First pain event related potentials to argon laser stimuli: recording and quantification

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Abstract
Argon laser induced event related responses to pricking pain (first pain) were recorded. The different recording parameters (recording site, filter setting, averaging technique), quantification parameters (amplitude, power) and variability between successive recordings were studied. The single responses were large, and averaging of 16-32 single trials was sufficient to obtain reliable averaged responses. The power (0.5-7.5 Hz) of the averaged vertex recorded response was, for the range of intensities considered, the most sensitive parameter to quantify the stimulus and hence the intensity of pain perceived.

When event related responses are evoked by selective pain stimuli without activation of mechanosensitive afferent fibres, they are assumed to reflect the state of the nociceptive system. The use of electrical or tactile nociceptive stimuli to skin is therefore not optimal because other receptors rather than nociceptors are activated. Electrical stimulation of the cutaneous Aß-fibre innervated nociceptors have been used to produce selective activation of these afferents and to elicit experimental pain of short duration. The advantages of electrical stimulation have been the easy control of intensity and pulse duration. High energy CO212 and argon10 lasers can deliver pulses of various durations and intensities to the skin without touching it. Lasers have therefore been adopted for experimental pain stimulation. High energy CO2 laser pulses can increase the temperature in the superficial skin layer with a rate of about 600°C/s.11 Heat dolorimeters produce a rate of change in skin temperature of about 19°C/s.12 The fast temperature increase induced by lasers is sufficient to generate time locked synchronous afferent activity and pain event related responses. The peak-to-peak amplitude of the averaged laser induced pain related response correlates to the distinct Aß-fibre mediated pricking first pain.13

In this study two experiments were designed to elucidate the problems related to recording and quantification of first pain related responses evoked by argon laser stimuli. In the first experiment optimal recording conditions and quantification techniques were determined. In the second experiment variability in amplitude between successive recordings was investigated when optimal recording conditions were used.

Methods
Volunteers
All volunteers gave their written consent. During the experiments the volunteers were resting comfortably and wore protective goggles. To avoid any acoustical interference at the time of the stimulus, white noise was given through earphones. The volunteers were asked to keep their eyes open and fix their gaze on a black spot on the ceiling. Skin fold thickness and skin reflectance were measured from the skin areas to which laser pulses were applied according to the description by Arendt-Nielsen and Bjerring.10 The surface skin temperatures in the test areas (hand and foot) were monitored by a digital thermometer and kept at 33°C±2°C by radiant heat source. The room temperature was kept at 24°C±2°C.

Laser stimulation
The visible light output from an argon laser (Sectra Physics, model 168, USA) was transmitted to the skin via a quartz fibre and a handle with adjustable beam diameter (0.4 to 6 mm). The stimulus parameters were standardised to a stimulus duration of 200 ms and a laser beam diameter of 3 mm. The laser was operated in TEMoo mode. Output power could be adjusted from 50 mW to 3.5 W. The wave lengths were 0.488 μm (blue) and 0.515 μm (green); the distribution of laser intensity at each wavelength was 33% and 66%, respectively. This distribution remained constant (+/-2%) for laser intensities above 50-100 mW. An external laser power meter (Ophir, Israel) was used to measure the dissipated output power. The coefficient of variance for the dissipated power remained with +/−6%. A continuous, low energy beam (50 mW) from the argon laser visualised the stimulation site.

EEG recording
The event related responses were recorded with platinum needle electrodes (Disa 25C04), inserted over C4, C3 and Cz (vertex) with reference to linked earlobes. The EEG was filtered by second order filters (0.5-30 Hz), amplified 200,000 times (Disa 5C01), sampled at 64 Hz by a computer, and stored on a hard disc.

Artifact rejection
Artifacts from eye movements (EOG) are similar in shape to single pain related responses, and the following method was used to eliminate the artifacts from the EEG: the horizontal eye movements could be detected optimally by horizontally aligned surface elec-
trodes and vertical movements by vertically aligned electrodes. In this study we wanted to detect vertical and horizontal movements in one recording and aligned the electrodes along a line of 45° to the vertical and horizontal lines as suggested by Thom and Andresen.  

