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SHORT COMMUNICATION

Chronobiology of Nasal Chemosensitivity: Do Odor or Trigeminal Pain Thresholds Follow a Circadian Rhythm?

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Abstract

Odor and trigeminal pain thresholds were studied four times each at 24:00, 04:00, 08:00, 12:00, 16:00 and 20:00 h in randomized order on different days in five healthy male volunteers. No circadian rhythm of olfactory or trigeminal thresholds were observed. However, the variability of odor, but not pain thresholds, increased from 04:00 h (thresholds between 0.4 and 1.2 p.p.m.) to 16:00 h (thresholds between 0.1 and 2 p.p.m.). It is hypothesized that environmental influences contribute to this increase in variance. **Chem. Senses 22: 593–598, 1997.**

Introduction

Chronobiological rhythms have been described for many biological systems. Little is known, however, about the chronobiology of nasal chemosensitivity. Two physiological systems are involved in the perception of chemicals: the trigeminal system that mediates pain and the olfactory system that mediates smell. Thus, a circadian rhythm of nasal chemosensitivity can be present in one or both systems and should therefore preferably be assessed separately for each system. From those few studies that have investigated chronobiological aspects of nasal chemosensitivity, conclusions about diurnal variations can only be drawn indirectly, because chemosensory function was studied together with other, potentially confounding variables. For example, a general circadian rhythm of olfactory function cannot be definitely concluded from a study which assessed food effects on olfactory sensitivity throughout the day, reporting diurnal variations in some individual subjects (Koelega, 1994). The same applies to an investigation that could correlate the variation of odor thresholds to the nasal cycle, an ultradian side-to-side rhythm of nasal engorgement

(Doty and Frye, 1991). Similarly, it is known from an investigation of the analgesic effects of opioids on trigeminal pain produced by stimulation of the nasal mucosa with CO_2 that analgesic effects differ in relation to the hour of administration (Hummel *et al.*, 1994), and trigeminal sensitivity tends to be higher at night (Lötsch *et al.*, 1992). Applicability of these findings to the chronobiology of nasal chemosensitivity, however, is limited because (i) that study was focused on analgesic drug effects and not on circadian rhythms of sensitivity to pain itself; and (ii) only two periods of the day—morning and evening—were investigated. In addition, the existence of chronobiological rhythms in the trigeminal system cannot be postulated on the basis of observations of diurnal changes in pain sensitivity induced in other body parts. The data reported so far have failed to produce a consistent picture. The individual findings appear to be closely dependent on the characteristics of the painful stimuli (electrical pain stimuli, thermal pain stimuli, clinical pain etc.) and the experimental conditions (Hildebrandt *et al.*, 1982; Strian *et al.*, 1989; Procacci, 1993). This makes it difficult to apply these findings to nasal chemosensitivity. For example, in animal studies the acrophasis of the circadian cycle of sensitivity to pain has been observed by some investigators at night-time (Kavaliers and Hirst, 1983) and by others at daytime (Frederickson *et al.*, 1977). Similarly, in man sensitivity to pain has been reported to be highest either in the early morning (Hildebrandt *et al.*, 1982) or in the afternoon (Davis *et al.*, 1978). Other investigators found the interindividual differences in the temporal pattern of sensitivity to pain too large to allow for a conclusion regarding a circadian rhythm (Strian *et al.*, 1989).

Considering the specific situation of intranasal chemoreception, the present study aimed to examine diurnal variations in both the trigeminal and olfactory systems. For specific stimulation of the trigeminal system CO_2 was used that produced a clear sensation of stinging pain. In contrast, H_2S was used for olfactory stimulation. From other investigations we know that H_2S is a specific olfactory stimulant that cannot be perceived by anosmics (Hummel *et al.*, 1991; Kobal and Hummel, 1991). Circadian variations of smell and trigeminal pain were investigated by measuring individual thresholds in healthy young male adults. The design of the study was based on the assumption that (i) only rhythms of 24 h or integer fractions of 24 would be practically relevant. All other rhythms would appear like unsystematic fluctuations in the normal 24 h rhythm of

Table 1 Randomization of the determinations of thresholds among subjects (1–5), nostrils (L, left; R, right) and test hour (00:00, 04:00, 08:00, 12:00, 16:00 and 20:00)

