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# Aδ nociceptor response to laser stimuli: selective effect of stimulus duration on skin temperature, brain potentials and pain perception

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**Abstract**

**Objective**: To disclose a possible effect of duration of pulsed laser heat stimuli on Aδ nociceptor responses, skin temperature profiles, brain evoked potentials and pain perception.

**Methods**: We used a laser stimulator which works in the millisecond range and allows us to change the duration of the pulse while keeping the total energy of the stimulus constant. In 10 healthy volunteers, we measured the intensity of perceived pain with a 0–10 scale and the latency and amplitude of the early N1 and late N2 components of the scalp potentials evoked by laser pulses of equal energy and three different stimulus durations (2, 10, and 20 ms). Using a specifically developed pyrometer with a temporal resolution lower than 1 ms we also measured stimulus-induced changes of skin temperature.

**Results**: Stimulus duration significantly influenced temperature rise times, pain perception, and brain potentials. Shorter stimulus durations yielded steeper slopes in the skin temperature profiles and higher pain ratings, shortened the latency of the N1 and N2 components, and increased the amplitude of N1.

**Conclusions and significance**: The shorter stimulus duration shortens receptor activation times and yields a more synchronous afferent volley, thus providing a stronger spatial–temporal summation at central synapses that enhances intensity of first pain and brain potentials. This may prove useful in clinical applications.



*Keywords:* Laser stimulation; Skin temperature; Evoked potentials; Pain perception

## 1. Introduction

The effect of changing stimulus parameters on the neurophysiological and psychophysical responses to noxious inputs has been widely investigated at different levels of the animal and human nervous system (e.g. Chen et al., 1979; Pertovaara, 1999; Price et al., 1989). Increasing the duration of laser- or thermode-induced noxious stimuli lowered the heat pain threshold (Arendt-Nielsen and Bjerring, 1988; Pertovaara, 1999) and increased the magnitude of perceived pain intensity in humans and the spinal neural responses in rats (Nielsen and Arendt-Nielsen, 1998; Pertovaara, 1999). In these studies, however, stimulus-duration changes always entailed an associated change in delivered energy (the longer the duration the higher the energy); hence a selective assessment of duration-induced effects on nociceptive responses was impossible.

Brief radiant heat pulses, generated by laser stimulators, selectively excite free nerve endings in superficial skin layers and thus activate Aδ and C fibres (Bromm and Treede, 1984). The brain responses evoked by standard laser stimuli (late laser-evoked potentials, LEPs) are related to the activation of type II Aδ mechano-heat nociceptors (AMH II units), small-myelinated primary afferents, and spinothalamic tract neurons (Bromm and Treede, 1991; Treede et al., 1995). The largest signal is a vertex negative–positive complex (N2–P2), probably generated by the posterior portion of the anterior cingulate gyrus

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(Bromm and Chen, 1995; Garcia-Larrea et al., 2003; Tarkka and Treede, 1993). An earlier negative component (N1) has been consistently described. This is a lateralized response with a scalp maximum over the Sylvian fissure that originates from the operculoinsular cortex (Frot and Mauguiere, 2003; Garcia-Larrea et al., 2003; Treede et al., 1988; Vogel et al., 2003). These Aδ-related LEPs are used in physiological and clinical studies in patients with peripheral or central lesions, to assess function of nociceptive pathways (Bromm and Treede, 1991; Garcia--Larrea et al., 2002; Iannetti et al., 2001; Kakigi et al., 1991; Spiegel et al., 2003; Treede et al., 2003).

In this study, in order to investigate specific effects of stimulus duration on nociceptor activation and signal transmission along the Aδ pain pathway, we measured skin temperature, psychophysical responses, and cerebral potentials after heat pulses generated by an Nd:YAP laser stimulator working in the millisecond range and allowing us to change the stimulus duration without altering the total energy delivered in one single pulse.

## 2. Methods

### 2.1. Subjects

Ten healthy volunteers (6 men and 4 women) aged 24–54 years (mean 29.7 ± 7.6 years) participated in the study. All subjects gave their informed consent, after the local ethics committee approved the procedures.

### 2.2. Laser stimulation

Painful heat stimuli were generated by an infrared neodymium yttrium aluminium perovskite (Nd:YAP) laser with a wavelength of 1.34 μm (Electronical Engineering, Florence, Italy). A He–Ne pilot laser pointed to the area to be stimulated. The laser beam was transmitted through an optic fibre and its diameter was set at 5 mm (~20 $mm^2$) by focussing lenses. This kind of laser stimulator allows adjusting the stimulus duration between 1 and 20 ms (steps of 1 ms) without changing the total energy delivered and its rise time (unlike the rise time of skin temperature, which is indeed affected by stimulus duration). Laser pulses produced by Nd:YAP stimulators do not induce damage to the irradiated skin, not even the transient dyschromic spots sometimes produced by high-intensity $CO_2$-laser pulses (Cruccu et al., 2003; Iannetti et al., 2003).

