Clinical Neurophysiology 116 (2005) 821–826

www.elsevier.com/locate/clinph

Laser-evoked potentials: normative values

A. Truini[a], F. Galeotti[a], A. Romaniello[a], M. Virtuoso[a], G.D. Iannetti[a,b], G. Cruccu[a,*]

[a]*Department of Neurological Sciences, La Sapienza University, Viale Università30, 00185 Rome, Italy*
[b]*Department of Human Anatomy and Genetics, University of Oxford, UK*

Accepted 14 October 2004
Available online 18 January 2005

Abstract

Objective: Laser-evoked potentials (LEPs) currently represent the most reliable and widely agreed method of investigating the Aδ-fibre pathways. Many studies dealt with the usefulness of LEPs in peripheral and central nervous system diseases. We aimed at gaining normative values for LEP data.

Methods: Using a CO_2 laser stimulator we recorded LEPs after face, hand, and foot stimulation in 100 normal subjects. We measured the perceptive threshold, latency and amplitude of the main vertex components, and their side-to-side differences. We also studied the correlations between LEP data and age and body height, as well as gender differences.

Results: Laser perceptive threshold increased and LEP amplitude decreased from face to foot ($P<0.0001$). The latency of hand and foot-LEPs correlated significantly with body height ($P<0.0001$). The amplitude, though not the latency, correlated with age ($P<0.0001$). LEP data did not significantly differ between genders ($P>0.1$).

Conclusions: This study provides normative values for the main LEP data and their absolute and side-to-side limits, highlighting the physiological differences related to, body height, age, gender and stimulation site.

Significance: Our data may help to improve the clinical reliability of LEPs as a diagnostic tool.

Keywords: Laser-evoked potentials; Nociceptive pathways; Aδ fibres

1. Introduction

Laser-generated radiant heat pulses selectively excite free nerve endings in the superficial skin layers and activate Aδ and C nociceptors (Bromm, 1985). Low-intensity pulses directed to the hairy skin evoke pinprick sensations and brain potentials (LEPs), both related to the activation of type II AMH mechanothermal nociceptors; the afferent volley is conducted along small-myelinated (Aδ) primary sensory neurons, and relayed to spinothalamic neurons and brain (Treede et al., 1995).

Although several types of laser stimulators are now available, most studies in patients used a CO_2-laser. This device has the advantage of a wavelength (10.6 μm) that closely matches the thermophysical properties of the skin (Arendt-Nielsen and Chen, 2003). Radiation with this wavelength has a negligible skin reflectance and is fully absorbed within the most superficial skin layers; nociceptors located in deeper skin layers are stimulated via heat conduction (Bromm and Lorenz, 1998).

The main LEP signal that is usually measured in a clinical setting, is a widespread negative–positive complex (N2–P2) that reaches its maximum amplitude at the vertex (Bromm and Lorenz, 1998). This complex is mostly generated by the anterior cingulate gyrus, with a possible contribution from the bilateral insular regions (Garcia-Larrea et al., 2003; Valeriani et al., 2004). The N2–P2 complex is preceded by an earlier, far smaller negative component (N1) which is lateralized, bilateral, and probably generated by the secondary somatosensory cortex (Spiegel et al., 1996). This component, being less reproducible and requiring far more trials than the N2–P2 vertex complex, is used only in experimental studies (Bromm and Lorenz, 1998).

* Corresponding author. Tel.: +39 6 49694209; fax: +39 6 49914758.
E-mail address: cruccu@uniroma1.it (G. Cruccu).

1388-2457/$30.00
doi:10.1016/j.clinph.2004.10.004

More than 100 LEP studies have been published. LEPs are widely used in patients with peripheral and central nervous system lesions (Garcia-Larrea et al., 2002; Spiegel et al., 2003; Truini et al., 2003), and LEPs have been acknowledged by the European Federation of Neurological Societies as the most reliable laboratory tool for assessing pain pathways (Cruccu et al., 2004). Although several studies reported normal values of LEPs (Arendt-Nielsen and Bjerring, 1988a; Devos et al., 2000; Spiegel et al., 2003), they have been collected only in small sample of normal subjects, which were in a narrow age range. Furthermore, the effects of important clinical variables, such as body height, age, and gender have not been studied.