Subject	Nostril	Test hour					
		00:00	04:00	08:00	12:00	16:00	20:00
1	L	8,23	10,22	2,19	5,11	1,13	4,9
	R	14,16	12,24	17,21	6,15	3,20	7,18
2	L	3,8	10,23	7,9	12,15	2,5	4,6
	R	14,17	13,18	11,16	19,20	21,24	1,22
3	L	9,12	1,3	7,18	4,10	2,8	5,13
	R	15,22	6,17	23,24	11,21	16,20	14,19
4	L	1,15	5,7	2,6	10,16	3,12	4,8
	R	19,22	11,18	9,14	17,23	21,24	13,20
5	L	5,17	10,11	1,4	2,6	8,9	3,12
	R	18,23	15,22	13,14	7,19	16,24	20,21

Each subject took part in 24 measurements (numbers 1–24). To avoid sequence effects, the 24 tests were randomized. The minimum intersession interval was 18 h except for the 24:00 and 04:00 sessions, which required a 36 h intersession interval to ensure return to normal diurnal rhythm. Using a balanced, pseudo-randomized design, each of the subject's nostrils was tested twice at each of the six test hours, starting either with measurements of the odor threshold or with measurements of the pain threshold.

daily life. Furthermore, (ii) a practically relevant circadian rhythm should be evident in measurements taken at random on different days, excluding sequence effects (e.g. effects of habituation or adaptation) between measurements taken subsequently on the same day. Therefore, measurements were taken in a randomized order. Instead of collecting single measurements from a relatively large number of subjects, we preferred to collect a large number of measurements from a relatively small sample of participants. Thus, the study was designed to obtain data distributed across the subjects' range of normal variation in pain and odor thresholds.

Methods, results and conclusions

Pain and odor thresholds were measured twice each per test hour and nostril at 24:00, 04:00, 08:00, 12:00, 16:00 and 20:00 h in a randomized order (Table 1) on different days. Each of the five healthy male volunteers aged between 23 and 34 years (mean = 27.2) took part in 24 sessions with a minimum intersession interval of 18 h. The study was conducted in accordance with the Declaration of Helsinki (Tokyo Amendment 1989). All subjects reported normal smell and taste sensitivity, and none of them had a history

of nasal/sinus disease or extensive exposure to chemicals with potential olfactory or trigeminal toxicity. The subjects were in excellent health as ascertained by clinical examination. Normal olfactory function was ensured by applying a validated olfactory test (Hummel *et al.*, 1997). Using a balanced, randomized design, each of the subject's nostrils was tested twice at each of the six test hours, starting either with measurements of the odor threshold or with measurements of the pain threshold. An individual session lasted for ~45 min.

Pain and odor thresholds were determined by employing 12 concentrations of CO_2 (30–60% v/v; 0.027 log steps) and 12 concentrations of H_2S (0.1–2 p.p.m.; 0.018 log steps) respectively. Stimuli (duration 200 ms) were applied by means of a dynamic olfactometer that allowed for accurate control of the concentrations of the stimuli and excluded concomitant alteration of mechanical or thermal conditions at the mucosa (Kobal, 1981). Pain threshold was determined after subjects had been instructed (stressing the word 'pain') to distinguish between non-noxious sensations (pre-pain sensations) and painful sensations evoked by the CO_2 stimuli. The threshold was determined using a forced-choice, single-staircase method. When a non-painful sensation was reported a higher concentration was subsequently used, whereas two consecutive reports of painful sensations prompted stimulation at a lower concentration. The test was terminated after seven reversals in the staircase procedure had been obtained. The threshold used for further analyses constituted the geometric mean of the concentrations of the last four reversals. To keep effects of adaptation/habituation small, the interstimulus interval (ISI) was 30 s. Odor thresholds were determined similar to pain thresholds as described above, except for triplets of stimuli being presented at each trial instead of a single stimulus. Each triplet was composed of one odorous stimulus and two blanks presented in a randomized order. Subjects were instructed to report which stimulus in the triplet evoked an odor sensation. The ISI between stimuli of the triplet was 3 s and the interval between triplets was 30 s.

Tiredness (continuous visual-analogue scale, length 100 mm, ranging from 'not tired' to 'very tired'), nasal patency [acoustic rhinometry, measured using a Rhinoklack RK 1000 (Stimotron, Wendelstein, Germany)], body temperature, heart rate and blood pressure were analyzed before and after assessment of the thresholds.

Data were analyzed in three steps using SPSS computer programs (version 7.0 for Windows; α-level 0.05).

