Original Reports

Awareness of Temperature and Pain Sensation

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Abstract: Evoked potentials (EPs) to radiant or contact heat pain stimuli reflect the synchronization of brain activity to noxious inputs. However, we do not know how they relate to conscious awareness (AW) of a sensation. In healthy volunteers, we determined the time of AW for thermal noxious and non-noxious sensory inputs and examined its correlation to parametric measures of vertex EPs. Subjects had to report the position of the hand of a Libet’s clock at the moment they perceived either a laser or a thermode stimulus. AW was determined after subtracting the position of the clock hand at the moment of stimulus delivery from the one reported by the subject, in ms. Subjects estimated AW in all single trials, including those in which no EPs could be identified. Mean AW was estimated earlier than the corresponding EP latency for both types and intensities of stimuli. There was a weak but significant negative correlation of AW to EPs amplitude, which was higher than the correlation of AW to EPs latency. Our results indicate that the timing of AW is influenced by the subjective relevance of sensory inputs. This feature could be used for the analysis of cognitive aspects of pain processing. Perspective: This article presents a way to measure the subjective awareness of the sensation induced by a noxious heat stimulus, either radiant or contact, in healthy human subjects. This method could be used for the analysis of cognitive aspects of pain processing.

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Key words: Pain, laser-evoked potentials, contact heat-evoked potentials, subjective awareness, small fiber conduction, sensory evaluation.

Presently, the 2 most commonly used stimulation methods for neurophysiological assessment of nociceptive pathways are radiant heat, delivered by laser stimulators,8,42 and contact heat, delivered by thermodes equipped with fast temperature rising systems.10,24,56 In both types of stimulation, recording of evoked potentials (EPs) through scalp electrodes is the usual way to assess the conduction time of the afferent volley up to the central nervous system (laser-evoked potentials [LEPs]; contact heat-evoked potentials [CHEPs]). Although the size of the EPs may correlate with the intensity of the stimulus and the subjective evaluation of the amount of pain,1,7,17,24 the EPs do not contain direct information about conscious perception of the stimulus-induced sensation. Reaction time (RT) is often used as a surrogate of subjective perception. However, although RT depends on sensorimotor integration of afferent inputs,25,35 subjects do not need to be fully conscious of the sensation to react to stimulus presentation.26 At present, there is no method adequate for quantitative recording of the time at which the subject becomes aware of a sensory input. Nevertheless, such function of the human central nervous system is of physiological interest and may occasionally be of clinical relevance. Indeed, the feeling of pain does not

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only depend on the characteristics of the stimulus activating the nociceptors but also on cognitive aspects and accompanying emotional reactions, which certainly modulate the conscious awareness (AW) of our sensations.\textsuperscript{11,54}

One important component of the nociceptive pathway, the unmyelinated C fibers, remains difficult to evaluate using EPs.\textsuperscript{6,40,55} The ingenious methods proposed by various authors\textsuperscript{6,46} are not sufficiently reliable in generating EPs to C-fiber input for their clinical use, except for facial stimulation.\textsuperscript{55} For instance, perception of the second pain sensation to laser stimuli\textsuperscript{6} may exist without a clear EP correlate even when attention is selectively focused on the stimulus.\textsuperscript{44} Indeed, pain has a cognitive-evaluative component, requiring learning, recall of past experiences, and active decision making,\textsuperscript{43} which should be borne in mind when assessing patients with pain complaints.

For all the above reasons, we considered it pertinent to quantify the conscious subjective perception of temperature and pain to expand our knowledge on the cognitive aspects of nociception. In the study reported here on healthy subjects, we aimed at defining quantitative measures of the timing of AW to warm and heat-pain inputs generated by either radiant or contact heat stimuli, using the method devised by Libet et al.\textsuperscript{38}

Methods

Subjects were 12 right-handed healthy volunteers (6 male and 6 female) recruited among staff members and students of our department. Their mean age was 35.3 years, ranging from 23 to 56 years. None had signs of peripheral neuropathy or central nervous system disorders. None had chronic pain and none were under any medication regime. They were informed about the procedures and signed a consent form. The experiments were approved by the Ethical Committee of the Hospital Clinic of Barcelona.

