Neuroscience Letters 489 (2011) 122–125

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Diurnal time course of heat pain perception in healthy humans

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ARTICLE INFO

Article history:
Received 26 October 2010
Received in revised form
29 November 2010
Accepted 30 November 2010

Keywords:
Laser-evoked potentials
Heat pain
Circadian variation
Thermal sensitivity

ABSTRACT

Rapid skin heating by infrared lasers can be used to investigate the integrity of the nociceptive system by activating A-delta and C fibers. The aim of our study was to analyze if healthy humans exhibit any clinically relevant diurnal variations in their heat pain sensitivity. Circadian A-delta fiber function was analyzed by studying N2 and P2 components of laser-evoked potentials (LEP) and pain thresholds evoked by laser stimulation of the foot every 2 h from 8 a.m. to 10 p.m. in 15 healthy subjects. Heat stimuli were generated by an infrared Tm-YAG laser and were delivered to an area of 4 cm × 4.5 cm on the dorsum of the right or left foot in 3 runs of incremental and decremental intensities. After each stimulus subjects were asked to classify the intensity of pain with a numeric rating scale (NRS). LEPs were recorded with fixed stimulus intensities that were 1.5× of the pain threshold. Data were collected with the SynAmps System (Neuroscan, El Paso, USA) and averaged across 35–40 trials. Laser-induced heat pain thresholds and circadian latencies of LEP did not significantly vary during the day. Our results correspond with previous studies that did not detect any consistent significant diurnal variations in perception of heat pain perception using contact thermodes. The intensity of pain perception did not demonstrate any correlation with mood or sleep parameters as measured with the Beck Depression Inventory (BDI), the subjective sleep scales Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS).

A variety of syndromes associated with painful dysaesthesias show diurnal variations, e.g., diabetic polyneuropathy [23], fibromyalgia [16], restless legs syndrome [2], rheumatoid arthritis [8,29]. However, only few methodical studies [19,21,27] investigated temporal variations of thermal pain perception in healthy humans and demonstrated conflicting results: Rogers and Vilkin [21] detected an increase in pain sensitivity as reflected by lower pain thresholds in the early evening when they induced pain experimentally with cutaneous electrical stimulation of the forearm of healthy volunteers. In contrast, Strian et al. [27] observed no significant circadian variations in heat pain sensitivity using contact thermodes. Different methodologies with distinct types of heat pain application may have contributed to these different outcomes. The perception of pain is likely affected by a variety of determinants, e.g. former pain-related experiences and anxiety [30], hormones [1], gender, ethnicity, and personality trait characteristics interacting with single nucleotide polymorphisms in TRPV1 and OPRD1 genes [11]. Consequently, pain perception may be influenced by variations of these determinants rather than by fluctuations that are specifically pain- or stimulus-related. Rapid skin heating by infrared lasers can be used to investigate the integrity of the nociceptive system by selectively activating small A-delta and C-fibers without simultaneous activation of mechanoreceptors [5,11,12]. Our current study aimed to assess the course of circadian heat pain perception of healthy humans by combining an objectifiable method such as LEP to assess the function of the A-delta and C fiber-mediated temperature nociceptive system with subjective pain sensations classified with a numeric rating scale (NRS) [3]. As the ability to detect noxious stimuli is an important protective function, we hypothesized that healthy individuals would not exhibit any clinically relevant diurnal variations in their heat pain sensitivity.

Fifteen healthy subjects (11 female, 4 male, mean age: 32.8 years; age range: 28–59 years) were identified based on a normal peroneal sensory and motor nerve conduction velocity and normal peroneal nerve somatosensory evoked potentials on both sides. A

Abbreviations: BDI, Beck Depression Inventory; EOG, electrooculography; ESS, Epworth Sleepiness Scale; LEP, laser-evoked potentials; NRS, numeric rating scale; PSQI, Pittsburgh Sleep Quality Index.

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doi:10.1016/j.neulet.2010.11.080

total of 70 applicants were screened to finally select 15 healthy subjects. These did not show any relevant lab chemistry abnormalities (HbA1c, vitamins B6, B12, folic acid, protein electrophoresis, aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma-glutamyltransferase [GGT], creatine kinase [CK], lactate dehydrogenase [LDH], urea, uric acid, electrolytes, blood count, iron, ferritin and thyroid stimulating hormone [TSH]). A comprehensive physical examination was conducted by a neurologist and a questionnaire was completed by each subject to exclude any relevant previous or concomitant psychiatric or neurological diseases or any condition associated with acute or chronic pain or somatosensory abnormalities, namely, mono- or polyneuropathy, myelopathy, herniated or protruded intervertebral disk, migraine, chronic or recurrent headache, transient ischemic attack, stroke, epilepsy, previous surgery of the lower limbs, dermographism, urticaria, regular consumption of alcohol or drugs, depression, psychosis or somatoform disorder. All subjects were able to understand the instructions. Furthermore, none of the subjects received regular medication except for oral contraception. All participants gave their informed consent and completed the Epworth Sleepiness Scale (ESS), Pittsburgh Sleep Quality (PSQI), and the Beck Depression Inventory (BDI). Measurements were carried out in a light- and noise protected room. Skin temperature was held constantly at 33–35 °C. The subjects sat in a reclined chair were awake, relaxed and asked to keep eyes closed. They were blinded for the order of stimuli applied. The study was performed in accordance with the Declaration of Helsinki and approved by the ethics committee of the Georg August University, Goettingen.