In this study recording LEPs in 100 normal subjects, we aimed at gaining normative values for perceptive threshold, latency, and amplitude of LEPs after face, hand, and foot stimulations, as well as at studying the possible influence of important clinical variables, such as body height, age, and gender.

2. Methods

One hundred healthy volunteers (50 female, 50 male), aged 14–82 years (mean 47 years) participated in the study. None had neurological disorders or were receiving medication. All subjects gave their informed consent to undergo the procedure, and the local Ethics Committee approved the research.

2.1. Stimulation and recording technique

Using a CO_2-laser stimulator (Neurolas, Electronic Engineering, Florence, Italy) we delivered brief radiant heat pulses (wavelength 10.6 μm, intensity 1.5–15 W, duration 15 ms, beam diameter 3 mm) to the skin of the perioral region, the back of the hand, and the dorsum of the foot (Fig. 1). To determine laser perceptive threshold (PTh) we delivered series of stimuli at increasing and decreasing intensity, and defined the perceptive threshold as the lowest intensity at which the subjects perceived at least 50% of the stimuli (Agostino et al., 2000a; Cruccu et al., 1999). In LEP recordings we used a stimulus intensity of twice the perceptive threshold. To avoid damage to the skin, fatigue or sensitisation of nociceptors, and central habituation, the interstimulus interval was varied pseudorandomly (10–20 s) and the irradiated spot was slightly shifted after each stimulus. The N2–P2 LEP complex was recorded through disc electrodes from the vertex (Cz) referenced to linked earlobes (A1–A2), i.e. the most widely used method in clinical studies (naturally this derivation does not allow recording the early N1 component). Electroculographic recordings monitored possible eye movements or blinks. For each site of stimulation, two series of 10–12 trials devoid of artefacts were averaged off line. We measured

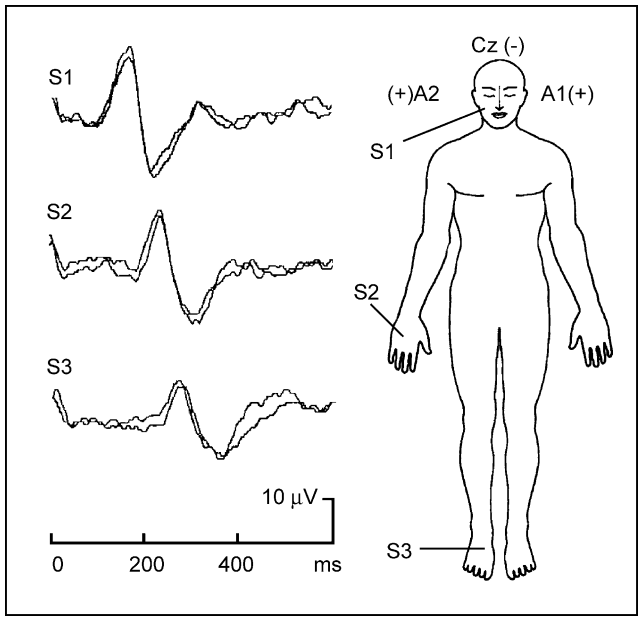

Fig. 1. Laser-evoked potentials (LEPs) in a representative normal subject. Two series of 12 trials devoid of artefacts, collected and averaged after perioral (S1), hand (S2), and foot stimulation (S3). Recordings from the vertex (Cz) referenced to linked earlobes (A1–A2).

the latency of the main negative and positive components and their peak-to-peak amplitude, and their side-to-side difference.

