP signal. The figure shows channels C1 (a), C3 (b) and CCP3h (c). The average response for all subjects is plotted in black, whereas the averaged response for all trials per subject is plotted using different colours. All amplitudes lay within a range of 8 μV.

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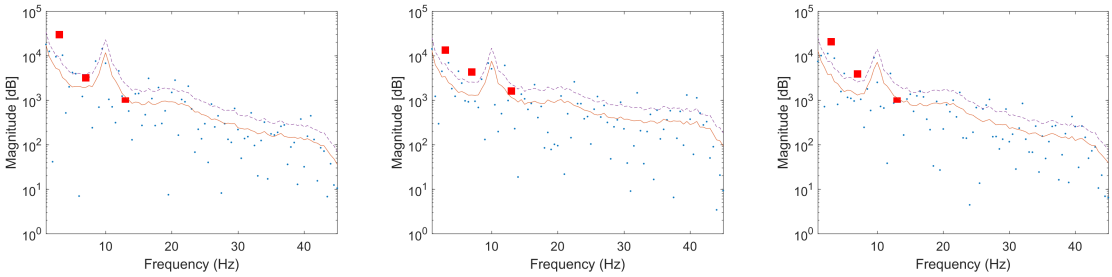

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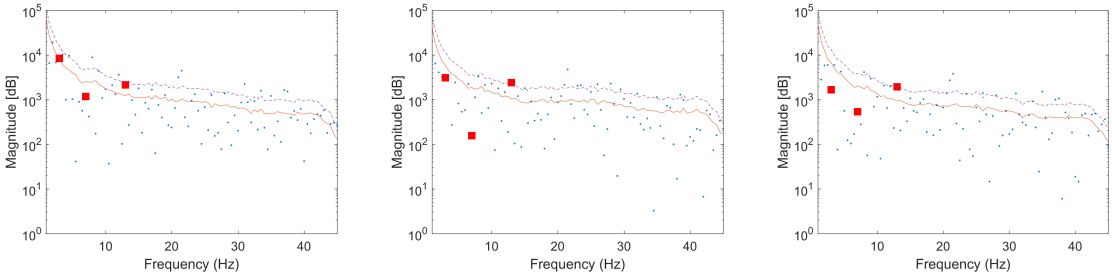

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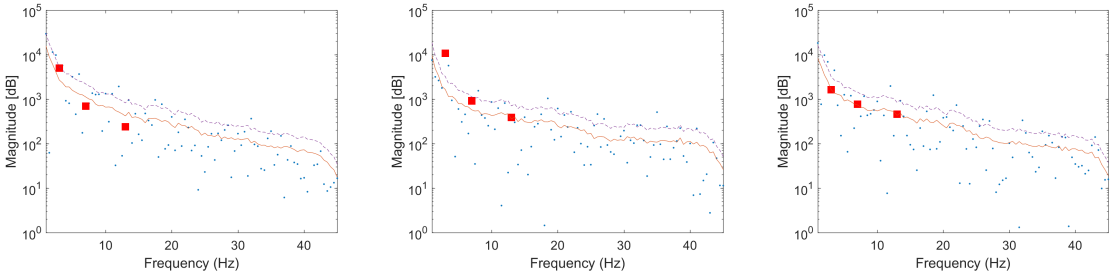

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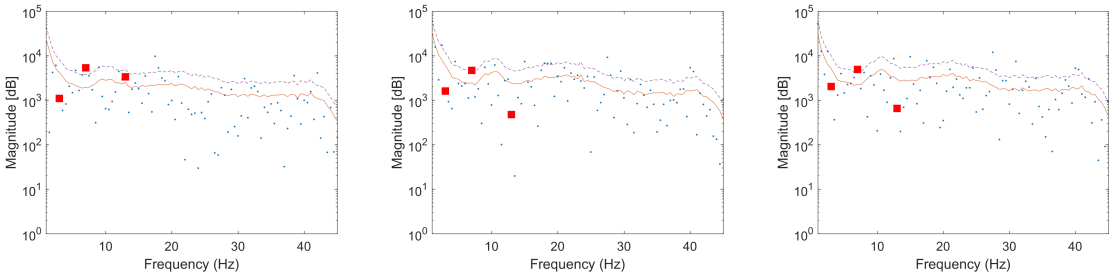

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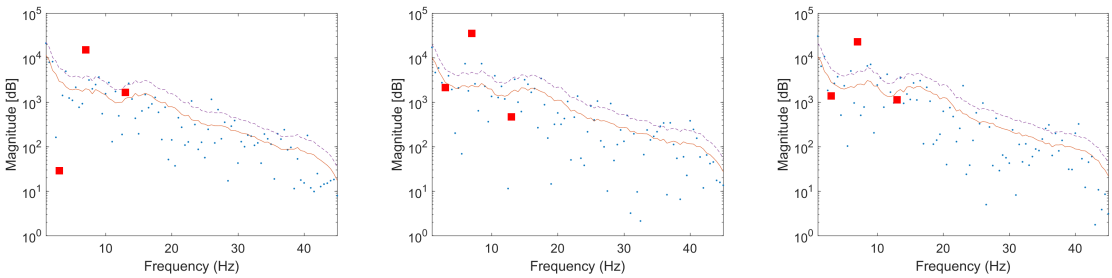