The electro-oculogram, recorded from this electrode configuration, was assigned as EOG\_{raw}. The estimated artifact free EEG (EEG\_{\text{noise}}) as a function of time \(t\), was calculated by using the linear model described by Verlanger et al.  

\[
\text{EEG}_{\text{noise}}(t) = \text{EEG}_{\text{measu}}(t) - k \text{EEG}_{\text{measu}}(t).
\]

The factor \(k\) was calculated from the regression coefficient between the EOG and EEG signal.  

Artifacts from muscle contractions were detected by the frequency content of the EEG signal. If the ratio between EEG power from 15–30 Hz and EEG power from 0–2 Hz exceeded a certain threshold, the signal would be rejected.  

**Frequency domain analysis**  
The spectral components of EEG activity can be calculated by a Fast Fourier Transform (FFT) algorithm or by an autoregressive model. A FFT spectrum may for many purposes be too noisy. Averaging FFT spectra has been used to increase the spectral signal-to-noise ratio. The alternative is to estimate the spectra by an autoregressive (AR) model. The information in this spectrum depends on the order of the model. AR spectra were calculated from a 1/2 to 1s EEG segments and no window function was used.  

**PROTOCOL EXPERIMENT 1 AND 2**  
Two experiments were designed to elucidate the properties of the argon laser evoked response. In the first experiment the optimal recording, analysis and quantification parameters were found. In the second experiment the variability between successive recordings was determined, using the optimal recording conditions found in experiment 1.  

**EXPERIMENT 1**  
**Volunteers**  
A total of 12 males (mean age 23 years, range 20–26 years) and seven females (mean age 24 years, range 19–26 years) participated in the experiment. None had neurological or psychological disorders or were receiving medication.  

**Classification of laser stimuli**  
The volunteers were initially trained to rate the intensity of the laser induced pain. The pain was classified as follows: Rating 1 distinct pinprick (moderate pain); Rating 2 intense pinprick (strong pain); Rating 3 very intense pinprick followed by a burning after-sensation (severe pain). Fifteen seconds after each stimulus the pain was rated, and the single responses were automatically stored in three databases according to the three pain ratings. The mean laser intensities related to each pain rating were determined for each volunteer.  

These laser intensities were used for subsequent stimulation.  

Each volunteer participated in two sessions. In the first session, 90 stimuli were applied to the dorsum of the hand (C7-dermatome) and in the second session 90 stimuli were applied to the lateral part of the foot (S1-dermatome). The 90 stimuli were randomly distributed between the three pain ratings with 30 in each group. The intervals between stimuli were randomly distributed with a mean of 30s (range 20 to 40 s).  

**Optimal recording site**  
It has been suggested that pain event related responses are largest at the vertex. It is, however, not known if the best signal-to-noise ratios for the single responses are obtained from the vertex since the EEG background activity vary across the scalp. Signal-to-noise ratios were estimated for the three recording positions C3, C4 and Cz with reference to linked earlobes, and for the three pain ratings. The signal-to-noise ratio was calculated by the method proposed by Bershad and Rockmore, in which the variance of the signal plus noise was estimated from the EEG segment 200 ms to 700 ms after stimulation, and variance of the noise was estimated from a 500 ms EEG epoch containing only background activity. The mean signal-to-noise ratio was calculated from the average of the signal-to-noise ratios for the individual single responses.  

**Effect of low pass filtering on the peak-to-peak amplitude of the averaged responses**  
No recommendations exist concerning filter settings for recording of laser evoked responses. The peak-to-peak amplitude of the averaged response was measured for different settings (2 to 13 Hz) of the second order Butterworth low pass filter.  

**Conventional averaging versus latency corrected averaging (Woody filtering)**  
Several reports have indicated that latency corrected averaging (Woody-filtering) might be helpful for estimating laser induced evoked potentials. The technique involves alignment of the single responses before averaging and the averaged responses are therefore not smeared by the latency variations of the single responses. To find the limitations for this technique the difference between conventional averaged responses and latency corrected averaged responses was investigated on a total of 114 evoked responses to different pain intensities.  