2.2. Statistics

Whereas LEP latency and amplitude values had a normal distribution, PTh values did not. LEP latency and amplitude differences between stimulation sites were evaluated with analysis of variance (ANOVA) for repeated measures and post test for linear trend; we used the Wilcoxon sign test for nonparametric paired data to analyse PTh differences between stimulation sites.

To evaluate gender differences we split males and females in two samples matched for age and body height; then PTh differences were evaluated with Mann–Whitney test and latency and amplitude differences with unpaired *t*-test. Because body height and age values had a normal distribution, we used linear regression and Pearson r^2 coefficient to evaluate their correlations with LEP values, and Spearman R coefficient to evaluate correlations with PTh. The normal limits of LEP values were calculated as the mean ± 2.5 SD (covering 99% of a normally distributed population and thus leaving a 1% probability that a sample value exceeding these limits still belonged to the normal population); for PTh we took the whole range (i.e. the lowest and highest value found in our 100 subjects). All results are reported as mean $\pm$ SD.

3. Results

3.1. Differences between stimulation sites

As the conduction distance lengthened from face to foot, LEP latency progressively increased (Table 1). The stimulation site also influenced LEP amplitude and PTh. LEP amplitude progressively decreased from face to foot ($P<0.0001$); PTh was lower after face than after hand stimulation ($P<0.0001$) and lower after hand than after foot stimulation ($P<0.001$).

Of the 100 subjects studied, in 17 (all older than 69 years), laser stimulation of the foot failed to evoke reproducible brain potentials bilaterally. In no subject did we find foot-LEPs absent unilaterally.

3.2. Correlations with age and height

For all LEPs, regardless of the stimulation site, LEP amplitude correlated negatively with age ($P<0.0001$), whereas LEP latency did not ($P>0.2$), (Fig. 2).

The latency of hand and foot-LEPs, though not that of face-LEPs, strongly correlated with body height (Fig. 3). The correlation between N latency and height was best fitted with a linear function: hand-LEPs, $Y=1.2X+26$, $r^2=0.44$, $F=124$, $P<0.0001$; foot-LEPs, $Y=1.4X+47.5$, $r^2=0.4$, $F=110.7$, $P<0.0001$ (Y being the latency in ms and X the height in cm). Both these functions yielded steep slopes: the mean foot-LEP latency increased with height (from 258 ms for subjects 150 cm in height to 307 ms for subjects 185 cm in height).

3.3. Normal limits

For clinical applications, when dealing with patients with unilateral dysfunction, the intraindividual side-differences yield the narrowest limits (making it unnecessary to adjust for the patient's height and age). In our subjects, the 99% normal limit of side asymmetry in N latency was 7.5% for face and 8% for limb-LEPs; the 99% normal limit of the side asymmetry in amplitude was within 50% (45% for face, 47% for limb-LEPs).

The normal limit of absolute latency (useful in patients with bilateral or diffuse dysfunction) was 200 ms for face-LEPs (which were uninfluenced by body height). Because limb-LEPs were strongly influenced by body height, the normal limit of latency should be calculated from the regression line function (mean expected value + 2.5 SD). The normal limit of foot-LEP latency varied from 300 ms (258 + 42) in subjects 150 cm tall, to 349 ms (307 + 42) in subjects 185 cm tall.

LEPs for all stimulation sites varied so widely in amplitude, and were so intensely affected by age, that the 99% lower limit often approached zero.

3.4. Gender differences

After matching males and females for body height and age, we analysed gender differences in 70 subjects. PTh was slightly lower in females than in males: this difference, which was greatest after foot stimulation (6.9 vs 7.8 mJ/mm^2), approached but did not reach statistical significance ($P>0.1$). Neither the latency nor the amplitude of LEPs differed between males and females ($P>0.5$).

4. Discussion

Besides providing the normal limits for the main LEP data after stimulation of the three body sites that are most useful for assessing nociceptive pathways in neurological disease, we also found site-related differences and correlations with body height and age that are important for establishing the normal limits and interesting from the physiological point of view.