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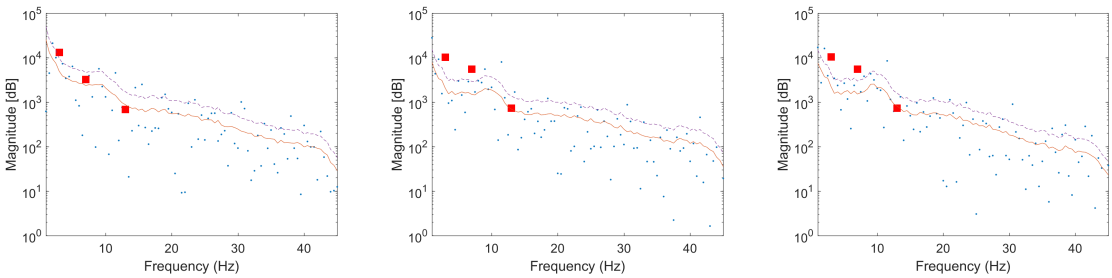

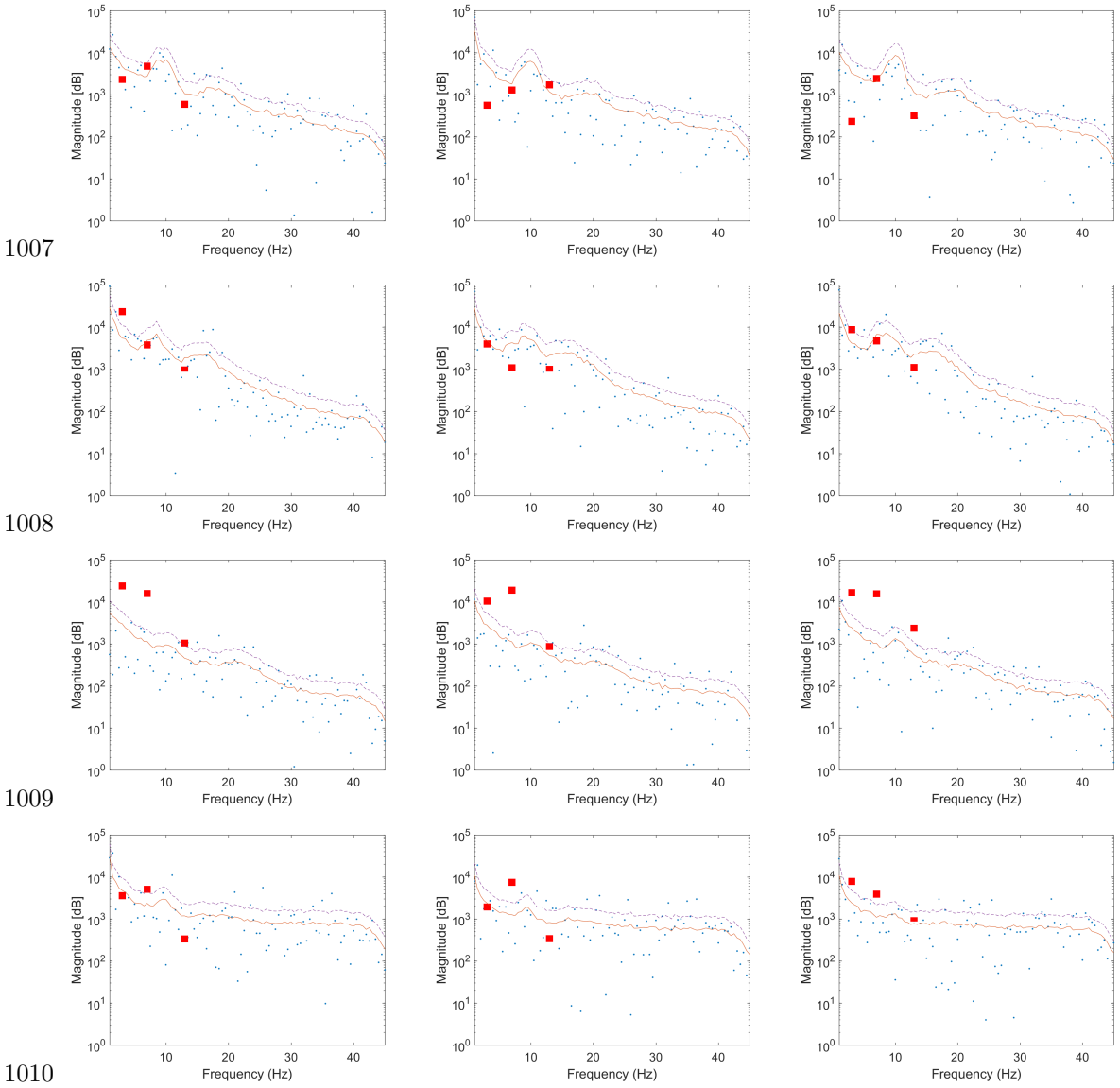

Figure 14: Power spectra per subject to assess presence of 3, 7 and 13 Hz activity per subject. Using the autospectral density and the noise variance, the power in each frequency is plotted for respectively channel C1 (a), C3 (b) and CCP3h (c). Using a confidence interval of 1.96 SD, all frequencies present above the purple dashed line are considered significant. The frequencies 3, 7 and 13 Hz have been highlighted by the red squares. Subjects 1001, 1003, 1006, 1008, 1009 and 1010 show significant 3 Hz activity, subjects 1001, 1004, 1005, 1006, 1009 and 1010 show significant 7 Hz activity and subjects 1003 and 1009 show significant 13 Hz activity.

