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SHORT COMMUNICATION

Trigeminal Chemosensitivity: Differences in Relation to the Time of the Day

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Abstract

Day–night differences of trigeminal chemosensitivity were investigated in 18 healthy volunteers employing both pain-related cortical potentials and pain ratings in response to stimulation of the nasal mucosa with CO_2. Day–night differences were found with N1P2 amplitudes, P2 latencies and pain ratings. It is concluded that the time of the day must not be ignored when human chemosensitivity is investigated at suprathreshold levels.

Introduction

Numerous studies in animals and humans have reported circadian changes of pain sensitivity. However, no consistent picture of diurnal variation of pain has emerged. For example, in animals the acrophase of the circadian cycle has been observed by some at night (Kavaliers and Hirst, 1983; Pickard, 1987) and by others at day (Frederickson et al., 1977; Kavaliers and Ossenkopp, 1988), but these differences cannot always be explained by the sleep–wake cycle of the animals. Similarly, results of human studies regarding the circadian rhythm of nociceptive changes are far from being unequivocal. Pain sensitivity has been reported to be highest in the early morning (Hildebrandt et al., 1982; Pownall et al., 1986; Sandrini et al., 1986; Gobel and Cordes, 1990) or in the afternoon (Davis et al., 1978), and there are studies which did not find a circadian rhythm at all (Domzal et al., 1983; Strian et al., 1989). The outcome of these investigations appears to be strongly dependent on both the characteristics of the painful stimuli (e.g. thermal, chemical, electrical, mechanical) and the specific experimental design. As intranasal trigeminal perception is an integral part of the nociceptive system, possible chronobiologic changes also apply to nasal trigeminal sensitivity.

We recently reported the absence of a circadian rhythm for psychophysical measures of trigeminal sensitivity at threshold level (Lötsch et al., 1997). The present study aimed to complete the former investigation by employing pain-related evoked cerebral potentials as a non-verbal electrophysiological measure of trigeminal sensitivity. Nasal

nociceptors were specifically stimulated with short pulses of gaseous CO_2 (Kobal and Hummel, 1988; Steen et al., 1990) at concentrations above threshold. The usefulness of the thus evoked cortical potentials as a specific nociceptive measure is emphasized by the localization of their generators in the somatosensory area S_{II} (Huttunen et al., 1986; Hari et al., 1997), which is assumed to be a primary projection area for nociceptive afferents (Chudler et al., 1985).

Methods and results

Eighteen healthy volunteers (eight males, ten females; mean age 26.5 years) participated in the study. The study was conducted in accordance with the Declaration of Helsinki (Tokyo Amendment 1989). All participants gave written consent after having been informed in detail about the purpose and enrollment of the study. None of them had a history of nasal/sinus disease or extensive exposure to chemicals with potential olfactory or trigeminal toxicity. Subjects were in excellent health as ascertained by clinical examination.

The experiments started at either 7:30 or 19:30 h. The interval between two experiments in the same subject was at least 3 days. Each experiment was divided into five sessions, which took place either at 07:30, 09:00, 10:00, 12:00 and 14:00 h (day experiments) or at 19:30, 21:00, 22:00, 24:00 and 02:00 h (night experiments). In a randomized order, half of the subjects started with the day experiments, the other

half with the night experiments. Trigeminal stimulation was produced by short pulses of gaseous CO_2 embedded in a constantly flowing airstream (8 l/min) with controlled temperature and humidity (36.5°C, 80% relative humidity) applied to the mucosa of the left nostril (stimulus duration 200 ms, stimulus rise-time <20 ms, interstimulus interval ~30 s). During each session a total of 40 stimuli of three concentrations (eight stimuli of 55% v/v CO_2, 16 of 60% v/v CO_2 and 16 of 65% v/v CO_2) were applied in a randomized sequence. The differences of the three concentrations were above the just noticeable differences for CO_2 stimuli of 60% v/v at 200 ms (3.4 ± 0.8% v/v CO_2; unpublished observation), and the interval was long enough to keep habituation/adaptation low (Hummel et al., 1994). Only responses to the two stronger stimuli were analyzed. The lowest concentration was used to span a wide range of possible sensations, thereby making it more difficult for the subjects to predict the stimulus intensity. The EEG was recorded from five positions of the international 10/20 system (Cz, C3, C4, Fz and Pz; see also Figure 1) referenced to linked earlobes (A1+A2). Possible blink artifacts were monitored from an additional site (Fp2/A1+A2). Stimulus-linked EEG segments of 2048 ms duration were sampled with a frequency of 250 Hz (band pass 0.2–70 Hz, prestimulus period 512 ms). Trigeminal event-related cortical potentials were obtained by averaging the digitized EEG records separately for each CO_2 concentration, recording both the position and the session. Records contaminated by eye-blinks (>40 µV in the Fp2 lead) were excluded from this process. Subsequently, base-to-peak amplitudes P1, N1, P2, their latencies and peak-to-peak amplitudes P1N1 and N1P2 of the trigeminal event related potentials (ERPs) were analyzed.