In previous experiments, we found that Nd:YAP laser pulses of high intensity (up to a 2 J energy directed to a skin area of about 20 $mm^2$) were optimal to elicit painful pinprick sensation (Aδ input) and evoke late LEPs after stimulation of different body districts, without inducing damage to the skin (Cruccu et al., 2003). In the present study, laser pulses were directed to the skin of the dorsum of the right hand. To avoid nociceptor fatigue or sensitisation, the irradiated spot was slightly shifted after each stimulus, and stimuli were delivered arrhythmically with long (8–15 s) intervals to minimize central habituation. While the energy (1 J) of radiation and the size (~20 $mm^2$) of the irradiated spot were kept constant across runs and subjects, three different stimulus durations (2, 10 and 20 ms) were used. With these parameters, the stimuli elicited a pinprick, moderately painful sensation that the subject could tolerate across 60 stimuli.

### 2.3. Measurement of skin temperature

In nine subjects, we measured the skin temperature and studied the time-course of the laser-induced heating with a radiation pyrometer. The KT22 pyrometer (Heitronics, Wiesbaden, Germany) detects the infrared emitted by hot bodies, within the 8–14 μm range. Hence, it is completely blind to radiation reflected by the skin during our Nd:YAP laser emission (wavelength 1.34 μm). Its response range is 0–200 °C, with a time resolution of 500 μs. The size of the reading area depends on the distance from the skin; with the aid of He–Ne laser guidance we took care to keep the laser and pyrometer spots coincident. The pyrometer output was A/D converted and PC displayed by means of LabView 7.0 (National Instruments, Austin, TX, USA). In each subject, three trials for each of the three durations (nine trials in total) were collected and analyzed off-line. The skin temperature was monitored with the pyrometer, and kept constant at about 36 °C. The trials belonging to each stimulus duration were averaged together, giving three mean time-courses for each subject. A grand average from all subjects was also calculated. To investigate the effect of changing the baseline temperature, in three subjects, we repeated the same stimulation at 27, 33 and 39 °C of baseline temperature. For each recording we measured the onset and the peak latency of the skin heating, and the difference ($\Delta t$) between peak and baseline temperature.

### 2.4. Scalp recording

Participants were seated in a comfortable chair, wore protective goggles and were asked to stay awake and relax their muscles. They were instructed to keep their eyes opened and gaze slightly downwards. Acoustic isolation was ensured using earplugs and headphones. Brain electrical activity was recorded with silver disc electrodes from Fz, Cz, and Pz referenced to linked earlobes (A1A2), and from T3 and T4 referenced to Fz according to the international 10–20 system. The electrode impedance was always kept below 5 kΩ. Signals were amplified, filtered (bandwidth of 0.3–50 Hz), and A/D converted (sampling rate 1024 Hz, conversion on 12 bit) with a final resolution of 0.195 μV/digit (SystemPlus, Micromed, Treviso, Italy). In order to monitor ocular movements or eye-blinks and discard contaminated trials, electroculographic signals were simultaneously recorded from the orbicularis oculi muscle

by surface electrodes, with the active electrode over the mid lower eyelid and the reference 2–3 cm lateral. In two subjects, we also recorded the electromyographic (EMG) activity from the orbicularis oculi, masseter and cervical muscles.

In each subject, two series of 30 artefact-free trials (20 trials for each of the three stimulus durations in total) were collected and averaged off-line. The window analysis time was 2 s (500 ms pre-stimulus + 1500 ms post-stimulus). Within each series, stimuli with different durations were given in a randomized order. Between 4 and 8 s after the stimulus, the subjects were visually prompted to rate verbally the intensity of the evoked sensation on a 11-point numerical rating scale ranging from 0 to 10, where 0 was 'no pain' and 10 'pain as bad as it could be' (Jensen et al., 1986). The subjects were also instructed to signal any perception different from a clear pinprick sensation, and were unaware of any variation in the stimulus parameters.

We measured the peak latencies of the lateralized early latency response (N1 wave) and the negative (N2) wave of the late vertex response. The amplitude of the N1 response was measured at the contralateral temporal electrode (T3), from baseline to peak; the amplitude of the vertex response (N2–P2) was measured peak-to-peak at Cz.