Step 1: Intraindividual uniformity in the temporal pattern of the thresholds was assessed by calculating the Pearson coefficients of correlation between the two repeated determinations of individual thresholds per nostril and test hour (five individual correlations). The individual coefficients of correlation (r) were normalized with Fisher's Z-transformation. The resulting Z-values were averaged (separately for each stimulus duration) and then re-transformed into correlation coefficients by solving the Fisher's Z-transformation for r. The influence of nasal patency, body temperature and tiredness was estimated by means of partial correlations.

Step 2: Interindividual consistency of diurnal variation was assessed with the Friedman test applied to the pooled values from all subjects and test repetitions to check for differences between test hours.

Step 3: Differences of threshold variance between test hours were assessed by means of F-tests applied to the pooled values from all subjects and test repetitions.

Neither absolute values of pain thresholds nor those of odor thresholds showed a circadian rhythm (Figure 1). The tests for intraindividual uniformity in the temporal pattern of the thresholds failed to reach statistical significance (mean coefficients of correlation $r = 0.08$ and 0.24 for pain and odor thresholds respectively, $P > 0.05$, $n = 5$). Partial correlations controlling for tiredness, nasal patency and body temperature increased the coefficient of correlation to 0.39 for pain thresholds and to 0.65 for odor thresholds, indicating the influence of these physiological parameters on nasal chemosensory thresholds. There was no interindividual consistency of diurnal variation of the thresholds (Friedman test not significant). Thresholds varied unsystematically, regardless of the hour of determination.

In contrast, differences between test hours were observed in the variability of odor thresholds. To be specific, variability was smallest at 04:00 h, when thresholds ranged from 0.4 to 1.2 p.p.m. It increased continuously until 16:00 h, when odor thresholds ranged from 0.1 to 2 p.p.m. (F-test, $P < 0.05$; Figure 1). Other than odor thresholds, variance of trigeminal pain thresholds remained stable throughout the test hours (F-test not significant).

These results indicate that circadian rhythms play a minor role in nasal chemosensitivity. As the study was based on a carefully performed randomization of variables, influences between single measurements, especially effects of
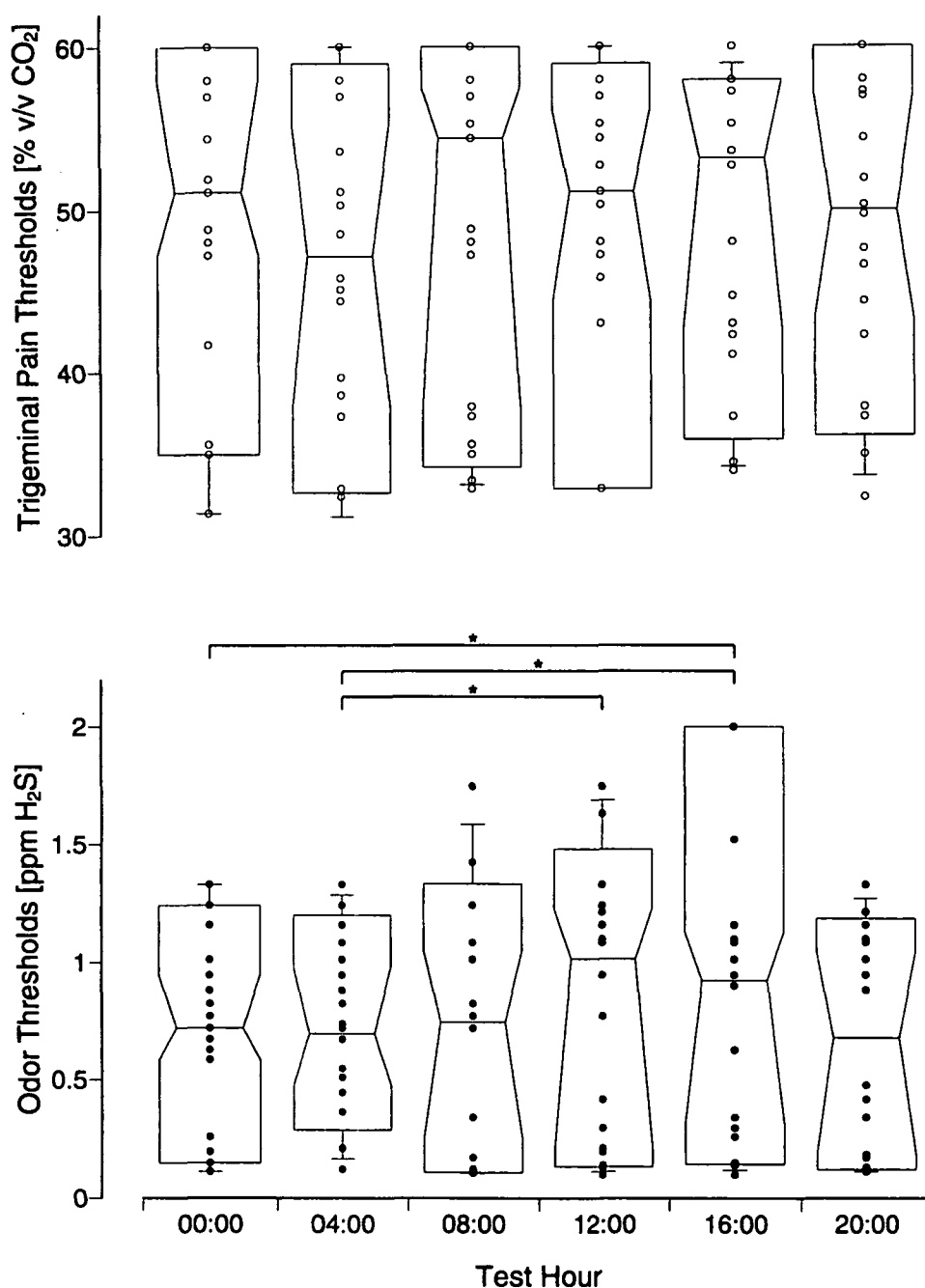
Figure 1 Individual thresholds of trigeminal pain (upper panel) and odor (lower panel). While the variance of pain thresholds remained stable over the test hours, the variance of odor thresholds was smallest at 04:00 h and largest at 12:00 and 16:00 h (*significant differences between variances of pooled thresholds from all subjects and test repetitions at different test hours). Box-whisker chart: sample data are marked with either open (trigeminal pain) or closed (odor) circles. Boxes on each side of the notched area include data from the 10th to the 25th and the 75th to the 90th percentiles. Whiskers show data from each box end to the 5th and 95th percentiles respectively. The horizontal lines at the notches represent the median of the data.