Within 3–4 s after presentation of each CO_2 stimulus, subjects estimated its pain intensity relative to a standard (60% v/v CO_2) which had been presented at the beginning of the first session of each experiment. The ratings were performed by means of a continuous visual analogue scale displayed on a computer monitor [horizontal bar without numbers; length changed by means of a joystick; intensity of the standard was defined as 100 estimation units (EU); see also Figure 2]. For statistical analysis, single ratings were averaged separately for each CO_2 concentration and session. Additionally, subjects rated their tiredness after each session by means of a 100 mm visual analogue scale ranging from 'very alert' to 'very sleepy'.

Statistical evaluation (SPSS® release 7.5.2 for Windows™) consisted of multivariate analyses of variance for repeated measures, carried out separately for each parameter, and, in the case of the ERP data, EEG recording position [within-subject factors 'time of day' (i.e. 'day-time' or 'night-time'; df = 1), 'session' (i.e. the five sessions of each experiment, df = 4)] and 'intensity' (i.e. 60 or 65% v/v CO_2; df = 1); between-subject factor 'gender' (df = 1); α-level = 0.05]. Degrees of freedom were adjusted with the Greenhouse–
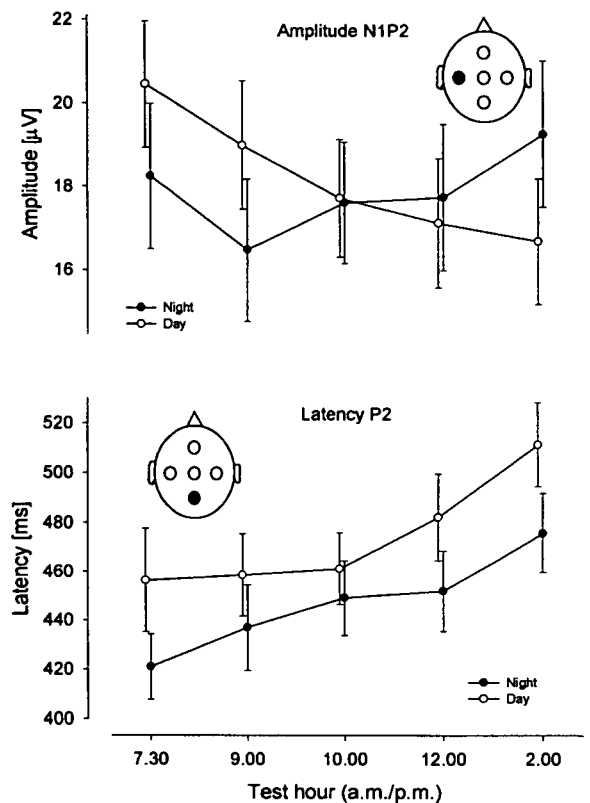

Figure 1 Time course of the ERP amplitude P2 and its latency in response to the 60% v/v CO_2 stimuli. For the amplitude N1P2 at recording position C3, there was a significant interaction between 'time of day' and 'session', and for latency P2 at recording position Pz a significant main effect of the 'time of day' was found. Open symbols show results for measurements taken during the day; closed symbols indicate those taken at night (means, standard error).

Geisser epsilon where indicated. Bonferroni t-tests were used for post-hoc comparisons of statistical main effects. Multivariate post-hoc comparisons of sequence effects between the time of day were performed by calculating within-subject contrasts on 'daytime', 'session' and 'daytime by session' ['simple contrasts' as implemented in SPSS; df = 1,16; for statistical methodology see also (Sharma, 1996)].