### 2.5. Data analysis and statistics

Trials with the same stimulus duration were averaged together off-line. One-way analysis of variance (ANOVA) for repeated measures, post test for linear trend and the post hoc Tukey's multiple comparison test were used to analyze the differences between the three stimulus durations used (2, 10, and 20 ms) for the following parameters: onset and peak latencies, slope and temperature increase ($\Delta t$) of skin heating; LEP latency and amplitude; pain ratings. Correlations between different variables were tested with Spearman's $R$ index and deviation from zero of regression lines with the $F$ test. The slope of the temperature increase was calculated from the regression line between temperature and time in the interval between onset and peak latency.

For all statistics and graphs, we used Prism 4.0 (GraphPad, Sorrento Valley, CA, USA).

## 3. Results

### 3.1. Quality and intensity of sensation

Regardless of stimulus duration, laser stimuli elicited a clear pinprick sensation in all subjects. Only occasionally (12 trials in total) three subjects either reported a 'burning' or a 'difficult to describe' sensation.

In contrast, although the total energy of the laser stimuli delivered was constant during the whole experiment, the stimulus duration did influence the intensity of pain perception.

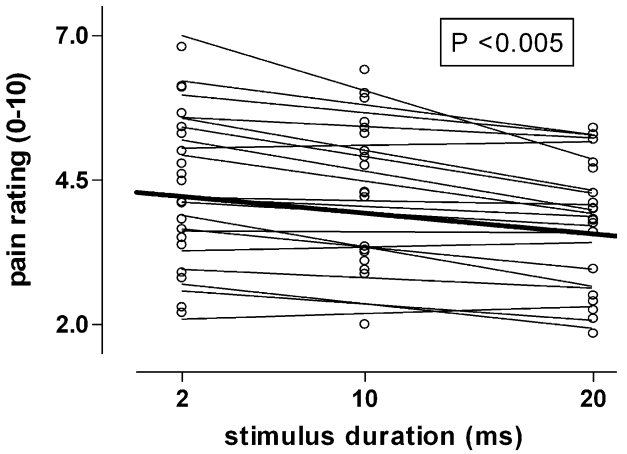


Fig. 1. Scatterplot of individual pain ratings to 2, 10 and 20 ms laser stimuli, with equal total energy (1 J). Each symbol indicates the mean pain rating obtained from one run of each subject. Thick and thin lines represent mean and intraindividual regressions of pain ratings, respectively. Trend analysis revealed significantly higher ratings to stimuli with shorter duration ($P < 0.005$). Note that the lines showing intraindividual pain rating changes tended to converge as the stimulus duration increased indicating a lower between-subject variability with longer-lasting stimuli.

Subjective pain ratings to 20 ms stimuli were significantly lower than those to 2- and 10 ms stimuli ($P < 0.01$; Tukey's test). Post test for linear trend showed a significant negative correlation between stimulus duration and pain rating ($P < 0.005$, Fig. 1).

### 3.2. Time-course of skin temperature

Although varying in duration, laser stimuli of equal energy produced a similar increase in temperature, and this increase remained constant independently from the baseline temperature. Mean temperature increases for stimulus durations of 2, 10 and 20 ms were 11.9 ± 2.03, 12.53 ± 1.85 and 13.4 ± 1.40 °C, respectively, i.e. temperature did not increase with stimulus duration (ANOVA, $P > 0.20$; Fig. 2A). Changing the baseline temperature did not influence the temperature increase or the duration-dependent variations of the temperature rise times (Fig. 2B). Similarly, the delay between stimulus and onset of the temperature rise was similar for the three stimulus durations. In contrast, the only temperature parameter affected by stimulus duration was the rise time, which increased with longer-lasting stimuli: mean slope values for durations of 2, 10 and 20 ms were 1.92 ± 0.043, 1.13 ± 0.022 and 0.79 ± 0.024, respectively (ANOVA, $P < 0.0001$; Fig. 2A).

### 3.3. Laser-evoked potentials (LEPs)

In all subjects, Nd:YAP laser stimulation of Aδ afferents easily evoked clear and reproducible late LEPs. EMG recordings showed that no reflex response was elicited in the orbicularis oculi, masseter, or cervical muscles. The earliest