The laser perceptive threshold was progressively lower and LEP amplitude higher after face than after hand and foot stimulations. Whereas all studies dealing with trigeminal-LEPs reported that facial stimulations yielded lower-threshold and higher-amplitude LEPs (for a review see Romaniello et al., 2003), the reports about possible

Table 1
Comparison between LEPs from different stimulation sites in 100 subjects (mean ± SD and range)

LEP data	Face	Hand	Foot[a]	P[b]
Perceptive threshold (mJ/mm^2)	4.6 ± 0.8 (3–9.2)	5.7 ± 2.6 (4.6–16.4)	7.3 ± 4.4 (4.6–18.4)	Significant[c]
N2 latency (ms)	164 ± 13.8 (130–200)	236 ± 18 (200–277)	275.4 ± 16.7 (228–314)	Significant[d]
Asymmetry ratio (%)	5 ± 1	4.9 ± 1.3	5.1 ± 1.4	NS
P2 latency (ms)	241.3 ± 20.2 (192–288)	315.4 ± 23.1 (248–380)	361 ± 26.3 (292–416)	Significant[d]
Asymmetry ratio‡ (%)	5.9 ± 3.8	6.1 ± 4	6 ± 4.4	NS
Amplitude (μV)	21.9 ± 8.5 (7–50)	18.3 ± 8.5 (6–45)	16 ± 5.5 (7–32)	Significant[d]
Asymmetry ratio‡ (%)	15.2 ± 12.1	15.6 ± 12.4	15.9 ± 12	NS

[a] $n=166$.
[b] Statistical significance of the differences between stimulation sites.
[c] Wilcoxon between face and hand, $P<0.0001$, between hand and foot, $P<0.001$.
[d] ANOVA repeated measures, $P<0.0001$. Post test for linear trend, $P<0.0001$.
‡ Side-to-side asymmetry ratio in percentage.