The averaged pain related response was used as a template for the Woody filtering algorithm in two different ways. In the first approach a fixed template was used throughout the latency corrected averaging, whereas in the other approach a fixed template was used for the first three latency corrections and the template was then substituted with the continually modified averaged response. The minimal latency corrections accepted were +/− 63 ms from the mean latency, and responses exceeding these limits were rejected. The correlation coefficient between the single responses and the
Table 1  The estimated mean (+/- SD) signal-to-noise ratio (SNR) for single evoked responses versus spontaneous EEG activity. Recordings of laser evoked pain related responses were made from Cz (vertex), C3, and C4 with reference to linked earlobes.

<table>
<thead>
<tr>
<th>Recording site</th>
<th>Moderate pain (SNR)</th>
<th>Strong pain (SNR)</th>
<th>Severe pain (SNR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cz</td>
<td>0.04 +/- 0.40</td>
<td>1.26 +/- 0.28</td>
<td>3.15 +/- 0.22</td>
</tr>
<tr>
<td>C3</td>
<td>0.03 +/- 0.39</td>
<td>1.09 +/- 0.26</td>
<td>1.16 +/- 0.20</td>
</tr>
<tr>
<td>C4</td>
<td>0.04 +/- 0.48</td>
<td>1.10 +/- 0.35</td>
<td>1.15 +/- 0.26</td>
</tr>
</tbody>
</table>

template was determined, and a response was rejected if the correlation coefficient was below a certain limit (for example, 0.8). In this investigation, the EEG was filtered from 0-5-12 Hz before averaging.

Influence of pre-stimulus EEG on the single responses
Scharein et al. found that the power of the alpha-rhythms in the EEG segment before the stimulus was related to the power of the evoked responses.

It has therefore been suggested that to improve the signal-to-noise ratio the single responses should be evoked when the general EEG activity was of low amplitude. To test this the relation between the power of the EEG segment before the stimulus and the power of the event related response was compared. For each volunteer the single responses to severe pain stimuli (rating 3) were selected and linear regression analysis was performed between the power (0.5-7.5 Hz) of EEG activity before the stimulus, and the power of the evoked response.

The most sensitive parameter for quantification of laser induced pain
When pain related responses are used for monitoring purposes the parameter used for quantification should be as sensitive as possible. Three quantification parameters were investigated:
A) The peak-to-peak amplitude of the major negative peak of the averaged response.
B) The power of the frequency intervals 0.5-2.5 Hz, 0.5-5.0 Hz, 0.5-7.5 Hz, and 0.5-10.0 Hz, calculated from the averaged response in the interval from 200 to 700 ms after stimulation.
C) The averaged power in the frequency intervals 0.5-2.5 Hz, 0.5-5.0 Hz, 0.5-7.5 Hz, and 0.5-10.0 Hz, calculated from the single responses in the interval from 200 ms to 700 ms after stimulation.

EXPERIMENT 2
Variability between successive recordings
In this experiment the inter-individual variations in peak-to-peak amplitude between successive recordings were determined for different intervals between recordings. Only the vertex recording site was used, because this was found in experiment 1 to give the best signal-to-noise ratio. The peak-to-peak amplitude of the major negative complex was used for quantification. Strong pain stimuli were used, conventional averaging was performed, and 30 single responses averaged. The laser pulses were applied to the dorsum of the hand. Five groups of volunteers who had not previously been exposed to laser stimulation participated in the investigations.

Investigation 1  There was a five minute interval between the end of the first recording and the start of the second. Ten volunteers participated (four male, mean age 24 years, range 21-27 years, six female, mean age 23 years, range 20-28 years).

Investigation 2  Sixty minutes elapsed between the recordings. Ten volunteers participated (five male, mean age 24 years, range 20-26 years, five female, mean age 24 years, range 20-30 years).

Investigation 3  A series of responses were recorded 15, 30, 45, 60, 90, and 120 minutes after the initial recording. Ten volunteers participated (six male, mean age 25, range 22-32 years, four female, mean age 25 years, range 21-31 years).

Investigation 4  The day-to-day variation was determined from a group of 12 volunteers (seven male, mean age 26 years, range 22-30 years, five female, mean age 26 years, range 24-33). The volunteers came twice and one to seven days elapsed between the recordings.

Investigation 5  Responses were evoked from the left and right hands to study whether there were any systematic differences between responses. The interval between recordings was 15 minutes and in half of the volunteers the left hand was stimulated first. Thirty-four right handed volunteers without known differences in sensibility between right and left hand participated (14 male, mean age 24, range 20-29 years, 20 female, mean age 26 years, range 21-32 years).