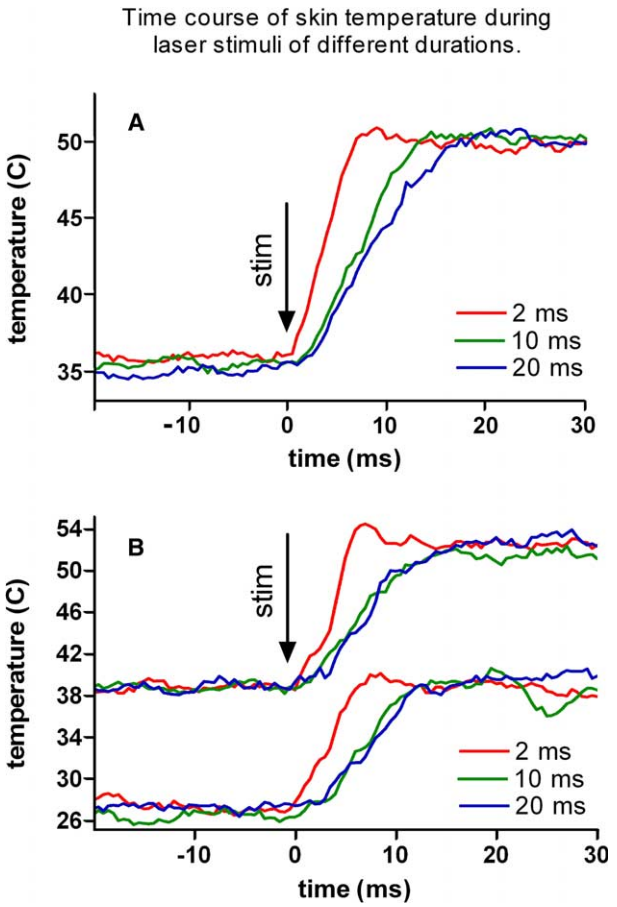


Fig. 2. Effect of stimulus duration on skin temperature. Skin temperature profiles after laser pulses with equal energy (1 J) and different durations (2, 10 and 20 ms). (A) Normalized grand averages from nine subjects; mean baseline and peak temperatures are taken as upper and lower limits (absolute values and statistics reported in the text). (B) Effect of baseline temperature on temperature increase. Averages of three, non-normalized trials in a representative subject. *y*-axis: surface skin temperature. *x*-axis: time. Arrows indicate stimulus onset. Temperature rise times significantly increased with longer-lasting stimuli ($P < 0.001$), whereas the increase of skin temperature was similar and constant, independently from the baseline temperature.

identifiable scalp component was the early latency negative wave (N1) visible in both the right and left temporal leads, with a latency of about 180 ms and an amplitude of about 7 μV (Fig. 3). The N1 component usually appeared after a few averaged trials in all subjects. The contralateral N1 had a shorter latency (174 ± 10 vs 182 ± 12 ms) and a higher amplitude (7.5 ± 2.9 vs 6.3 ± 3.4 μV) than the ipsilateral.

The N1 component was followed by the late negative–positive complex (N2–P2) visible in the midline (Fz, Cz and Pz) leads with a mean N2 latency of 228 ± 17 ms and a peak-to-peak amplitude of 29.2 ± 9.7 μV. Even single trials often yielded a clear N2–P2 complex on the midline electrodes, and its peak latency and shape became stable after few averaged trials (Fig. 3).

### 3.4. Effect of stimulus duration on LEPs

Despite the total energy of the laser stimuli delivered being constant during the whole experiment, the three examined stimulus durations (2, 10 and 20 ms) influenced significantly both the pain perception and brain potentials.

All LEP data were affected by stimulus duration, with the exception of the amplitude of the late N2–P2 vertex complex. A briefer stimulus duration shortened the latency of both N1 and N2 and increased the amplitude of N1 (Fig. 3). The N1- and N2-latencies significantly correlated with stimulus duration (N1: $R = 0.1762$, $F = 11.76$, $P < 0.005$; N2: $R = 0.1103$, $F = 6.697$, $P < 0.05$; Fig. 4, upper row). The N1 amplitude showed a negative correlation with stimulus duration ($R = 0.1361$, $F = 6.697$, $P < 0.05$; Fig. 4, lower left). In contrast, the correlation between N2–P2 amplitude and stimulus duration was not statistically significant ($P > 0.05$; Fig. 4, lower right).