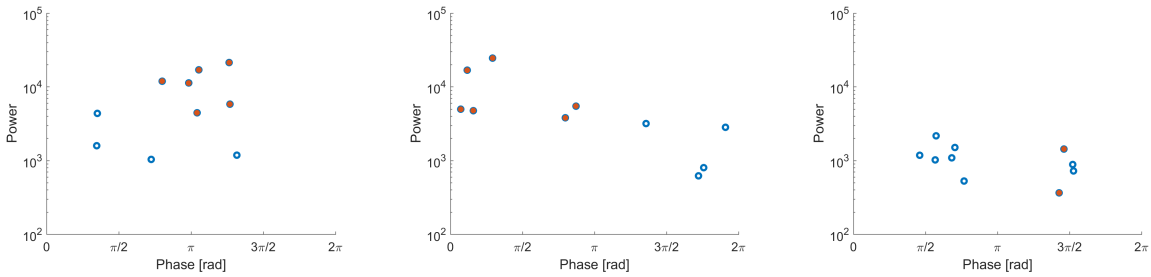

Figure 15: Phase difference plots for respectively 3 Hz, 7 Hz and 13 Hz for all subjects to show the spread of Φ_{Diff}. The phase difference was plotted against the power present in that same frequency showing all participants in blue circles. Subjects showing significant 3, 7 and/or 13 Hz activity were marked with a red asterisk inside the blue circle. Both 3 Hz and 13 Hz show very similar Φ_{Diff} between significant subjects, whilst 7 Hz shows a large spread.

B Experimental protocol

On the following pages the experimental protocol as used during the present study is presented.

Experimental setup

Following my thesis on steady-state evoked potentials following nociceptive stimulation, this document will allow for future research to follow the same protocol and setup. This document will describe all the materials used, the experimental setup and how the experiment is performed.

Table of contents

Scope

Following my thesis on steady-state evoked potentials following nociceptive stimulation, this document will allow for future research to follow the same protocol and setup. This document will describe all the materials used, the experimental setup and how the experiment is performed.

Background

Little is yet known about the nociceptive system. To investigate some of the properties of the this system, an EEG study using electrical stimulation is used in the following study. Electrical stimulation has already proven to allow for discriminating between different block wave stimulation in the resulting EEG signal [ref]. After several pilot experiments testing the discriminatory behavior of sinusoidal waves using frequency modulation, this experiment will use a frequency modulated sinusoidal signal. Electrical stimuli are generated with a custom built stimulator (Nocicept) from the University of Twente. Stimulus trains composed of the frequencies 3, 7 and 13 Hz combined in a multisine wave are applied. The setup can be found in figure 1.

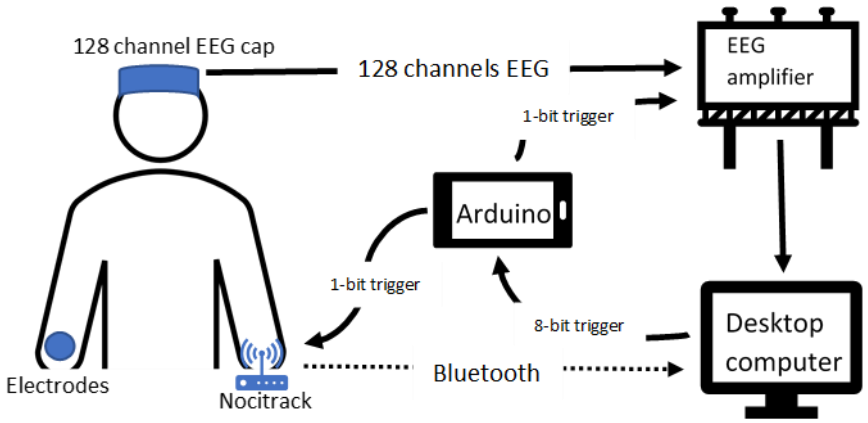

Figure 1: Example setup EEG measurement of nociceptive stimuli

Required materials

Description	Number	Specification
General		
Desktop Computer	1	Computer connected to EEG amplifier and Arduino. Requirements: - Asalab - Labview.exe program
Laptop	1	Computer used for analysis, Matlab 2017 or higher
Tape	0.5 m	Non-allergenic skin-friendly medical tape (eg Leukosilk)
Scrub gel	1 cl	Medical abrasive gel for removal of dead skin cells (Nuprep)
Non-stick compress small	2	Used for scrubbing + removal scrub gel
Non-stick compress medium	4	Used for alcohol cleaning of the hair
Cleaning alcohol	2 ml	Removing access dirt from the hair
Stimulus related		
Stimulator	1	NociTRACK AmbuStim single-channel stimulator, capable of generating a minimum current of 8 µA and a maximum current