General Procedure

The experiments were divided in 2 sessions separated by at least 1 week. In 1 session, we examined heat pain inputs and in the other warmth inputs to radiant (laser) and contact heat (thermode) stimulation. The order of the sessions was random. The experiments were done in a warm (22°C), semidarkened room. Subjects were sitting comfortably in an armchair in a semireclining position, with the head supported by cushions. The experimenters present in the same room were all situated at the back of the subject to avoid distraction. They were asked to keep their eyes open, refrain from blinking and relax their head, face and neck muscles, in order to avoid artifacts. Their hands were resting on top of a portable and adjustable table, with the left forearm exposed for stimulation purposes (Fig 1). Subjects faced a computer monitor showing the Libet’s clock\textsuperscript{38} and were instructed about the task to be performed. All subjects were already acquainted with noxious stimulation from previous experiments.

**Thermoalgesic Stimuli**

For each stimulus modality (laser and thermode), we chose 2 different stimulation intensities: one inducing painful pinprick sensation, and another inducing warmth sensation. Stimulus intensities were established in the course of a preliminary evaluation. Subjects were asked to evaluate the sensations induced by each type of stimulus using a verbal numerical rating scale (NRS) score from 1 to 10, in which the intensity of warmth ranged from 1 to 5 and the intensity of pain ranged from 6 to 10. Stimulus intensities were adjusted in such a way that subjects scored an NRS value to stimuli applied through the contact thermode similar as to those of the laser stimuli. Care was taken for the stimulus intensity used in the second session to match the NRS value reported to the stimuli applied in the first session.

Laser stimuli were delivered with a CO\textsubscript{2} laser stimulator (AGM, Barcelona). For laser-induced pain sensation (laserPS), we used a laser beam of 5-mm diameter directed to the skin overlying the first dorsal interosseous space. We determined the individual’s pricking pain sensation threshold intensity by adjusting stimulus duration and power, and set the stimulus intensity for the experiment at 10% above threshold. For laser-induced warmth sensation (laserWS), we used a system of lenses that spread the laser beam in such a way that, when applied at a distance of 25 cm from skin surface, the stimulated area was about 2 cm\textsuperscript{2}. Duration and power were adjusted for the subject to feel a sensation of warmth but no pain. We
checked that peak temperature was limited to 42°C for laserWS stimulation, measured with a thermometer probe (Panlab TMP812RS) attached to the skin. The site of laser stimulation was slightly changed after each stimulus in order to prevent habituation. Contact heat stimuli were delivered by a Pathway temperature stimulator (Medoc, Ramat Yishai, Israel), equipped with a fast heating/fast cooling probe of an area of 5.7 cm². All contact heat stimuli were delivered at the fastest available ramp rate of 70°C/second from a baseline temperature of 32°C. We set the peak temperature as to deliver a stimulus that was felt as pricking pain (thermodePS), or to induce warmth but no pain sensation (thermodeWS). The thermode was attached to the skin overlapping the dorsum of the hand for thermodePS and the ventral side of the distal third of the forearm for thermodeWS. To prevent habituation, the position of the thermode was slightly changed after each stimulus and left in the same place for at least 10 seconds before delivering the actual stimulus. A total of 12 stimuli were delivered for each stimulus type.

**EP Recording and Measurement**

Surface recording electrodes were attached to previously prepared skin at the scalp vertex (Cz), referenced to linked earlobes. Impedance was kept below 5 kOhms. The EEG signals were band-pass filtered (1 to 20 Hz) and sampled at a rate of 200 Hz. We recorded also from the orbicularis oculi to identify on-line the traces in which reflex blinking could have interfered with the recording of EPs. If that was the case, the trace was excluded and the trial repeated. After delivering 12 stimuli for each modality, with an interstimulus interval of at least 60 seconds, the resulting traces were superimposed and averaged to define relevant characteristics of mean EPs. Then we used the average trace as a template model for identification of single trace EPs. Two investigators (JVS and JMC) independently measured the latency and amplitude of EPs in each trace. Responses were not considered when there was no agreement between the 2 measurements, expressed as latency difference larger than 10 ms or amplitude difference larger than 3 μV.