## 4. Discussion

In this experimental study in healthy volunteers, we investigated the selective effect of the duration of noxious

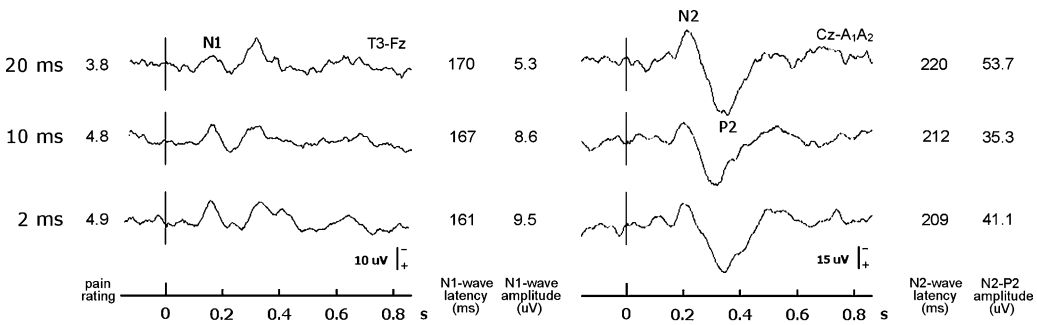


Fig. 3. Enhancement of brain potentials and pain perception by stimulus duration. Early (N1) and late (N2–P2) components of brain responses, and mean pain ratings evoked by laser stimuli of 2, 10 and 20 ms duration, in a representative subject. Negativity upwards. Average of 20 trials per stimulus duration. N1 and N2 latencies and pain ratings are affected by stimulus duration, with shorter latencies and higher ratings after shorter-lasting stimuli. The amplitude of the early latency component N1 (left column) is larger after stimuli of shorter duration, while the amplitude of N2–P2 complex (right column) is unaffected by the duration of the stimulus.

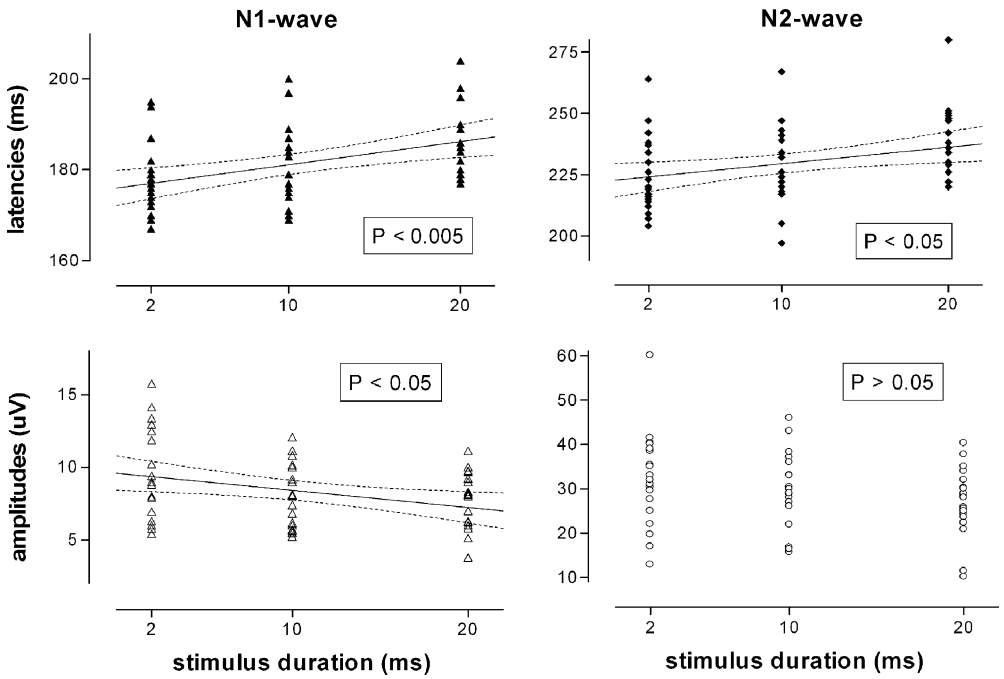


Fig. 4. Stimulus duration–LEP correlations. (Upper row) Correlation with latency. A shorter duration of the laser stimulus significantly shortens latency of N1 and N2 components of LEPs. *y*-axis: peak latency. *x*-axis: stimulus duration. Each symbol indicates the mean peak latency obtained from one run of each subject. The lines are the mean regressions calculated on all latency values (N1-wave: $n = 56$; N2-wave: $n = 60$); dashed lines indicate the 95% confidence limits. (Lower row) Correlation with amplitude. A shorter duration of the laser stimulus increases the amplitude of N1 component of LEPs, while does not affect significantly the peak-to-peak amplitude of the N2–P2 complex. *y*-axis: amplitude. *x*-axis: stimulus duration. Each symbol indicates the mean amplitude obtained from one run of each subject. The line on the left graph is the mean regression calculated on all amplitude values (N1-wave: $n = 56$; N2–P2 complex: $n = 60$); dashed lines indicate the 95% confidence limits.

laser stimuli on skin temperature changes, subjective pain perception and brain evoked potentials, having controlled for variances in energy delivered by use of a laser stimulator that produced equal energy for all stimuli durations. A shorter stimulus duration yielded steeper slopes in skin heating profiles and increased perceived pain intensity; accordingly, brain evoked potentials were enhanced. Probably, the same energy delivered in a shorter time reduces receptor activation times and yields a more synchronized afferent volley, thus producing a more efficient summation at central synapses and a stronger input to the brain.