Results
EXPERIMENT 1
Optimal recording site
For both stimulation sites (foot, hand) the vertex (Cz) recorded responses were 25%, larger in peak-to-peak amplitude and 36% larger in power (0.5-12 Hz) than the corresponding ipsi- and contralateral recordings. The power (0.5-12 Hz) of the EEG segments before stimulation was 29% larger for the vertex recording than for the ipsi- and contralateral recordings. The larger response amplitude over the vertex could therefore be a reflection of the generally higher EEG activity of relevant frequency components in this region. It was therefore necessary to estimate the signal-to-noise ratio of the responses to verify the best recording position. The signal-to-noise estimation technique showed that for the three pain ratings, the best result was always obtained from the vertex recording (table 1). The signal-to-noise ratios for vertex recorded responses were 10%, 13%, and 14% higher than ipsi-/contralateral recordings for moderate, strong and severe pain, respectively.

Effect of low pass filtering on the peak-to-peak amplitude of the averaged responses
The maximal energy of the pain related complex was between 1 and 4 Hz with a main peak around 2.5 Hz (fig 1B). The effect of low pass
filtering on the major peak-to-peak amplitude of the negative (N400) complex was found for different cut-off frequencies. Cut-off frequencies of 10 Hz, 8 Hz, 6 Hz and 4 Hz reduced the amplitude by 3·9%, 6·9%, 14·8% and 30·1%, respectively (fig 1C).

Conventional averaging versus latency corrected averaging (Woody filtering)

The number of averaged trials was varied systematically from two to 30 to find the number needed to obtain reliable averaged responses. By superimposing traces it was found that 16–30 averaged responses normally were sufficient to give a good match between superimposed traces. This depended on the intensity of the induced pain, because the signal-to-noise ratio increased for higher pain intensities (table 1). The responses evoked from the dorsum of the hand contained three negative peaks (N250, P300, N400). The first peak (N250) was smaller and of higher frequency than N400 (fig 1A), and furthermore N250 was largest over the contralateral recording site. Using the Woody filter each single response was cross-correlated to a fixed or continuously modified template. The time difference required for the single response to obtain maximal correlation with the template represents the latency correction.

The latency corrections of the single responses were +/−47 ms, +/−42 ms, and +/−37 ms (+/− SD) for moderate, strong and severe pain, respectively. The latency variations around the mean were normally distributed. Theoretically the Woody filtering should therefore give the best improvement of the averaged responses to low laser intensities due to the largest latency variations. Unfortunately, the single responses to weak laser stimuli are small (signal-to-noise ratio close to 1), and a reliable estimation of the latency correction can be difficult to obtain. The amplitude improvements of the Woody filtered averaged responses were very similar for fixed and modified templates. The mean amplitude improvements for both techniques were 42%, 40% and 28% for moderate, strong and severe pain, respectively. These amplitude improvements could, however, possibly be erroneous. If EEG segments before stimulation were used as input to the Woody-filter, it was possible to obtain responses with a shape similar to the template and hence the laser induced evoked responses (fig 2). Both the continuously modified and the fixed template technique could generate erroneous responses. For high laser intensities the signal-to-noise ratio of the single responses became better and the Woody filter gave more reliable improvements of the responses (fig 2). The latency corrected averaged responses were of
higher frequency than the conventional averaged responses, because latency variations normally smear (low pass filter) the averaged response. The applicability of Woody-filtering is very much restricted, because reliable improvements are obtained when the signal-to-noise ratio of the single responses are already large.

Influence of pre-stimulus EEG on the single responses
For all pain ratings linear regressions were calculated between the power from 0.5–12 Hz of the EEG segments before the stimuli and the power of the event related response. Non-significant correlation coefficients between −0.14 and +0.3 were found.

The most sensitive parameter for quantification of laser induced pain
For monitoring purposes it is important to use the parameter with the best dynamic properties. Both time and frequency domain parameters were studied.

For increasing pain intensity the peak-to-peak amplitude in general increased less than the calculated power parameter (fig 3). For both the averaged responses and for the averaged spectra of the single responses the frequency interval from 0.5 to 7.5 Hz gave the strongest relation to the pain rating. The power of the averaged response increased 421% from rating 1 to 3, and the averaged spectra of the single responses increased 137%.