**Assessment of AW**

Subjects viewed the Libet’s clock face on a screen situated at eye level at a distance of 1 m (Fig 1). This clock has conventional 5 minute markings and a single hand, 1.3 cm long, which makes a full rotation every 2,560 ms. Subjects were asked to assess the position of the clock hand at the moment they felt the sensation, with an accuracy to the 60th division, corresponding to a resolution error of 42.67 ms. An experimenter entered the number given by the subject at the end of each trial, while the computer automatically generated the actual clock’s hand position at the time of stimulus delivery.

**Data Reduction and Statistical Analysis**

The main variable under study was AW, calculated as the difference between the subjective appraisal of the clock’s hand position and its actual position at the time of stimulus delivery, transformed in ms. Note that, obviously, this estimated time included not only the time needed for perceptual processing but also the peripheral and central conduction times of the afferent volley. In the EP recordings, we determined the latency of the negative peak (N2 latency) and the positive peak (P2 latency) as well as the peak-to-peak amplitude (N2-P2 amplitude). We also noted the actual numerical score that subjects gave for each stimulus-induced sensation. Average values among the 12 recordings obtained for each individual and each 1 of the 4 types of stimuli (laserWS, laserPS, thermodeWS, thermodePS) were used for statistical analysis. Repeated measures 1-factor analysis of variance were used to ascertain the effects of stimulus types on AW, N2 latency, and N2/P2 amplitude. Data were also compared between male and female subjects.

Individual AW data from each trial were represented against time to analyze the temporal distribution of events. We were specifically interested in examining the correlation between AW and EP. For this, we normalized the data according to subject and condition. We first calculated the mean value of AW, EP latency, and EP amplitude for subject and condition, which was assigned 100%. Then, each value was transformed to a percentage of the individual’s mean. For all subjects, trials were numbered according to the AW value, linked to the value on EP latency, EP amplitude, and the numerical rating score. Finally, we averaged the values for each condition from all subjects, according to trial order. In this way, we ended up with 12 independent values (the number of trials), normalized as percentages of the parameters chosen for assessment of correlation (Pearson’s correlation coefficient).

**Results**

All subjects were able to comply with the task and produce the information requested about the Libet’s clock. The characteristics of the stimuli delivered during the experiment were as follows. For laserWS, the mean stimulus duration was 68.2 ± 4.6 ms and the power was 15.3 ± 3.8 watts (note that the stimuli for laserWS were delivered at a distance of 25 cm from the skin and spread with lenses over an area of about 2 cm²). For laserPS, the mean stimulus duration was 13.2 ± 1.3 ms and the power was 14.2 ± 2.0 watts. With contact heat stimulation, mean peak temperature was 42.4 ± 4.2°C for thermodeWS and 53.2 ± 2.1°C for thermodePS. We repeated on-line only a few trials because of artifacts in the EP recording or subjects’ transient lack of attention (less than 2% of the total number of trials). The mean NRS scores of the individuals’ reports of subjective sensation were 1.5 ± .2 for laserWS, 1.3 ± .3 for thermodeWS, 6.2 ± .2 for laserPS, and 6.0 ± .1 for thermodePS.

**EPs**

EPs were identified in a large proportion of trials in which the subjects evaluated their sensation as painful. However, it was impossible to identify individual EPs...
amid other brain oscillations in 11 laserPS trials (9.2%), 13 thermodePS trials (10.8%), 33 laserWS trials (27.5%), and 37 thermodeWS trials (30.8%), which were excluded from statistical analysis. The percentage of trials excluded was not different when comparing the 2 stimulation types (paired t-test; \( P > .05 \)). Fig 2 shows examples of EPs from a representative subject for each type and intensity of stimuli.