Both the intensity of the CO_2 stimuli and the session number had significant influences on almost all pain-related parameters. As expected, the higher CO_2 concentration produced significantly stronger pain, higher ERP amplitudes and shorter ERP latencies. During the daytime, the ERP amplitudes decreased continuously (Figure 1). In contrast, amplitudes at night increased starting from session 2 (21:00 h) following an initial decrease from session 1 to session 2 (Figure 1). A significant main effect of the factor 'time of day' was observed for the latency of P2 at recording position Pz, which was longer during the day than at night ($F = 4.75$, $P < 0.05$, observed power = 0.54; t-test, $P < 0.05$; confidence interval for differences between day and night, 0.6–46.8 ms). Additionally, an interaction between the factors 'time of day' and 'session' in the ANOVA indicated
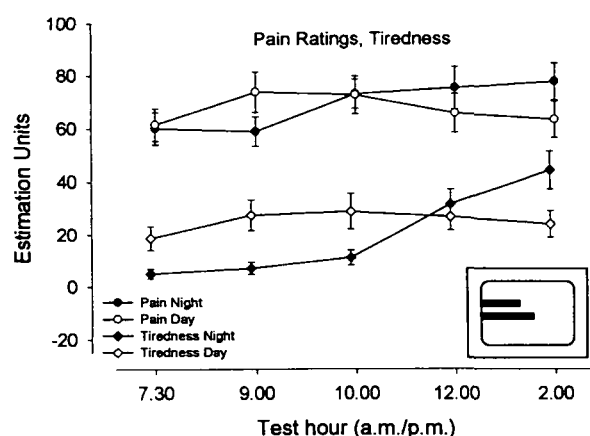
Figure 2 Pain ratings and estimates of tiredness during the day (open symbols) and at night (closed symbols). A significant interaction between the factors 'time of day' and 'session' was observed for both parameters. The inset shows the computer monitor with the visual analogue scale used for pain intensity ratings. The black horizontal bar represented the intensity of the standard stimulus. Subjects encoded the intensity of the actual stimulus in relation to the standard by changing the length of the gray horizontal bar.

the statistical significance of sequence effects for the amplitude N1P2 at recording position C3 ipsilateral to the stimulated nostril ($F = 3.24$, $P < 0.05$, observed power = 0.8). Within-subject contrasts specified that differences were most pronounced between sessions early in the afternoon and late at night, i.e. between the sessions at 14:00 and 02:00 h (C3: $F = 6.02$, $P < 0.05$, observed test power = 0.64).

Similar to ERP amplitudes, pain ratings exhibited a different time-course at day than at night. During the daytime pain ratings decreased from session 2 after an initial increase from session 1 to session 2. In contrast, they continuously increased during the night (Figure 2). The significance of this observation was indicated by an interaction between factors 'daytime' and 'session' in the ANOVA ($F = 3.7$, $P < 0.01$, observed test power = 0.86). As with ERP amplitudes, within-subjects contrasts identified the last sessions as being the most different, i.e. the sessions at 14:00 and 02:00 h ($F = 12.7$, $P < 0.01$, observed test power = 0.92; differences to session 1: $F = 9.9$, $P < 0.01$, observed test power = 0.84). In addition, a significant between-subjects effect of 'gender' was observed ($F = 10.58$, $P < 0.01$). Specifically, men tended to rate the pain intensity lower than women.

The time-course of tiredness was similar to that observed for the pain ratings (Figure 2), though they did not have a statistically significant correlation with each other. Significant effects of 'tiredness' consisted of an interaction between 'time of day' and 'session' ($F = 10.45$, $P < 0.05$). Within-subjects contrasts revealed significant differences between sessions at 14:00 and 02:00 h ($F = 18.05$, $P = 0.01$).