		of 16 mA
Charger	1	NociTRACK charger for AmbuStim stimulators
Trigger generator	1	Arduino-based trigger generation system, which can be connected to the: - Computer (input) via USB A to B cable - NociTRACK AmbuStim stimulator (output 1) via a BNC cable - EEG amplifier via a DB25 parallel cable
USB A to B cable	1	Cable for connection of the trigger generator to the computer
BNC Cable	1	Cable for connection of the trigger generator to the NociTRACK AmbuStim stimulator, which should be at least 2 meters long
Parallel cable	1	DB25 parallel cable for connection of the trigger generator to the EEG Amplifier, which should be at least 1 meter long
Stimulation electrode	1	Sterile IES-5 RVS electrode for intra-epidermal electrocutaneous Stimulation
Grounding electrode	1	Technomed's Disposable Adhesive Surface Electrode, which will serve as a ground during the stimulation
Stimulator-to-electrode cable	1	A custom-made double cable for connecting the stimulation and grounding electrode to the stimulator
USB B extension cable	1	Cable for bluetooth adapter, which should be at least 3 meters long
Bluetooth adapter	1	Bluetooth adapter to allow for communication between the desktop computer and the Nocitrack
EEG Measurements		
EEG amplifier	1	TMSI Refa 136-channel amplifier, with 128 unipolar, 4 bipolar and 4 auxiliary input channels
Fibre-to-usb converter	1	TMSi optical fibre-to-USB converter
Optical fibre	1	TMSi optical fibre, used to communicate between the EEG amplifier and the computer via the TMSi fibre-to-USB converter
USB A to B cable	1	USB A to B cable for the connection of the TMSi fibre-to-USB converter to the computer
EEG caps	2	TMSi 128-channel low-noise actively shielded caps with an EBA multi-connector, a medium and a large sized cap for varying subject head sizes
EBA multi-connectors	4	EBA multiconnectors from 1-32 Hirose to micro coax cables, to connect the head cap to the amplifier
Measurement lint	1	Tape for measuring the required EEG cap size
Syringes	1 or 2	10 ml Luer-Lok tip (BD) syringe for the injection of EEG gel into the head cap electrodes
Blunt needles	1 or 2	Blunt needle (JG15-0.5”) for the injection of EEG gel into cap electrodes and for scratching of the skin
EEG electrode gel	200 ml	Electro-gel (ECI), to be injected into the EEG cap electrodes
Towels	1	A towel for the test subjects to dry their hair after the experiment

ECG electrodes	1	Foam, hydrogel pre-gelled disposable electrodes (H124SG, Kendall) with a diameter of 30 mm x 24 mm for the ground at the fore head
Shielded unipolar cable	1	TMSi ExG shielded unipolar cable with 1 snap connector, to attach the ground electrode
Comfortable chair	1	Comfortable chair that allows participants to relax during the experiment and provides rest to head and neck muscles, since contraction of those muscles might disturb the measurement
Pain rating scale	1	Small scale made to allow for pain ratings to every first stimulation train

Procedure

General Preparation

Time: >1 day before session

1. Confirm potential candidates after having sent them the official patient information letter. This email should contain:
    - ✓ Date, time and location of the experiment
    - ✓ Meeting point with student
    - ✓ Contact information
    - ✓ Wear glasses if they normally do
    - ✓ Eat and drink as normal
2. Inform Mana and Elias about when the experiment is taking place
3. Make sure you have the emergency contact information readily available.
4. Book the lab well in advance and notify the other users.

Materials Preparation

From 1 day to 1 hour before session

1. Ensure the Nocitrack is charged. However, do not leave it on the charger for more than 12 hrs as this may damage the battery.
2. Ensure there are sterilized electrodes available, if not contact Michelle Minneboo about sterilization of the electrodes.
3. Ensure the EEG amplifier is connected.
4. Check by connecting an EEG cap and starting asalab if any signal is recorded.
5. Prepare all the materials for the experiment:
    - ✓ EEG cap(s) + measurement lint
    - ✓ Scrub gel + two small compresses
    - ✓ Rubbing alcohol + 4 medium compresses
    - ✓ ECG electrode for ground measurement
    - ✓ Sterile IES-5 electrode + Technomed electrode
    - ✓ Syringe with blunt needle and EEG electrode gel

System Start-up and Check

Less than 1 hour before session

1. Turn on computer
2. Turn on EEG amplifier
3. Connect tv to desktop computer via a HDMI cable (standard setting)
4. Connect Arduino and Nocitrack, see figure 2
5. Launch config.ini (Multisine/MS_Data/config.ini) and check the following settings:

   Channel=1
   NoP=1
   PW=0.5
   IPI=10
   Stepsize=0.025
   InitLow=0.0
   InitHigh=2.0
   MSRelAmp=2.0
   MSPW=0.5
   MSDur=8500
   MSOffset=110
   MSAmp1=30
   MSFreq1=3
   MSAmp2=30
   MSFreq2=13
   MSAmp3=30
   MSFreq3=7
6. Open asalab and fill in participant data, create new study and show impedances on screen, see figure 3
7. Launch frontpanel.vi, see figure 4
8. Turn phone to airplane mode