Mean latency and amplitude of the EPs were obtained for each subject after averaging all traces with a recognizable response. The grand mean values among all subjects are reported in Table 1. As expected, N2 latency was shorter for laserPS than for thermodePS as well as for laserWS than for thermodeWS (\( P < .001 \) for both comparisons). The P2 peak deflection followed the N2 peak in all stimulation conditions at a relatively fixed mean time lag. N2-P2 amplitude was larger in pain than in warmth trials (\( P < .001 \) for both, laser and thermode stimuli), but no differences in EP amplitude were detected between laserPS and thermodePS or between laserWS and thermodeWS (\( P > .05 \) for both comparisons).

AW

Subjects assessed AW in all trials, including those in which no EPs could be identified. AW had a roughly Gaussian temporal distribution with respect to the stimulus for each of the conditions, with relatively more dispersion in stimuli of warmth than pain modalities (Fig 3). No significant differences were found when comparing AW between male and female subjects for any sensory modality (t-test; \( P > .05 \) for all comparisons).

Correlation of AW to EP Parameters and Subjective Rating of Sensation

As a general rule, AW had a larger dispersion than the N2 latency. The mean coefficient of variation was 21% for AW and 10% for N2. Fig 4 shows the values of AW plotted against N2 latency values (A) and N2/P2 amplitude values (B) for each of the 12 trials in each condition, represented as percentages of the mean. There was a weak, nonsignificant, positive correlation of AW to N2 latency (\( R^2 = .11; P = .08 \)) and a stronger, statistically significant, negative correlation of AW to N2/P2 amplitude (\( R^2 = .42; P = .003 \)). Even though our experimental setup required similar intensities for all stimuli, subjects reported slight differences in the numerical ratings of stimulus-induced sensation, which correlated positively with the N2/P2 amplitude (\( R^2 = .27 \)).

Discussion

In this study, we showed that conscious awareness of warmth or heat-pain sensations induced by radiant or

Table 1. Data on All Variables

<table>
<thead>
<tr>
<th></th>
<th>LaserPS</th>
<th>ThermodePS</th>
<th>LaserWS</th>
<th>ThermodeWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2-P2 amplitude (( \mu V ))</td>
<td>31.3 (12)</td>
<td>25.3 (9)</td>
<td>8.6 (2)</td>
<td>8.7 (2)</td>
</tr>
<tr>
<td>N2 latency (ms)</td>
<td>217.1 (35)</td>
<td>412.4 (44)</td>
<td>751.6 (104)</td>
<td>689.4 (98)</td>
</tr>
<tr>
<td>P2 latency (ms)</td>
<td>382.3 (32)</td>
<td>575.1 (91)</td>
<td>889.5 (124)</td>
<td>828.2 (99)</td>
</tr>
<tr>
<td>AW (ms)</td>
<td>139.9 (36)</td>
<td>298.9 (57)</td>
<td>456.8 (111)</td>
<td>493.3 (116)</td>
</tr>
<tr>
<td>N2 – AW (ms)</td>
<td>83.2 (28)</td>
<td>113.5 (46)</td>
<td>294.8 (90)</td>
<td>196.1 (93)</td>
</tr>
<tr>
<td>P2 – AW (ms)</td>
<td>248.4 (31)</td>
<td>276.2 (44)</td>
<td>432.7 (88)</td>
<td>334.9 (91)</td>
</tr>
</tbody>
</table>

NOTE. Data are expressed as the mean and standard deviation values among all 12 subjects of the study after averaging the individual values for each subject.
contact heat stimuli can be recorded by means of subjective evaluation of the time of occurrence of the event. Such measurement, when using heat-pain stimuli, shows a higher correlation coefficient of AW to the amplitude than to the latency of the EPs. As expected, we also found a weak positive correlation of N2/P2 amplitudes to the individual’s numerical scores of sensation, which was probably not higher because we aimed at having similar pain and warmth scores in our experimental setup. The correlation of AW was negative for both the EP amplitude and the score; ie, the shorter the AW, the larger the amplitude of the EP and the higher the subjective score.