We assessed circadian A-delta fiber function by studying N2 and P2 components of LEPs and pain thresholds evoked by laser stimulation of the foot every 2 h from 8 a.m. to 10 p.m. in a shielded chamber. Heat stimuli were generated by an infrared Tm-YAG laser (Wavelight Laser Technologies, Erlangen, Germany, wavelength 2,01 μm, pulse duration 1 ms, spot diameter 7 mm, penetration depth of 360 μm into the skin) and were delivered to an area of 4 cm × 4.5 cm on the dorsum of the right or left foot in 3–4 runs of incremental and decremental intensities in steps of 50 mJ, starting at a minimum of 100 mJ (2.6 mJ/mm^2) and reaching a maximum of 800 mJ (20.8 mJ/mm^2). After each stimulus subjects were asked to classify their sensation with a numeric rating scale [3], ranging from 0 to 10 as expressed in centimeter units; 0 cm indicating no pain and 10 cm indicating the strongest pain theoretically achievable. Probands were blinded for the order of increasing and decreasing stimulus intensity. The interstimulus intervals were varied between 8 and 12 s, as interstimulus intervals influence LEP amplitudes [25]. LEPs were recorded with fixed stimulus intensities as described by Spiegel et al. [26], and the spot was slightly moved for each stimulus within a rectangle of 4 cm × 4.5 cm (18 cm^2). LEP was assessed by electrodes positioned at Fz, Cz, Pz, T3, T4, A1, A2, and skin impedance of the scalp was maintained below 5 kΩ. Data were collected with the SynAmps System (Neuroscan, El Paso, USA) and automatically averaged across 35–40 trials, so that N2 and P2 values represent average values of 35–40 trials for each time point. Sweeps containing artifacts, as detected by parallel EOG recording, were discarded. Goggles were worn by all participants for eye protection. Each stimulus generation and laser beam emission was accompanied by a clicking sound. To avoid any acoustic interference, a constant white noise was presented to the probands via earphones. Before application of each laser beam, baseline skin temperature was checked and maintained between 30.0 and 32.0 °C with a heat lamp, as a decreased skin temperature affects processing of laser heat stimuli [18]. Initially we aimed to study also ultralate potentials in all healthy subjects with ischemic blockade of the peroneal nerve [6]. Briefly, 1.8 pounds were hung above the ankle. As soon as probands reported a tingling sensatio (normally between 75 and 105 min after initiation of the blockade) and cold sensation disappeared, LEP were applied. The C fiber response could be unmasked, but was evocable only in a minority of the subjects, which is in accordance with the literature [29]. Therefore, we decided not to continue to study ultralate potentials.

Cutaneous heat stimuli were applied in 3 runs of incremental and decremental intensities in steps of 50 mJ, starting at a minimum of 100 mJ and reaching a maximum of 800 mJ. Laser-induced pain stimuli are sensed as dual pain sensation attributable to the two different fibers' conduction velocities. After each stimulus, subjects were asked to classify their sensation on a NRS between 1 and 10 (0 = not perceived, 1 = mild sensation of warmth, 2 = non-painful pricking or itching, 3 = definitely painful pinprick sensation) [3], NRS 3 was chosen as pain threshold value or, alternatively NRS 4, if the rating NRS 3 was not chosen by the proband. Pain threshold stimulation intensities at NRS 3 and 4 (9.8–22.1 mJ/mm^2) were within the previously published range [9,26].