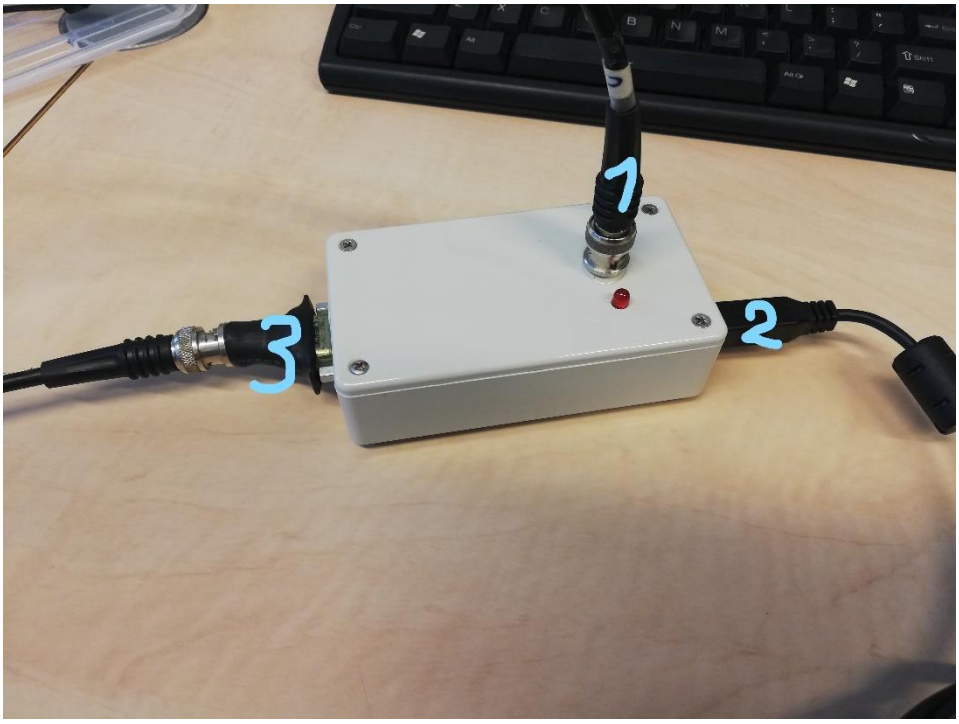

Figure 2: Attachments of Arduino. 1 attaches to the Nocitrack. 2 attaches to the computer. 3 attaches to the EEG amplifier

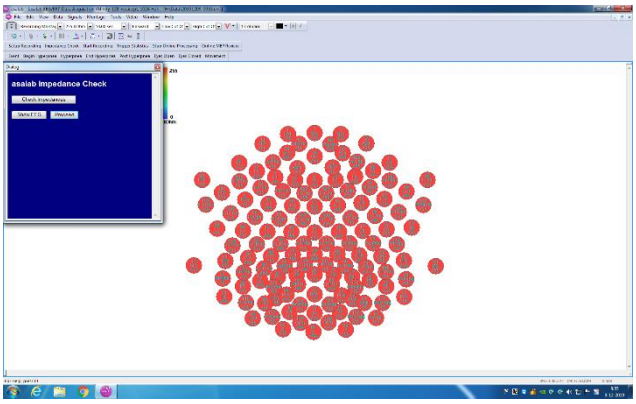

Figure 3: Impedance check in Asalab

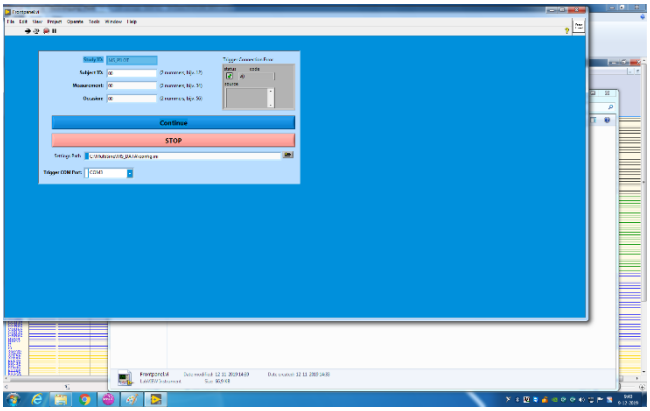

Figure 4: Initial screen of frontpanel.vi

Subject Reception and Preparation

Time: start of session

1. Meet with subject either at entrance building or entrance of the lab (be present at least 10 minutes prior to start experiment).
2. Give the subject a hard-copy of the information letter and the informed consent (preferably, the
   subject has already received and read the information letter in advance, via e-mail). Ask the subject:
   - **"Do you have any questions?"**
   - **"Would you like to participate in the study?"**
   - **"Do you want to sign the informed consent?"**
3. Explain to the subject what is going to happen. Ask the subject:
   - **"Please, set your mobile phone to airplane mode."**
   - **"Do you need to go to the toilet? The session will take approximately 2 hours."**
4. Ask the subject to sit down in the chair
5. Ask the subject: **"Are you left or right handed?"** and write down the answer
6. Scrub the right side of the head just below the ear (see figure 5)
7. Use the rubbing alcohol to clean the hair
8. Use the measurement lint to measure which capsize is required and place the EEG cap on the participants head (adjust if needed using the lint, making sure CZ is in the middle of the head)
9. Measure the distance between the nasion and the inion and place the Cz at half the measured distance
10. Measure the distance between the pre-auricular points and place the Cz at half the measured distance
11. Explain to the subject what is going to happen
    "You will not feel anything from the EEG measurements."
    - **"Gel will be injected into the electrodes and the skin will be scratched with a blunt needle, to improve conduction. This should not hurt, if it does, pleas say so."**
    - **"If you feel any discomfort, you can indicate this at any time."**
12. Take the needle and the syringe. Show the subject that you are working with a sterile needle and open the package in front of him/her, attach the needle to the syringe
13. Fill the syringe with EEG gel and scratch the skin underneath each electrode by putting the blunt needle into an electrode

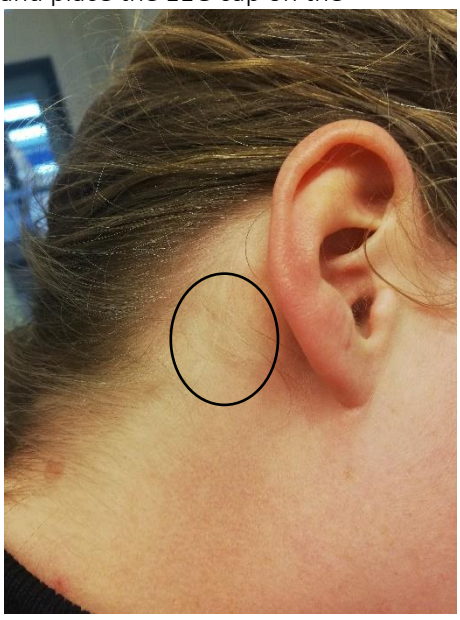

Figure 5: Scrub location for ground electrode

and moving the needle in a circular motion against the skin. Inject gel into the cap while slowly pulling the needle out