Figure 3. Temporal resolution of AW for each of the 4 conditions of the study. Each bar represents the number of occurrences (n) of AW in a time bin of 10 ms at the corresponding latency from the time that the stimulus was applied. (A) shows data for laserPS (filled bars) and laserWS (empty bars). (B) shows data for thermode PS (filled bars) and thermodeWS (empty bars). Note the superposition of data in time bins from 290 to 410 for the thermodePS and thermodeWS.

Conscious awareness of heat-pain sensation is an important aspect of the physiology of pain perception. It may indicate the relevance of nociception among other concomitant sensory inputs simultaneously reaching the central nervous system. The time in which we believe that a stimulus has occurred is a construct of our mind, made up from the memory of experienced events. \(^{13,41}\) Hence, quantification of that variable could be an important asset in the whole process of evaluation of nociception, bringing new information on pathophysiological aspects of chronic pain. Perception of heat-pain stimuli is usually assessed by means of psychophysical procedures. A common method is to ask subjects to perform a simple reaction time at perception of the stimulus and record the EMG activity related to muscle contraction or the actual execution of the requested task. \(^{30,44,45,57}\) However, this does not necessarily reflect that the stimulus has reached conscious perception. \(^{26}\)

The temporal profile of sensory perception can be estimated using several methods. One that is commonly used is to judge the temporal order between 2 stimuli presented separately. \(^{53,58}\) However, this technique has not been used so far with painful stimuli. Recording from the ventral postero-lateral nucleus of the thalamus, Pralong et al \(^{48}\) found a change in the degree of synchronization of stimulus-related neuronal discharges at a latency of 325 ms after contact heat-pain stimuli, which was correlated with pain intensity ratings. Although this has been reported so far in only 1 patient, the finding is of relevance. Babiloni et al \(^{2}\) described anticipatory suppression of the alpha rhythm in the contra-lateral sensorimotor cortex as a form of event-related desynchronization that correlated with the intensity of perceived pain sensation. None of these methods, however, reflects the time when subjects become consciously aware of a stimulus nor the temporal relationship between subjective appraisal of sensation and generation of electrical activity in the brain in the form of EPs. In fact, even though the method used here may not allow for the same temporal accuracy as the neurophysiological recordings or for the spatial resolution of neuroimaging, it is one of the simplest methods that allow measuring subjective awareness of a sensation with potential clinical applicability. \(^{39}\)

Libet’s clock has often been used to explore the perception of one’s own motor actions but seldom to evaluate stimulus-induced sensory perception. \(^{22,23,31,49}\) In the present study, the assessment was usually done a few seconds after stimulus delivery and, therefore, after short latency consequences of the stimulus were over. This allows for some introspection before delivering the verbal report, which could be obviously biased according to the importance attributed to the stimulus, and to body reactions the stimulus may have triggered in the subject. The relevance that a subject attributes to the sensory input among other experienced inputs is likely a key factor in the assessment of AW. In the present study we found no correlation between AW and EPs generated by warmth stimuli. On the other hand, we found a negative correlation of AW (ie, the estimated time of stimulus perception) to the.
amplitude of LEPs and CHEPs. Many factors can account for such a finding, including the size of the afferent volley and the attention that the subject paid to a given stimulus. In our subjects, the stimulus intensity was kept constant. However, we changed the stimulation site from trial to trial, a common procedure carried out in order to avoid fast habituation.20 This could have induced small differences in the actual size of the afferent volley, which, in addition to slight random variations in attention level, could have caused intertrial differences in the degree of subjective arousal derived from each individual stimulus.