### *4.1. General characteristics of solid-state lasers, nociceptor activation, and brain evoked potentials*

Although used less widely than $CO_2$ lasers, solid-state (YAG/YAP) lasers provide reliable pain-related brain responses (late LEPs), resulting from the activation of type II Aδ mechano-heat nociceptors (AMH II units); that the input yielded by these stimulators is as selective as the one yielded by $CO_2$ lasers has been demonstrated by similar evoked sensation (pinprick), conduction velocity (in the Aδ-fibre range), and findings in patients with dissociated sensory loss (Bromm and Treede, 1991; Bromm and Lorenz, 1998; Cruccu et al., 2003; Spiegel et al., 2000, 2003).

In this study, the Aδ-related brain responses following Nd:YAP laser stimulation of the hand dorsum showed an excellent signal to noise ratio. The N2–P2 vertex component was almost always visible in single trials, and the N1 lateral component became clear after a few averaged trials in all subjects (Fig. 3). These findings confirm that solid-state laser stimulation of the hairy skin provides an excellent peripheral input and thus is an optimal tool for the neurophysiological examination of the thermal-pain system.

The radiation of the Nd:YAP laser has a wavelength of 1.34 μm, and thus penetrates deeper than the $CO_2$ laser radiation (10.6 μm). Because of the diversity in radiation wavelength, $CO_2$ and solid-state lasers produce different spatial gradients of skin heating, with a higher decrease in temperature from the skin surface for $CO_2$ vs solid-state lasers (75 vs 40% at 200 μm in an agar model of the skin) (Bromm and Treede, 1983; Spiegel et al., 2000). Because of these characteristics, the radiation of our solid-state laser minimizes (with respect to $CO_2$ laser radiation) the heat conduction times through the skin, mainly activating nociceptors by direct heating.

If nociceptors are mainly activated directly, changes in psychophysical and electrophysiological responses should be mainly due to the physiological properties of nociceptors themselves.

### 4.2. Stimulus duration induced-effects on skin temperature

The assessment of the temperature of the skin irradiated by a laser pulse is by no means easy and represents a major problem in the LEP understanding and standardization. Most previous studies that tried to measure the skin temperature used thermocouples. Thermocouples have at least two drawbacks. Firstly, they must either be positioned over the laser-irradiated skin, and thus directly receive the laser radiation themselves, or be positioned close to the irradiated skin, and thus measure the heating of the nearby skin rather than that irradiated. Secondly, they have non-negligible thermal inertia, which, however small, will prevent a reliable assessment of fast temperature changes.

Radiation pyrometers, using semiconductors to detect infrared emission by heated bodies from a distance, avoid the problem of close contact (e.g. LaMotte and Campbell, 1978; Meyer et al., 1976); they have been used in a number of recent LEP studies, mainly dealing with the feedback-control of the temperature induced by a $CO_2$ laser stimulus (Arendt-Nielsen and Chen, 2003; Magerl et al., 1999; Plaghki and Mouraux, 2003; Treede et al., 1994). Also this method has some drawbacks: according to the wavelength of the laser radiation, radiation pyrometers may directly read the radiation reflected from the skin and are usually slow in reacting. But the wavelength of the Nd:YAP that we used was too short to affect our pyrometer and this was an extremely fast device, recently developed for industrial use, which yielded a time resolution of 500 μs.

Using this method, we could reliably and precisely measure fast temperature changes of the laser-irradiated skin spot. We found that, whereas the onset latency and temperature increase were not affected by the duration of the stimulus, this did influence the temperature rise-time: independently from the baseline temperature of the skin, longer-lasting stimuli of equal energy produced longer-lasting temperature rise-times (Figs. 2 and 5). Very similar results were reported by Greffrath et al. (2002) in a study investigating the effects of diode laser pulses on currents in dissociated primary nociceptive neurons. To estimate the time-course of temperature changes induced by heat laser stimuli with different characteristics they used open patch-clamp pipettes, and found that—changing simultaneously power and duration of two stimuli in order to reach a similar temperature (i.e. keeping the energy constant)—the slope to reach the same temperature was steeper with shorter (240 ms) than with longer-lasting (400 ms) stimuli (Fig. 1D in Greffrath et al., 2002).