habituation, adaptation and fatigue between succeeding measurements, were minimal. However, considering the long and uneven intervals between measurements, it seems unlikely that all these variables had remained stable over a longer period, and the uncontrolled influence of interfering variables may have increased. In fact, two of those variables

could be identified, namely nasal patency and body temperature. The observation of an influence of nasal patency on odor and pain thresholds is in line with previous reports of changes in olfactory thresholds with nasal engorgement (Doty and Frye, 1991). Regarding the influence of body temperature on odor thresholds, no such

clear-cut explanation is readily available. Possible explanations might be found in changes of local conditions at the mucosa. According to Lundqvist *et al.* (1993), increasing blood-flow might have stimulated nasal secretion, which in turn might have changed the diffusion time of odorant molecules. However, the exact mechanism of the relation between body temperature and olfactory function remains unclear.

Circadian changes could only be detected in the variance of odor, but not trigeminal pain thresholds. The variance of odor thresholds increased from early morning to afternoon. It may be speculated that the increase in variance over the day was produced by environmental factors which might have influenced the sense of smell. At 04:00 h, before the start of daily activities, subjects had experienced only a few environmental influences. In contrast, at 16:00 h the subjects' sense of smell might have been modified by various environmental factors encountered during their daily activities, resulting in this afternoon peak of variance of

odor thresholds. This interpretation is also supported by previous reports of an influence of environmental effects on the sense of smell (Berglund *et al.*, 1992; Cometto Muniz and Cain, 1992; Mergler and Beauvais, 1992; Schwartz *et al.*, 1989) and encourages further research in this area. Alternatively, in the afternoon subjects might have been distracted by their daily activities to a greater degree than in the morning and might have paid less attention to the presentation of the odor stimuli, which, in turn, might have produced more errors, resulting in an increase in variance. In contrast, the painful trigeminal sensations might be more easily detected, leading to a greater stability of the data.

To summarize, neither intranasal trigeminal pain thresholds nor odor thresholds seem to follow a circadian rhythm. However, variation of odor thresholds was shown to be lowest in the early morning and highest in the afternoon. It is hypothesized that environmental influences contribute to this increase in variance.

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