Statistical analysis was performed using the SPSS17 statistical package (SPSS Inc., Chicago, IL). The chronology of laser stimulation was the same for all subjects at every time point. If the stimulation scheme is the same and time influences the pain response, then the mean values of NRS should change over time. The primary dependent measure was the NRS scoring at fixed intensities of stimulation in mJ (50–800 mJ). Data were analyzed with general linear model analyses for repeated measures ANOVA with time and intensity as within, the side of stimulation as between subjects factor, and the dependent variable subjective scoring of pain (NRS). If the Mauchly-test of sphericity was significant, a Greenhouse–Geisser correction of the *F*-values was performed. To investigate the effect of sleep questionnaire outcomes (Epworth Sleepiness Scale: ESS; Pittsburgh Sleep Quality Index: PSQI) and the Beck Depression Inventory (BDI) results on the NRS ratings, we included them separately as covariates in the model. The values of N2 and P2 were analyzed via repeated measures ANOVA with time as within, the side of stimulation as between subjects factor, and the dependent variable N2 or P2, respectively (Figs. 2 and 3).

All data were obtained from measures either at the right ($n = 7$) or left ($n = 8$) dorsum of the foot in 2-h intervals from 8 a.m. to 10 a.m.. The group of seven subjects stimulated at the dorsum of the right foot consisted of seven right-handers (male: $n = 1$, female: $n = 6$). The group of eight subjects stimulated at the dorsum of the left foot consisted of seven right-handers (male: $n = 1$, female: $n = 6$) and one left-hander (female: $n = 1$).

Laser-induced heat pain ratings of laser-evoked potentials did not vary significantly during the observational period and showed no significant side differences ($F = 1.621$; $p = 0.139$): Fig. 1 shows the diurnal course of NRS ratings in boxplots.

Circadian latencies of laser-evoked potentials did not vary significantly during the observational period (for N2: $F = 0.652$, $p = 0.71$; for P2: $F = 1.498$, $p = 0.178$). Latencies of late potentials N2 and P2 were collected for each subject in 2-h intervals and automatically averaged across 35–40 trials by the SynAmps System (Neuroscan, El Paso, USA) (Figs. 2 and 3). Means and standard deviations of N2 and P2 were similar to previously published data [7,9,26].

Probands had normal mean scores for PSQI (3.3 ± 0.5), ESS (6.8 ± 0.8), and for BDI (7.8 ± 2.0). Furthermore, analysis of sleep questionnaires obtained no significant effect of the covariates Epworth Sleepiness Scale ($p = 0.952$), Pittsburgh Sleep Quality Index ($p = 0.990$) on NRS pain ratings. Also the BDI had no significant effect on NRS pain ratings ($p = 0.924$), making a major effect of the healthy control's mood on their NRS pain ratings unlikely.

The present report analyzes electrophysiologically and psychophysically the diurnal course of LEP and laser-induced pain in healthy humans. Analysis of LEP revealed no diurnal changes in LEP latencies. In addition, analyses of pain ratings performed with NRS

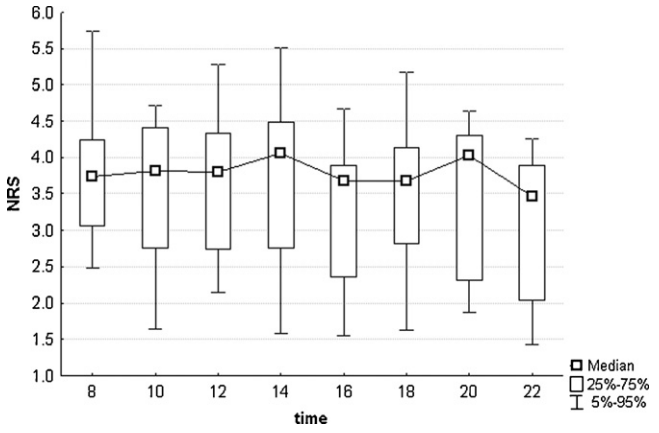

Fig. 1. Diurnal course of pain ratings on a NRS between one and ten. Heat pain ratings of laser-evoked potentials did not vary significantly during the observational period and showed no significant side differences. Data are expressed as median, quartiles and 5%-quantiles.

demonstrated no significant effect of time on the subjective pain ratings of the entire healthy humans group. Therefore, the outcome of the present study reconfirms our hypothesis that the important role of pain as a physiological protection makes significant diurnal alterations in pain perception in healthy humans unlikely. In line with this notion, we did not find any evidence pointing to pronounced subjective or objective diurnal variations. Our findings correspond with the results of Strian et al. [27], who analyzed circadian heat pain perception applied with contact thermodes to the right hand and foot in 11 healthy controls and observed a tendency to lower pain thresholds between 3.00 and 10.59 p.m., falling short of significance level for the whole group.