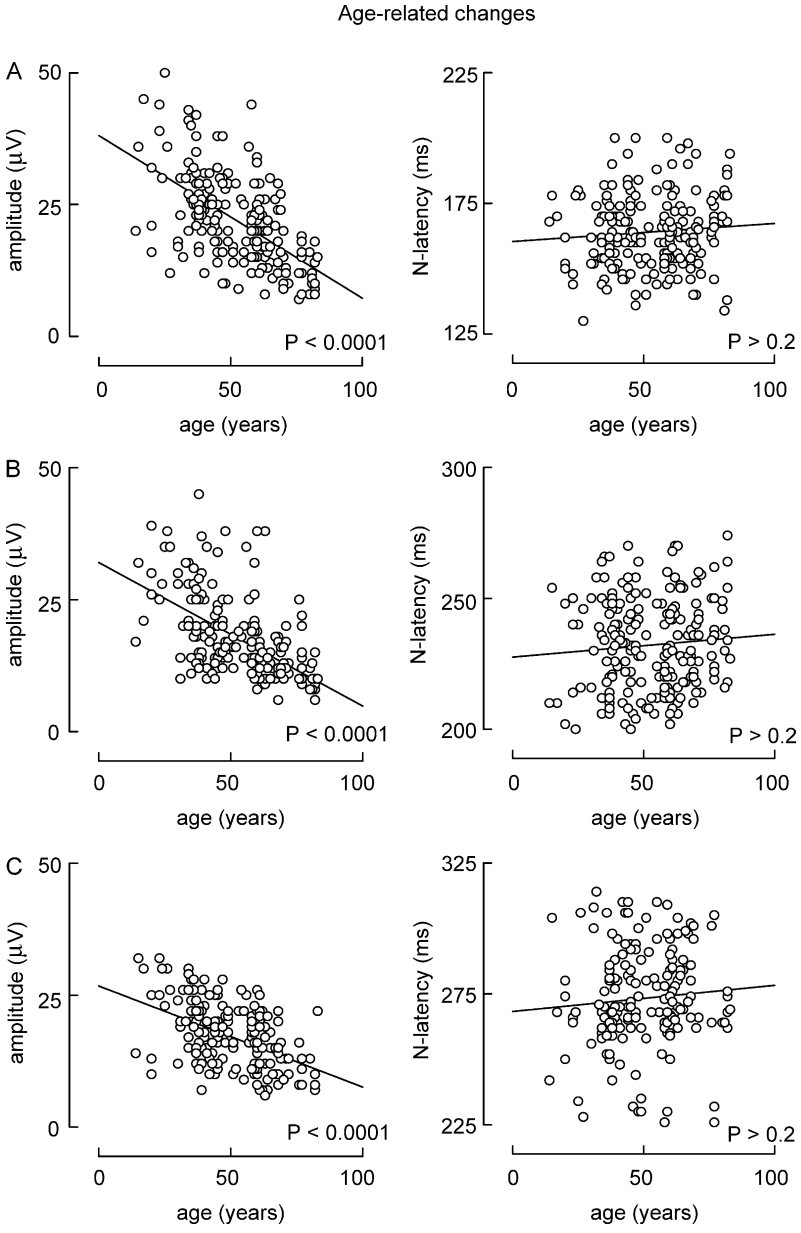

Fig. 2. Age-related changes. *y*-axis: amplitude and latency of LEPs after face (A), hand (B) and foot (C) stimulation. *x*-axis: age of subjects (years). Left panel: LEP amplitude correlated negatively with age ($P<0.0001$). Right panel: LEP latency did not correlate with age ($P>0.2$).

differences in LEP data between hand and foot stimulation are controversial. Some studies found significant differences between LEPs to hand and foot stimuli (Cruccu et al., 1999; Spiegel et al., 2000), whereas others did not (Agostino et al., 2000b; Rossi et al., 2002). These studies were conducted in small groups of normal subjects, however, with a narrow age range. Our findings, obtained in a wide age range (14–82 years), showing that LEP amplitude and threshold change with distance are consistent with findings from skin biopsy and psychophysiological studies. The threshold changes we observed receive support from skin biopsy investigations (Lauria, 1999; McArthur et al., 1998), demonstrating a higher density of epidermal free nerve endings in proximal than in distal body sites. A study on the topographical distribution of pinprick sensations related to CO_2-laser stimuli reported a positive correlation between distance from the brain and the laser perceptive threshold (Agostino et al., 2000a). A short distance from the brain probably reduces the signal dispersion along Aδ afferents. The lower signal dispersion along a shorter distance yields a highly synchronized volley that exerts a strong spatial-temporal summation at central synapses and thus provides higher-amplitude responses.

Conversely, the longer conduction distance engenders a high-signal dispersion which explains—together with the lower receptor density—why foot-LEPs are comparatively small and occasionally so small that they cannot even be detected, particularly in elderly subjects (17% in our sample).