It is known that the effective energy transmitted to the receptors with both radiant and contact heat stimuli may vary from trial to trial.28 The size of the EPs to noxious stimuli increases with the perceived intensity of pain1,10,17,27,32 as well as with the attention paid to the stimulus.35,36 Regarding AW, it is known that the subjective experience of time shrinks with high emotional involvement and lengthens with poor attentional engagement.19,52 Therefore, it is possible that our measure of AW contains an indirect measure of the arousal induced by feeling the stimulus, ie, those felt of high intensity led to a faster assessment of perception than those felt of lower intensity. These results fit well with the higher effectiveness of noxious than tactile stimuli in engaging motor reactions, as reported by Ploner et al.47 Premotor processes may be speeded up before conscious awareness but they will indeed contribute to define the characteristics of our recall of the whole experience. Insight of the timing of a nociceptive signal may not exactly match the precise moment at which the generated sensation actually reaches consciousness. Actually, AW occurred earlier than the EPs in practically all recordings. We think that this does not mean that our subjects activated specific brain circuits at that particular point in time. Instead, we believe that subjects made their judgment about AW in retrospect. Probably, subjects made an overall evaluation of the sensation and attributed it to a specific time: the higher the stimulus intensity the faster the process of evaluation, with the consequence of fewer errors and narrower distribution of the data.

Brain dipoles to noxious stimuli are similar for radiant and contact heat pain.46 However, CHEPs are of longer latency and smaller amplitude than LEPs, in accordance to the longer duration of contact heat stimuli.3,10,29 We found not only EPs but also AW of significantly longer latency for contact heat than for laser stimuli. Also, latencies to contact heat stimuli were more variable than those to laser stimuli, which is possibly due to the longer time available for subjective AW with contact heat than with laser stimuli.3 We found no significant correlation between AW and EP latencies, suggesting that these 2 phenomena may result from different processing circuits. Dissociation between perception of pain and latency of cerebral events has been reported for different pain levels with late somatosensory EPs to electrical stimuli.5,14 Latency and amplitude are easily measurable parameters in EPs following noxious single stimuli of both types.7,10,20,28,40 However, similar to other studies,10,40,55 we have been unable to identify EPs to warmth stimulation in about 30% of our subjects. In those cases, though, subjects were evaluating AW with no differences from trials containing identifiable EPs, indicating again a relative independence of the two measures. Another explanation for the discrepant correlations of AW to pain sensation and to EP amplitudes is that pain usually competes with other attention-demanding stimuli for limited cognitive resources.16,21 Therefore, pain stimuli may impair top-down attentional control mechanisms and impair task performance.37 Components of the pain matrix are also critical in the processing of cognitive information.42 Indeed, using fMRI, Klein et al33 demonstrated the role of the anterior cingulate cortex in the control of selective attention, working memory and error awareness.

Our study has some limitations. We did not study age-related differences and the fact that no differences were found between males and females may only be due to a small sample size. Whether age and gender have a strong influence in AW remains, therefore, unknown. Subjects had to pay attention simultaneously to 2 sources of information: to somatosensory input related to heat stimuli and to visual input from Libet’s clock. This might have caused a divided attention problem,4,51 which may have attenuated EP amplitudes.9,34,36 However, if this were the case, the effect should have similarly involved all trials and conditions and would likely cancel out in statistical analysis. Apart from that, data on AW were used to compare conditions within the same subjects, which partially accounts for possible deviations from the norm. Also, emotional states unrelated to the stimulus could have affected the results. This could have been the case, for instance, with anxiety.15,18 Therefore, the eventual application of the test in patients should take into account the possibility of hypervigilance or other types of stress derived from the disease. Another limitation is a possible oversimplification of the recording by obtaining responses to independent stimuli only from Cz. The EP in Cz is usually the largest event picked up from the scalp following noxious stimulation and may represent activity in the operculo-insular region.28 However, we cannot rule out the possibility that other scalp regions furnish different information on cognitive processes related to the noxious stimulus. It is also possible that earlier (N1) or late (P3) components of the EP to noxious stimuli contain additional information not gathered with the single recording site. However, it is difficult at present to obtain reliably N1 peaks in single traces, and cognitive potentials require a large number of averagings.

In conclusion, conscious awareness of the time that a stimulus is perceived depends on the relevance and salience of the sensory signal amid other coincidental inputs. Noxious stimuli, giving rise to pain, are among the most relevant sensory inputs in the somatosensory domain and, therefore, it may be clinically relevant to know about their subjective evaluation using a quantifying method such as AW. Psychophysical assessment of AW could be regarded as an indirect surrogate of cognitive aspects of pain and the subjective importance given to the sensation triggered by the stimulus.
References