Radiation pyrometers can only assess the temperature at the skin surface. Some previous studies used thermodynamic models to obtain information about temperature at receptor level (Plaghki, 1997; Spiegel et al., 2000). However, compared to any thermodynamic model of temperature at different skin depths, our surface measures have the great advantage of being genuine, and even if the absolute temperature value at the skin surface differs from that at 50–500 μm depth (i.e. where the relevant receptors are located), the relative difference across the three time-courses can hardly be affected by the thermodynamic properties of the skin layers.

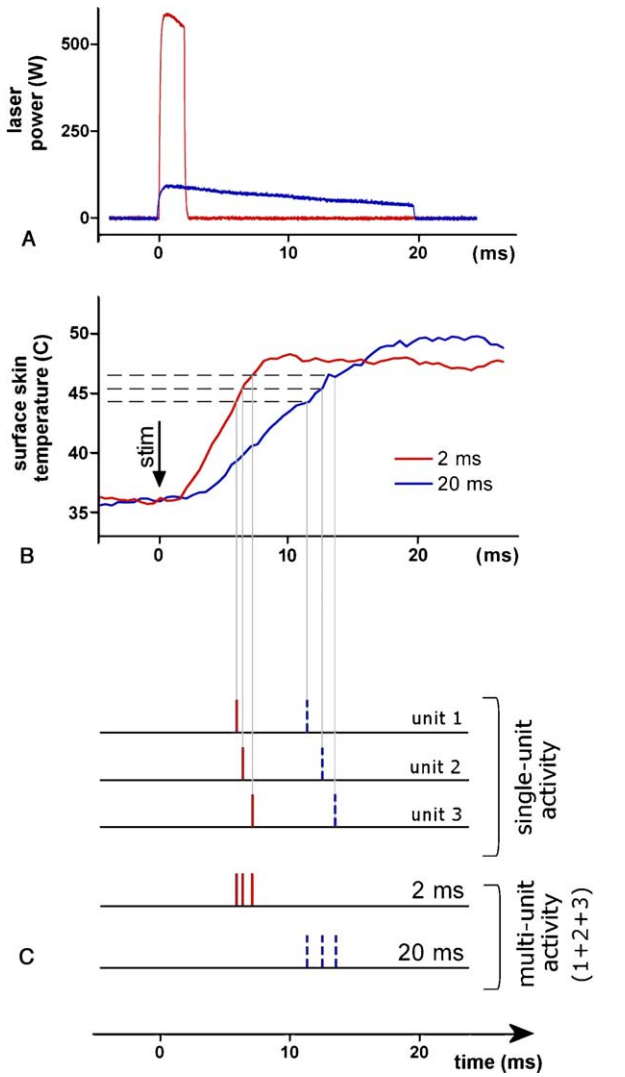


Fig. 5. Scheme of the possible effects of stimulus duration on receptor activity. A laser stimulus with shorter duration (2 ms) induces a more synchronized afferent volley in the excited type II AMH nociceptors. (A) Duration (ms) and power (W) of two laser stimuli that deliver the same energy (1 J) to the skin (the energy is expressed by the area under the two curves) have been measured using a photodiode sensitive to the wavelength delivered by the Nd:YAP laser (1.34 μm). (B) Pyrometric measure of temperature increases at the skin surface during laser stimuli of different durations. Even if the increase of the skin temperature is similar with short (2 ms) and long duration (20 ms) stimuli, the heating induced by the 2 ms stimulus is faster. Hence, the neural response in three hypothetical type II AMH nociceptors (units 1, 2 and 3, in C) with different heat activation thresholds will be closer to the stimulus onset if the stimulus has a shorter duration. (C) The upper traces illustrate the hypothetical action potentials generated by the three nociceptors with the heat thresholds depicted in B; the dashed spikes represent the activity induced by the 20 ms long stimulus; the lower traces show the total afferent volley; for clarity, the receptor activation time is not represented. Should these three afferent neurons have collaterals converging on the same synapses, spatial summation would be more effective with 2 than 20 ms stimuli.