14. Use the impedance display on the tv and make sure every electrode is below 5kOhm, retry electrodes until they reach this value
15. If a cap electrode is broken (remains at the same high value), disconnect the corresponding cables from the EEG amplifier (NOTE: Cz, C3, C4 should never be removed).
16. When finished, make a screenshot of the impedances at the start of the experiment
17. Shut down the tv and look in Asalab if you see any activity
18. Scrub the top side of the right hand fully and explain you are going to place two electrodes:
    - The first electrode has small pins and will activate the upper layer of the skin
    - The second electrode is a sticky electrode which serves as a ground
    - Mark 5 different locations on the hand
    - Place the two different electrodes (see figure 7), with the first electrode first placed at location 2 (see figure 6).
19. Attach the NociTRACK stimulator to the two different electrodes (see figure 8) and connect in the Bluetooth interface (see figure 9).
20. Ask the subject to hold the AmbuStim in his left hand.

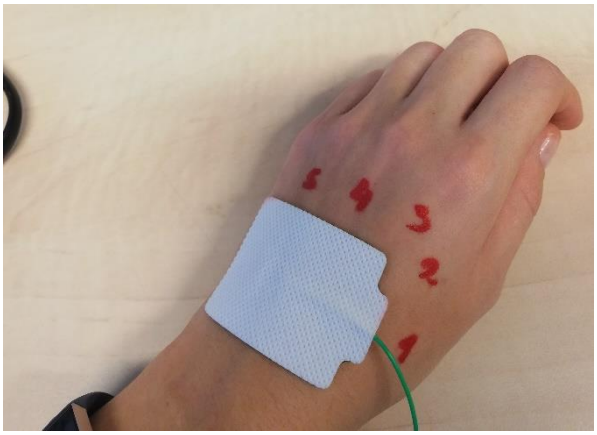

Figure 6: 5 different locations for the electrode. Follows the pattern 2, 4, 1, 3, 5.

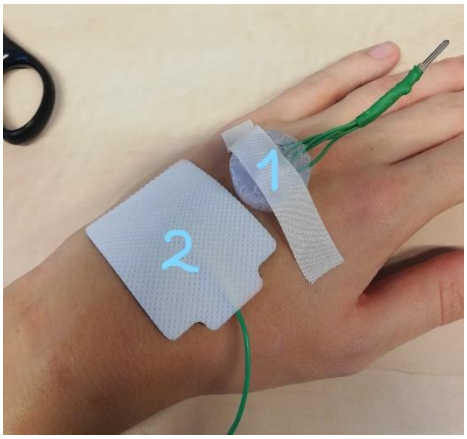

Figure 7: Ground electrode (2) and stimulating electrode (1)

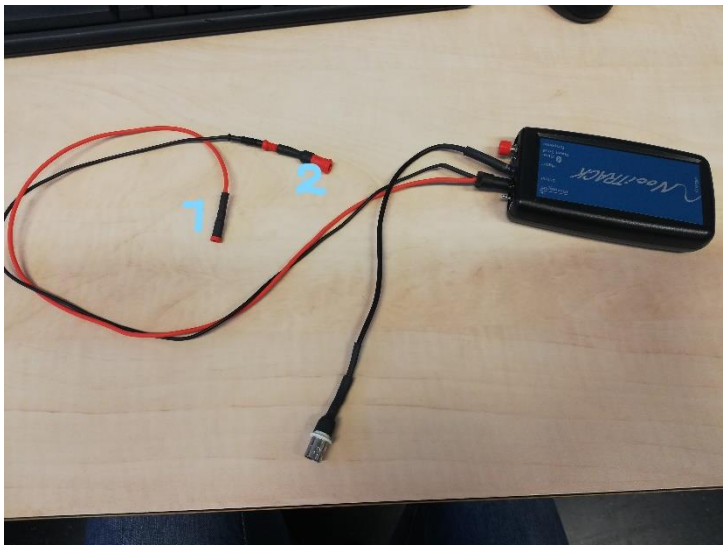

Figure 8: Nocitrack connections. Ending 1 connects with the stimulating electode (marked with a 1 in figure 7), ground electrode is connected to ending 2.

Familiarization and Threshold Determination

Time: 40 minutes after start of the session

1. Explain to the subject:
    - "First, a measurement will be done for you to get acquainted with stimulus sequence that will be used."
    - "I will press on start in the application. However, the measurement will not start until you press
      the response button. You can pause or stop the experiment by releasing the response button."
2. Ask the subject:
    - "Please, hold the stimulator in your left hand."
3. Explain to the subject:
   "The first measurement will just serve to get acquainted with the stimuli. Therefore, you should keep the button pressed until you are sure you felt several pulses"
4. Go to the initial threshold interface (see Figure 9). When the subject is ready, start the first measurement via the LabView interface by pressing 'Start'.
5. Tell the subject that he/she can start by pressing the response button.
6. After the subject has released the button a message will appear on the screen, asking if the subject is clearly familiar with the stimulus now.
7. Ask the subject:
   "Did you clearly feel the stimulus, or would you like to do another round of familiarization?"
8. If the subject wants to continue press "yes", if the subject would like to do another round of familiarization press "no" and repeat step 3 to 8.
9. Explain that:
   "The second measurement will serve to determine your initial detection/pain threshold. Therefore, you should release the response button as soon as you think that you feel something that could be ascribed to the stimulus. It is OK if you are not a 100 percent sure."
10. Tell the subject that he/she can start by pressing the response button.
11. After the subject has released the button a message will appear on the screen, asking if the subject has released the button at the right moment.
12. Ask the subject:
    - "Do you think you released the button at the correct moment, did you really only feel one stimulus?"
13. If the subject wants to continue press "yes", if the subject would like to do another threshold measurement press "no" and repeat step 9 to 13.
14. A new screen will appear (see Figure 13), which is the control interface for stimulus train administration.