Furthermore, our results are in line with several studies that did not detect any significant laterality differences in heat pain sensitivity [13,22,28]. Different methodologies with distinct types of heat pain application via a stimulation electrode [21], radiant heat [19], contact thermode [27], and hot water bath [14,24] may contribute to different outcomes of previous investigations. Furthermore, pain assessments were performed at different body parts with different sensitivities. Neurophysiological studies suggested that affect

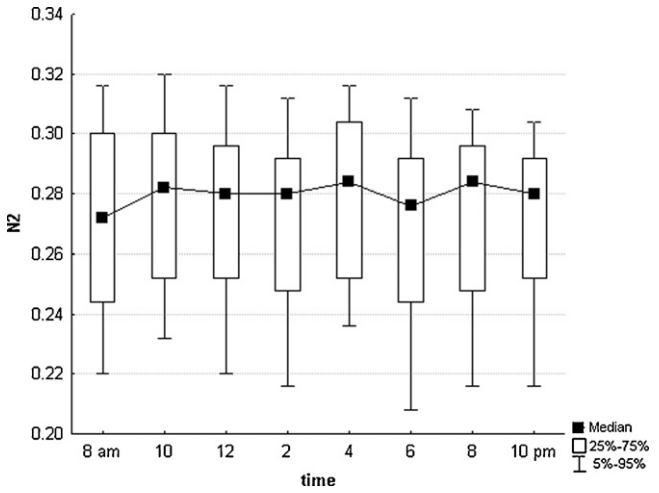

Fig. 2. Diurnal course of N2 latencies. N2 and P2 components of LEPs were evoked by laser stimulation with an infrared Tm-YAG laser of the foot every 2 h from 8 a.m. to 10 p.m. Latencies of late potentials N2 and P2 were collected for each subject in 2-h intervals and automatically averaged across 35–40 trials by the SynAmps System (Neuroscan, El Paso, USA) and did not vary significantly during the observational period. Data are shown as median, quartiles and 5%-quantiles.

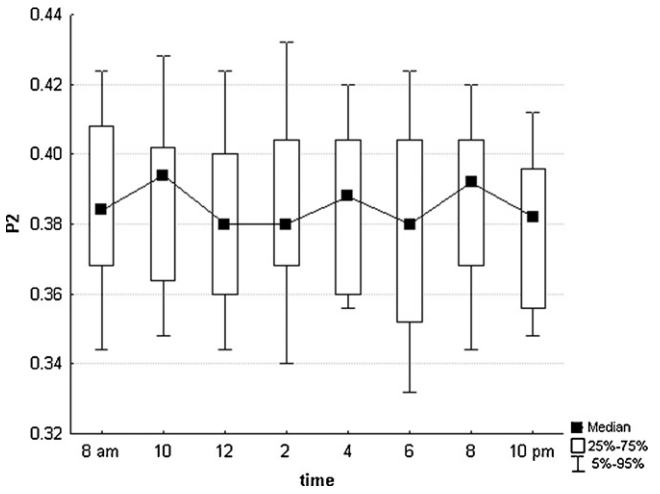

Fig. 3. Diurnal course of P2 latencies. N2 and P2 components of LEPs were evoked by laser stimulation with an infrared Tm-YAG laser of the foot every 2 h from 8 a.m. to 10 p.m. Latencies of late potentials N2 and P2 were collected for each subject in 2-h intervals and automatically averaged across 35–40 trials by the SynAmps System (Neuroscan, El Paso, USA) and did not vary significantly during the observational period. Data are shown as median, quartiles and 5%-quantiles.

can modulate pain processing [10]. In the present study, however, intensity of pain perception did not demonstrate any correlation with mood as measured with the Beck Depression Inventory. This finding is in accordance with earlier studies that did not detect any significant correlations between experimental pain measures in healthy controls and depression or mood questionnaires [4,21].

Sleep is one of the most important synchronizers of human chronobiology, and several studies have reported that particularly REM sleep deprivation may result in increased pain sensitivity [15,20]. The normal outcome PSQI and ESS questionnaires did not indicate any sleep disturbances of the probands participating in the current study. However, the use of only subjective sleep questionnaires – ESS, PSQI – instead of polysomnography to analyze particularly REM sleep, may explain that the outcomes of subjective sleep rating scales did not correlate with NRS ratings in our study.

In sum, the present study shows that healthy probands do not display significant diurnal variations in heat pain sensitivity emphasizing the important protective function of pain detection. The intensity of pain perception did not demonstrate any correlation with mood or sleep parameters as measured with the Beck Depression Inventory (BDI), the subjective sleep scales Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS). In this pilot investigation, a comparatively limited number of fifteen subjects was analyzed. Thus, larger consecutive trials should be performed to corroborate our results and analyze additional factors affecting heat pain, e.g., age or gender differences. In diseases that are associated with diurnal variations of pain sensitivity, e.g. RLS or diabetic polyneuropathy, the observed rhythms may be centrally mediated by melatonin [17].

Acknowledgements

The authors want to thank Dr. Antonia Zapf for preparing the figures and Alan Oberlin for discussion.

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