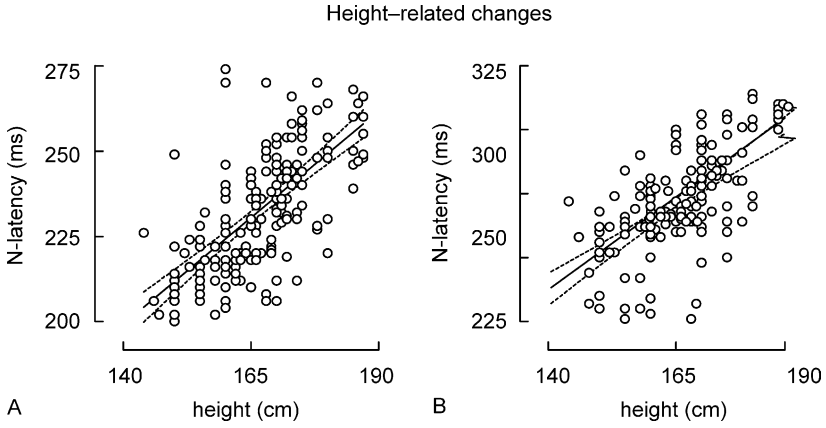

Fig. 3. Height-related changes. *y*-axis: N-latency of LEPs after hand (A) and foot (B) stimulation. *x*-axis: body height of subjects. The N latency significantly correlated with height ($P<0.0001$). The relationship between latency (expressed in ms) and height (expressed in cm) strongly fitted with a linear function: hand-LEPs, $Y=1.2X+26$ ($r^2=0.44$, $F=124$, $P<0.0001$); foot-LEPs, $Y=1.4X+47.5$ ($r^2=0.4$, $F=111$, $P<0.0001$).

Although increasing the stimulus intensity or the number of averaged trials might reduce the occurrence of absent foot-LEPs, still the finding of bilaterally absent foot-LEPs in elderly subjects—unlike unilaterally absent foot-LEPS—does not demonstrate a neurological dysfunction.

The LEP latency after hand and foot (though not after face) stimulations strongly correlated with body height. Although never reported previously, the correlation between limb-LEP latency and height (and thus conduction distance) is obvious. But owing to the low conduction velocity of these pathways—the relationship between latency and height is ruled by a steep linear function, which engenders such wide latency changes that height must necessarily be adjusted for in clinical practice, both for foot and hand-LEPs (Fig. 3).

In our healthy population, LEP amplitude correlated negatively with age. The few studies addressing this issue consistently found an age-related decrease in LEP amplitude (Cruccu et al., 1999; Gibson et al., 1991), possibly due to a mild neuronal loss or dysfunction in the peripheral nerves or in the brain with advancing age (Gagliese and Melzack, 2000; Gibson and Helme, 2001), as happens for other evoked potentials (Deuschl and Eisen, 1999). Indeed a subclinical dysfunction might have also contributed to the relatively high percentage of absent foot-LEPs that we found in elderly subjects. The age-related and distance-related decrease in LEP amplitude, together with its wide interindividual variability, make the LEP amplitude of scarce diagnostic value unless the patient has a unilateral dysfunction and normal LEPs on the contralateral side.

Conversely, age had no significant effect on LEP latency, even after foot stimuli. Clearly, if the age-related amplitude decrease arises from a reduced afferent input, this should affect latency as well. If the LEP attenuation is mostly caused by brain changes, amplitude (also depending on the number of healthy neurons in the brain areas that generate LEPs) may be affected more than latency.

Although Gibson et al. (1991) found an age-related increase in LEP latency after hand stimuli, their stimulus variables distinctly differed from ours and the correlation barely reached significance ($P<0.05$); we have no simple explanation for the contrasting results. Nevertheless, minor latency changes, and lack of statistical significance may be diagnostically unimportant (Fig. 2). Hence, even though age may affect latency, in a clinical setting we consider it unnecessary to correct the normal limits for age.

Whereas latency and amplitude of LEPs were almost identical (once matched for height) in males and females, PTh differed slightly (PTh lower in females). Although in our subjects this difference was not significant, a previous study using an argon laser stimulator reported a perceptive threshold after hand stimulation significantly lower in females than in males (Arendt-Nielsen and Bjerring, 1988a) and in general females have a lower threshold to a wide range of noxious stimuli (Keogh and Herdenfeldt, 2002). Nevertheless the finding of a minor gender difference in PTh is diagnostically negligible, particularly with respect to the strong height and age-related changes in LEPs.