In a preliminary study we have increased the laser intensity above the defined pain rating 3, and found that the higher laser intensities do not evoke corresponding larger responses. This indicates that there is a range of laser intensities, and hence pain intensities, where correlation between stimulus intensity (perception) and amplitude (power) exists.

EXPERIMENT 2
Variability between successive recordings
The peak-to-peak amplitude of the pain related response was dependent on the time between recordings. The mean decrease in amplitude between the first and second recording was 17%, 13%, and 10% for time intervals between recordings of 5, 15, and 60 minutes respectively. The amplitude variations between successive recordings (recording 2–3, 3–4, 4–5, 5–6, 6–7, separation in time 15, 15, 30, 30 minutes, respectively) were between 2%–8% (mean 5%). The day-to-day variation in amplitude varied from 10% to 32% with a mean of 24%. The mean amplitude variations between responses evoked from the left and right hand was 20%. The interval between recordings was 5 minutes and part of the 20% variation can be caused by the decrease (mean 17%) between the first and second recording (separated by 15 minutes).

Discussion
Optimal recording site
Pain related responses (vertex potential) to laser stimulation have been recorded for almost 12 years, but no guidelines for optimal recording conditions and quantification exist.

In one of the first studies on pain related responses to CO2 laser stimulation, vertex recorded responses were found to be 20–40% larger than responses recorded over the contra- and ipsilateral somatosensory areas. Following this study by Carmon et al9 vertex recorded responses have predominantly been used. It is known that the power of the EEG signal in various frequency bands may vary across the scalp and the maximal amplitude of the responses does therefore not necessarily represent the optimal recording site for the best signal-to-noise ratio. Several techniques exist to estimate the signal-to-noise ratio for evoked responses but the techniques differ in their methods for estimating the signal and the noise. This is very simple, in theory, but when working on biological signals neither the signal nor the noise is known. The values of the signal-to-noise ratios are therefore not necessarily comparable for the different techniques. The technique used in the present study gave mean signal-to-noise ratios from 0.93 to 1.35 for the amplitudes of the responses compared to the amplitude of the background EEG. For signals recorded simultaneously this estimation procedure can give a relative indication of where the best signal-to-noise ratio can be obtained, knowing that the absolute values might not be correct. Applied to pain related responses the best signal-to-noise ratio was obtained from vertex recordings, and as such confirms the previous findings.

For clinical and monitoring purposes the time required for recording of pain related responses should be as short as possible. This time can be reduced by reducing the number of trials averaged and it was found that 16–30 stimuli under normal conditions were sufficient to produce well defined and reproducible responses. In the literature the number of stimuli range from 20 to 128 (table 2).

Effect of low pass filtering on the peak-to-peak amplitude of the averaged responses
The filter parameters used in different studies when laser induced responses are recorded range from 0.1–30 Hz to 1–100 Hz (table 2), which makes it very difficult to compare response latencies and amplitudes reported from different laboratories. For comparisons it is very important to use filters with identical frequency and phase characteristics. The pain
related response contained mainly of frequencies below 12 Hz, and this frequency can be suggested as an upper frequency limit for the low pass filter. A reduction of the filter bandwidth decreases the noise and therefore a minimal bandwidth is required. A lower frequency range of 0.5 Hz is suggested.

Conventional averaging versus latency corrected averaging (Woody filtering)
The Woody filtering technique has previously been applied to first pain related responses. For the three pain ratings considered in this study the standard deviation (SD) of the single response latency around the mean was below 47 ms in all cases and the amplitude improvement did not exceed 42%. Bormann and Treede found a SD of 55 ms for CO₂ laser evoked responses, and an amplitude improvement of 50%. It is agreed that latency corrections can improve the response amplitude, but the possibility of generating faulty signals has not previously been discussed.