### 4.3. Stimulus duration induced-effects on LEPs and psychophysics

A number of studies investigated LEPs as nociceptive responses; among these, different laser sources and different stimulus durations were used. Despite differences among laboratories, generally the longer the stimulus duration, the longer the latency of the N2 LEP component; for example, after hand stimulation, the latency increased from 208 ms with 2 ms-long Th:YAG laser stimuli (Spiegel et al., 1996, 2000) to 243–249 ms with 20 ms-long $CO_2$ laser stimuli (Bromm and Treede, 1987, 1991) and to 400 ms with 200 ms-long Argon laser stimuli (Arendt-Nielsen and Bjerring, 1988). Also comparing studies that used $CO_2$ lasers, the latency seems to correlate with stimulus duration; for example, the latency increased from 201 to 240 and to 256 ms with stimulus durations of 10, 20, and 30 ms (Bromm and Treede, 1987; Kakigi et al., 1989; Towell et al., 1996). Treede et al. (1994) are the only investigators who directly compared the brain responses evoked by laser pulses with different stimulus durations and rise times, concluding that the rise time of the stimulus, rather than the duration of the plateau, is important to trigger an evoked potential. However, any physiologically meaningful comparison among these results is rather difficult, because the change in stimulus duration always entails a change in the delivered energy. For these reasons, we tried to clarify the issue of the selective effect of stimulus duration on nociceptor responses by designing a study that employs a laser source with a wavelength that activates nociceptors mainly directly, and equipped with a stimulus control software specifically designed to enable change in the stimulus duration only, without altering the total energy delivered; furthermore, we exploited a fast radiation pyrometer to control the skin temperature profiles during laser stimuli with different duration.

Considering the observed significant increase in steepness of skin temperature slopes with shorter-lasting stimuli (see Fig. 2 and above), two neurophysiological mechanisms explain the latency gain of LEPs. Firstly (direct effect), nociceptors reach the firing threshold and peak frequency of discharge earlier (Treede et al., 1994; Treede et al., 1995). Secondly (synchronisation effect), because the peak temperature is reached earlier, receptors with a slightly higher threshold are excited with a shorter delay and thus the afferent volley is more synchronised and exerts a stronger spatial summation at central synapses. The schematic drawing in Fig. 5 shows the effects of two laser stimuli with identical energy but different durations (2 and 20 ms) on the skin temperature rise times and possible nociceptor responses. Even if the maximal skin temperature is the same, the shorter-lasting stimulus causes a faster heating and, consequently, a more immediate receptor excitation and a more synchronous volley.

Besides the latency gain, shorter-lasting stimuli also induced a significant increase in pain ratings and amplitude of the early latency N1 component. While both the direct and synchronisation effects may explain the latency gain of brain responses, the enhancement of perceived pain intensity and N1 amplitude should be entirely due to the synchronisation effect at central synapses. The most obvious explanation is a stronger spatial summation, due to the synchronisation of the volleys from different afferents that converge on the same post-synaptic neurons. However, an additional contribution of temporal summation to the increased pain ratings and N1 amplitude cannot be excluded: microneurographic recordings in animals have shown that the peak discharge frequency of nociceptors increases with the stimulus ramp rate, indicating a rate-sensitive transduction mechanism (Tillman et al., 1995; Yarnitsky et al., 1992). Moreover, several investigators have shown that only an impulse frequency above a certain value on a human peripheral nerve is sufficient to be painful, thus demonstrating that temporal summation is required for pain perception (Torebjork and Schady, 1984; Van Hees and Gybels, 1981). All these mechanisms (heat conduction time, direct effect, and synchronisation effect) probably contribute to explain why the N2-wave latency is often shorter after solid-state (hand: 210–230 ms; face: 150–160 ms) than $CO_2$ laser stimulations (hand: 230–280 ms; face: 166–179 ms) (Bromm and Chen, 1995; Bromm and Lorenz, 1998; Cruccu et al., 1999, 2003; Plaghki, 1997; Spiegel et al., 2000).

In contrast with the amplitude modulation of the early latency N1 component, the amplitude of the late N2–P2 complex was not significantly affected by the stimulus duration (Fig. 4, lower right), possibly because the late N2–P2 components are more related to the attentional/cognitive processes than the intensity of sensory input (Beydoun et al., 1993; Garcia-Larrea et al., 1997). The different behaviour of the early and late LEP components supports the view that the early N1 component reflects a less integrated station of the cortical processing, and represents a more reliable neurophysiological correlate of the noxious input. Despite the effects of stimulus duration on LEPs and psychophysics are statistically significant and provide interesting physiological information, the size of their effect is relatively small, thus their clinical relevance may only be hypothesized.

In conclusion, our findings show that shorter-lasting stimuli, using laser pulses of equal energy, raise the skin temperature in shorter times and provide higher-amplitude brain signals. This gives some advantages that may prove useful in physiological and clinical studies aimed at assessing nociceptive pathway function. Because the greater synchronization yields a high-amplitude N1, the investigators may rely on a response that is more related to the sensory input and less sensitive to cognitive influences than the vertex N2–P2, thus a more reliable measure (Garcia--Larrea et al., 1997). Furthermore, since fewer trials are

sufficient to get reliable brain responses, the time spent, attention changes, and subject's discomfort are minimized.

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