Although most clinical studies have been performed with a CO_2-laser stimulator, several investigators have used other kinds of laser radiation. The argon laser (wavelength 488 and 515 nm) emits a radiation which penetrates into dermal structures, far deeper than the CO_2 laser. It has a weak output power. Stimulus durations, therefore, may be as long as 200 ms (Arendt-Nielsen and Bjerring, 1988ab). Since the peak temperature is reached at the end of a constant power laser pulse (Spiegel et al., 2003), long stimulus durations increase the latency of LEPs and their latency jitter. The thulium-YAG laser, a solid-state laser, emits near-infrared radiation (wavelength 2.01 μm) that has a penetration depth of 360 μm. Unlike CO_2 lasers, it may also directly activate nociceptors located more deeply, thus providing shorter LEP latencies than those obtained with CO_2 lasers

(Devos et al., 2000; Spiegel et al., 2003). In contrast, the amplitude does not differ significantly (Bromm and Lorenz, 1998).

Finally, it has been recently demonstrated with solid-state lasers that stimulus duration influences LEPs: the longer the stimulus duration the longer the LEP latency (Iannetti et al., 2004).

Although a direct study normalising the duration effect on CO2-laser evoked potentials is still lacking, a survey of the literature showed that, also with CO_2 lasers, the magnitude of this effect may not be negligible. For instance, after hand stimuli, the latency increased from 201 to 233 and to 256 ms with stimulus durations of 10, 20, and 30 ms (Kakigi et al., 1989; Kunde and Treede, 1993; Towell et al., 1996). Our data were collected with a stimulus duration intermediate between 10 and 30 ms.

Other investigators, if referring to our normative values, should always take into account the influence of their stimulus characteristics.

References

Arendt-Nielsen L, Bjerring P. Sensory and pain threshold characteristics to laser stimuli. J Neurol Neurosurg Psychiatry 1988a;51(1):35–42.

Arendt-Nielsen L, Bjerring P. Reaction times to painless and painful CO2 and argon laser stimulation. Eur J Appl Physiol Occup Physiol 1988b; 58(3):266–73.

Arendt-Nielsen L, Chen AC. Lasers and other thermal stimulators for activation of skin nociceptors in humans. Neurophysiol Clin 2003; 33(6):259–68.

Agostino R, Cruccu G, Iannetti GD, Romaniello A, Truini A, Manfredi M. Topographical distribution of pinprick and warmth threshold to CO_2 laser stimulation on the human skin. Neurosci Lett 2000a;285:115–8.

Agostino R, Cruccu G, Romaniello A, Innocenti P, Inghilleri M, Manfredi M. Dysfunction of small myelinated afferents in diabetic polyneuropathy, as assessed by laser evoked potentials. Clin Neurophysiol 2000b;111:270–6.

Bromm B. Evoked cerebral potentials and pain. In: Fields HL, editor. Advances in pain research and therapy, vol. 9. New York, NY: Thomas Springfield; 1985. p. 305–29.

Bromm B, Lorenz J. Neurophysiological evaluation of pain. Electroencephalogr Clin Neurophysiol 1998;107:227–53.

Cruccu G, Romaniello A, Amantini A, Lombardi M, Innocenti P, Manfredi M. Assessment of trigeminal small-fiber function: brain and reflex responses evoked by CO_2-laser stimulation. Muscle Nerve 1999; 22:508–16.

Cruccu G, Anand P, Attal N, Garcia-Larrea L, Haanpää M, Jørum E, Serra J, Jensen TS. EFNS guidelines on neuropathic pain assessement. Eur J Neurol 2004;11:153–62.

Deuschl G, Eisen A. Recommendations for the Practice of Clinical Neurophysiology: Guidelines of the International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol 1999;Suppl.:52.

Devos D, Creac'h C, Laureau E, Bourriez JL, Guieu JD. Thulium laser evoked potentials. Normative values for the arms and legs. Neurophysiol Clin 2000;30:313–22.

Gagliese L, Melzack R. Age differences in nociception and pain behaviours in the rat. Neurosci Biobehav Rev 2000;24:843–54.