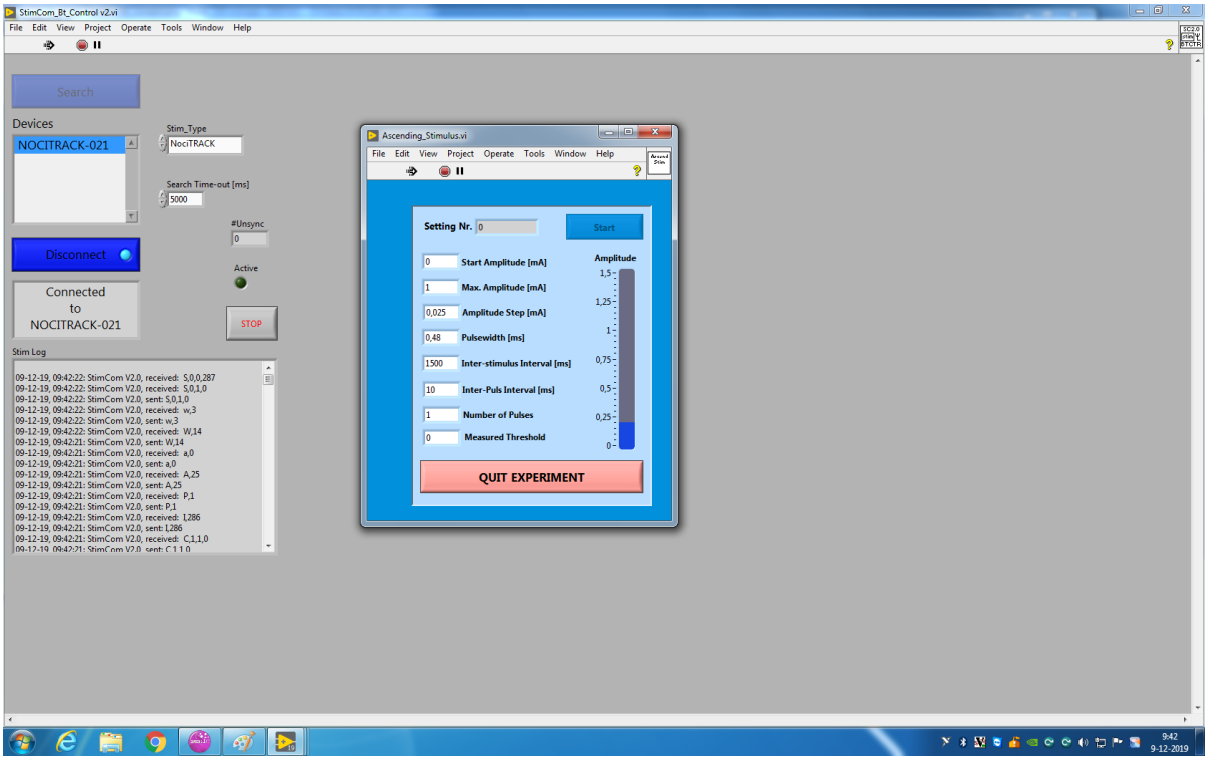

Figure 9: Bluetooth and Thresholding interface. In the grey screen the log file of the Bluetooth connection can be tracked, in the blue screen the slowly increasing amplitude of the pulse is visualized in the bar.