Discussion

Significant differences between day and night were observed for the late peaks of the evoked potentials. The shorter latency of P2 at night than during the day indicates a different, perhaps faster cerebral processing of nociceptive information at night. This conclusion is based on the assumption that late components of evoked potentials relate to cognitive processing, while earlier components reflect the characteristics of the peripheral input. This had been demonstrated for evoked potentials after painful stimulation of the tooth-pulp: earlier components of evoked potentials correlated with the physical stimulus intensity, while later components were related to the estimates of pain intensity (Chen *et al.*, 1979). Similarly, after CO_2–laser stimulation the amplitude P2 was reported to be most likely associated with pain-related cognitive function (Miyazaki *et al.*, 1994).

The statistical interactions between 'daytime' and 'session' found for both the ERP amplitude N1 and intensity estimates indicate that measurements at day or night were subject to different sequence effects. Since many study designs involve the investigation of pre- and post-treatment effects, these interactions are important. In practice this means that scheduling experiments should account for possible effects of the time of day. Hence, to avoid confounding of the results by diurnal effects, in a repeated measures design subjects should always be tested at the same time of day, unless circadian variations are the subject of research.

According to the design adopted from investigations of analgesic drug effects, subjects rated the pain intensity of the CO_2 stimuli in relation to a standard that was presented at the beginning of each experiment, i.e. once in the morning and once in the evening. The agreement between initial pain ratings during the day and at night (Figure 2) indicates that the subjects were able to relate the actually perceived pain to the standard stimulus, which was given at the beginning of the respective experiment. However, by relating the pain ratings to the respective standard, the experimental design is likely to have masked day–night differences of subjective pain perception in terms of absolute sensitivity. In contrast, our data clearly allow for a comparison of sequence effects between day and night.

The present findings contrast to our recent report of a circadian stability of trigeminal pain thresholds (Lötsch *et al.*, 1997). This might reflect a different information processing at threshold and at stimulus levels above threshold (Eccles *et al.*, 1989). However, the present findings of day–night differences in pain are not surprising considering the large body of literature in this field. As already stated above, the remarkable inconsistency in the literature, with even contradictory results depending on the kind of pain investigated or other specific experimental conditions, has made the present investigation necessary in terms of

trigeminal chemosensitivity. However, the present results represent the end-point of pain perception and processing.

There may be a multiplicity of underlying mechanisms. Using the present data, however, it is impossible to know whether portions of the effects are due to changes at receptor level or to changes at higher levels of nociceptive processing. Many of the physiological systems involved in perception and processing of pain undergo circadian rhythms. For nasal chemosensitivity, circadian processes start at a peripheral level, namely at the nasal engorgement, which undergoes a time-dependent rhythm, the nasal cycle (Krauchi and Wirz-Justice, 1994). Other factors with circadian rhythms potentially affecting local conditions of the nasal mucosa are temperature or the perception of blood circulation (Refinetti and Menaker, 1992; Marinelli *et al.*, 1994). Signal transmission and processing of trigeminal stimuli involves a large number of systems which are subject to circadian rhythms, e.g. the serotonergic, adrenocorticoid and opioidergic systems (Schlosberg and Harvey, 1978; Domzal *et al.*, 1983; Meyerson *et al.*, 1989). For example, an acrophase of serotonin levels at 14:00 h and decreasing levels during the night were observed (Tenen, 1968). When considering reports of a functional antagonism of morphine analgesia by serotonin antagonists (Mozzanica *et al.*, 1991), lower serotonin levels at night may relate to our observation of higher pain sensitivity at that time. Moreover, considering reports that the highest beta-endorphin levels are found in the morning and the lowest levels at midnight, our results are clearly in agreement with circadian rhythms of endogenous opioids (Farsang *et al.*, 1983).

Owing to the design of the study, a causal relationship between the present results and circadian rhythms must remain hypothetical. Therefore, having established day–night differences of trigeminal chemosensitivity, investigations of the underlying mechanisms have to follow. However, the present study clearly indicates the significance of the time of day regarding the perception of trigeminal stimuli. Since most odorants activate both the trigeminal and the olfactory system, circadian changes in trigeminal nasal chemosensitivity can also be assumed to directly affect the perception of odorants.

To summarize, the present data indicate day–night differences in the perception of suprathreshold trigeminal stimuli of the nasal mucosa. Similar differences were observed for both electrophysiological and psychophysical measures. It is concluded that day–night differences in pain processing are of critical importance when planning studies that employ chemical stimulation at levels above threshold.

Acknowledgements

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