Garcia-Larrea L, Convers P, Magnin M, Andre-Obadia N, Peyron R, Laurent B, Mauguiere F. Laser-evoked potential abnormalities in central pain patients: the influence of spontaneous and provoked pain. Brain 2002;125:2766–81.

Garcia-Larrea L, Frot M, Valeriani M. Brain generators of laser-evoked potentials: from dipoles to functional significance. Neurophysiol Clin 2003;33:279–92.

Gibson SJ, Helme RD. Age-related differences in pain perception and report. Clin Geriatr Med 2001;17:433–56.

Gibson SJ, Gorman MM, Helme RD. Assessement of pain in the elderly using event related cerebral potentials. In: Bond MR, Charlton JE, Woolf CJ, editors. Proceedings of the VIth world congress on pain, 1991. p. 527–33.

Iannetti GD, Leandri M, Truini A, Zambreanu L, Cruccu G, Tracey I. Aδ nociceptor response to laser stimuli: selective effect of stimulus duration on skin temperature, brain potentials and pain perception. in press Clin Neurophysiol 2004;115:2629–37.

Kakigi R, Shibasaki H, Ikeda A. Pain-related somatosensory evoked potentials following CO2 laser stimulation in man. Electroencephalogr Clin Neurophysiol 1989;74:139–46.

Keogh E, Herdenfeldt M. Gender, coping and the perception of pain. Pain 2002;97(3):195–201.

Kunde V, Treede RD. Topography of middle-latency somatosensory evoked potentials following painful laser stimuli and non-painful electrical stimuli. Electroencephalogr Clin Neurophysiol 1993;88(4): 280–9.

Lauria G. Innervation of the human epidermis. A historical review. Ital J Neurol Sci 1999;20:63–70.

McArthur JC, Stocks EA, Hauer P, Cornblath DR, Griffin JW. Epidermal nerve fiber density: normative reference range and diagnostic efficiency. Arch Neurol 1998;55:1513–20.

Romaniello A, Iannetti GD, Truini A, Cruccu G. Trigeminal responses to laser stimuli. Neurophysiol Clin 2003;33:315–24.

Rossi P, Morano S, Serrao M, Gabriele A, Di Mario U, Morocutti C, Pozzessere G. Pre-perceptual pain sensory responses (N1 component) in type 1 diabetes mellitus. Neuroreport 2002;13:1009–12.

Spiegel J, Hansen C, Treede RD. Laser-evoked potentials after painful hand and foot stimulation in humans: evidence for generation of the middle-latency component in the secondary somatosensory cortex. Neurosci Lett 1996;216:179–82.

Spiegel J, Hansen C, Treede RD. Clinical evaluation criteria for the assessment of impaired pain sensitivity by thulium-laser evoked potentials. Clin Neurophysiol 2000;111:725–35.

Spiegel J, Hansen C, Baumgartner U, Hopf HC, Treede RD. Sensitivity of laser-evoked potentials versus somatosensory evoked potentials in patients with multiple sclerosis. Clin Neurophysiol 2003;114: 992–1002.

Treede RD, Meyer RA, Raja SN, Campbell JN. Evidence for two different heat transduction mechanisms in nociceptive primary afferents innervating monkey skin. J Physiol 1995;483:747–58.

Truini A, Haanpaa M, Zucchi R, Galeotti F, Iannetti GD, Romaniello A, Cruccu G. Laser-evoked potentials in post-herpetic neuralgia. Clin Neurophysiol 2003;114:702–9.

Towell AD, Purves AM, Boyd SG. CO2 laser activation of nociceptive and non-nociceptive thermal afferents from hairy and glabrous skin. Pain 1996;66:79–86.

Valeriani M, Barba C, le Pera D, Restuccia D, Colicchio G, Tonali P, Gagliardo O, Treede RD. Different neuronal contribution to N20 somatosensory evoked potential and to CO2 laser evoked potentials: an intracerebral recording study. Clin Neurophysiol 2004;115(1):211–6.