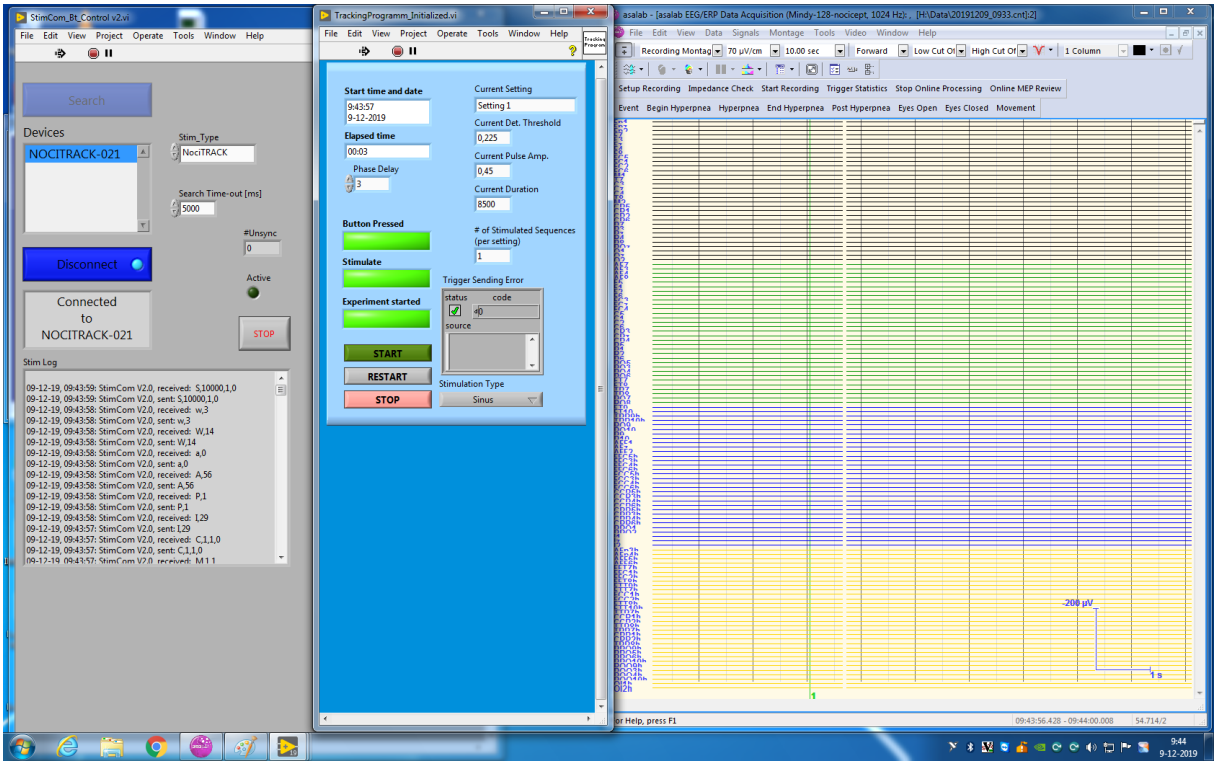

Figure 10: Interface seen on computer during the experiment. On the left, the Bluetooth connection can be seen. In the middle the current phase delay used in the multisine can be seen, as well as the number of trials already performed. On the right the EEG signal, showing the triggers of stimulation by a green vertical line indicated by a 1.

Experiment

Time: 50 minutes after start of the experiment

1. Explain the experimental procedure to the subject:
   - “To receive stimuli, you have to press the red button.”
   - “The upcoming measurement will consist of 20 stimulus sequences.”
   - “If you need a short break, you can release the red button at any time.”
   - “Data is only recorded while you are pressing the button.”
   - “After the first stimuli, rate the sensation on the magnetic board (see figure 11)
2. Ask the subject:
   - “Please, blink as few times possible while holding the response button.”
   - “Keep looking towards the wall and do not close your eyes while holding the response button.”
   - “Try to relax and do not move while holding the response button.”
   - “Do not talk while holding the response button.”
   - “Keep your attention focused on the detection of stimuli.”
   - “Doing this will greatly enhance the signal quality.”
3. Close the door, and press “Start” in the stimulus administration overview (see Figure 10).
4. Indicate the subject may now press the button to start the measurement via the intercom.
5. Closely watch the subject’s EEG signals in the signal interface (see Figure 10) and tell him/her to relax if artefacts arise.
6. Pay attention to the Bluetooth connection with the NociTRACK (see Figure 9 and 10) and ensure that stimuli information is sent to and received by it, as displayed in the “stimulus log”. If not, ask the subject to release the stimulator button, re-establish the connection and then continue with the experiment by asking the subject to press the red button again.
7. Wait until the EEG recording application indicates that 20 stimulus trains have been administered (see Figure 10).
8. Instruct the subject to release the button.
9. Press the “Stop” button in LabView during a period where no stimulus is administered. The program will close on its own.
10. Repeat steps 1 to 7 five times. In between these five blocks the following steps should be performed:
    - The MultiSine program must be restarted.
    - “FrontPanel.vi” has be filled in, now with increased measurement number.
    - The NociTRACK will have to be connected again.
    - “Familiarization and threshold determination” will have to be executed again to reset the threshold.

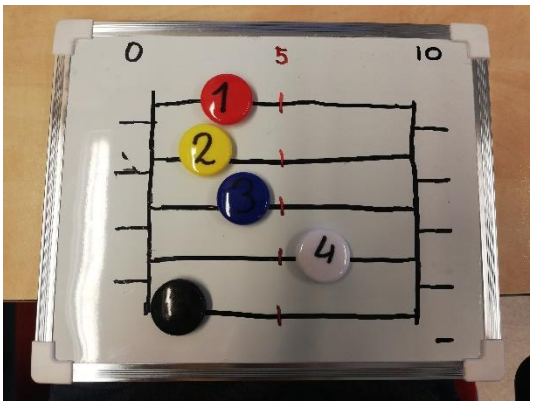

Figure 11: Pain rating scale. A measuring lint is used to measure the length of each magnet from the left.

Round-up

1. Inform the subject:
   - “The experiment was completed successfully.”
2. Turn-off the stimulator and disconnect the subject from all cables.
3. Take over the stimulator from the test subject, and temporarily put it away.
4. Remove the IES-5 electrode and Technomed electrode
5. Instruct the subject:
   - “I am now taking of your EEG cap.” (Place it in the white bucket)
   - “You can wash your hair in the sink in the lab, or upstairs in the shower, (E200, go up the stairs and walk through the grey door at E2, the middle door is the shower).
6. When the subject is ready to leave, tell the subject:
   - “Thank you for your participation in the experiment.”
7. Give the subject a present to thank him for his participation in the experiment.
8. Ask the subject if he/she would like to be informed about the result of the experiment.
9. Provide the subject with contact information in case he/she has any questions.

Clean-up

1. Turn-off the software, and the EEG amplifier.
2. Clean the EEG cap electrodes directly after the experiment.
3. Dry the cap on the tap
4. Clean the syringes that were used for gel injection.
5. Put all equipment back where it belongs.
6. Save all data that was gathered during the experiment.