It has been argued that “averaging of EEG-segments selected for maximum similarity with a given template would result precisely in the present waveform” and Bormann and Treede therefore “choose an adaptive procedure where the template is continually modified according to the waveforms present in the single trials”. We tried both procedures on baseline EEG and on the first pain related responses and found that both techniques could estimate responses from baseline EEG using less than 30 averaged trials. The artefactual responses were similar to responses generated by laser stimuli of low intensity. The generated responses could simply be modified by increasing the “limit for rejection” (the correlation coefficient between single response and the template), but was relatively insensitive to the shape of the template initially used. It has been suggested that the latency of the pain related evoked responses might be informative with respect to the quality of the pain perceived. Using latency corrected averaging the latency of the response is dependent on the latency of the template. It can be concluded that latency corrected averaging should only be used when the signal-to-noise ratio is controlled and found sufficiently high. For monitoring purposes where the responses can be reduced in amplitude, the technique cannot be recommended because artefactual signals might be generated.

Influence of pre-stimulus EEG on the single responses
It has been suggested that there exists a correlation between the amplitude of alpha-rhythms (7.5-12.5 Hz) in the baseline EEG activity before stimulation and the amplitude of the following response. The main energy of the laser induced pain related response was below 7 Hz and therefore expected to be relatively insensitive to changes in the alpha-frequency band. In this study the power of the argon laser evoked response from 0.5 to 7.5 Hz was not affected by the power of the pre-stimulus EEG activity. This indicates that the suggested relation between alpha-rhythms and response amplitude might be an indirect evidence for a relationship between alpha-rhythms and arousal-level, and hence arousal-level and response amplitude. Although there was no relationship between the power of the EEG activity before and after stimulation the best signal-to-noise ratio can be expected when the amplitude of the EEG activity before stimulation in the relevant frequency range is low. This advantage has previously been utilised by Bjerring and Arendt-Nielsen when single responses to argon laser stimulation have been analysed.

The most sensitive parameter for quantification of laser induced pain
The amplitude of the pain related complex has been found to correlate with the intensity of the pain perceived. The amplitude increased 87% when the intensity of the pain induced by the argon laser light was increased from moderate to severe pain. Due to this substantial increase, no attempts have previously been made to find a better and more sensitive parameter for pain quantification.

Carmon et al. rated CO₂ laser pulses from non-painful to strong pain on a five point scale and found an amplitude increase of 120% whereas the root-mean-square (RMS) value increased 273%. In another study by Carmon et al. the amplitude increased 100% when the pain intensity rated on a two point scale was increased from 1 to 2. In this present study the increased pain intensity increased the power of the averaged response in the range from 0.5 to 7.5 Hz by 421%, whereas the amplitude increased 87%. Comparisons between studies are difficult because different discrete pain rating scales have been used.

Arendt-Nielsen and Bjerring found a tendency to increased duration of the major negative (N400) pain related complex for increasing pain rating, but this information is not included in a simple measure for the overall amplitude. The power of the signal is influenced by both amplitude and duration and should therefore be more sensitive. From the present findings it is clear that a parameter describing the overall response shape is a better parameter for quantification than the amplitude alone. This observation might be applied to other electrophysiological signals such as compound sensory action potentials.

Variability between successive recordings
The intra-individual amplitude variation between successive argon laser evoked responses has not previously been discussed. This is extremely important if the present technique is to be used clinically for comparisons between affected and normally innervated skin areas. The amplitude of the evoked responses decreased 10-17% between the first and the second recording, and the reduction was related to the time between recordings.

One explanation for this reduction might be that the volunteers had not previously experienced laser stimulation or recording of pain related responses, and in the first session
were not familiar with the procedures. This might also explain why the variability in amplitude was reduced when successive recordings were performed. The level of attention has previously been found to influence pain related responses. We do not believe that changes in attention to the pricking pain perception could account for the variability because the intensity of the pain perceived was reported to remain almost constant during the experiments.

The day-to-day and hand-to-hand variations were 24% and 20%, respectively. The conditions in these experiments were not optimised because no initial response was recorded to acclimatise the volunteers before the sessions. In conclusion, it has been difficult to examine clinically the function of the thin myelinated Aδ-fibres quantitatively, and techniques for this purpose are needed. Laser stimulation activates the Aδ-fibre innervated nociceptors and evokes first pain event related responses. The amplitude and power of these responses do, for a range of laser intensities, contain information about the intensity of the stimulus and hence the pain perceived, and may be used in future studies to assess the function of these fibres.

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