Short Communications

Is there directional smelling?

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Summary. The aim of the present study was to establish the crucial precondition for directional smelling, i.e. the ability of humans to discriminate between odorous stimuli perceived either from the right or from the left side. When the 'pure' odorants hydrogen sulphide or vanillin were used as stimulants localization was random. On the other hand stimulation with carbon dioxide or menthol yielded identification rates of more than 96%. These results established the fact that directional orientation, considering single momentary odorous sensations, can only be assumed, when the olfactory stimulants simultaneously excite the trigeminal somatosensory system.
Key words. Nose; olfaction; directional smelling; orientation; dichotic stimulation.

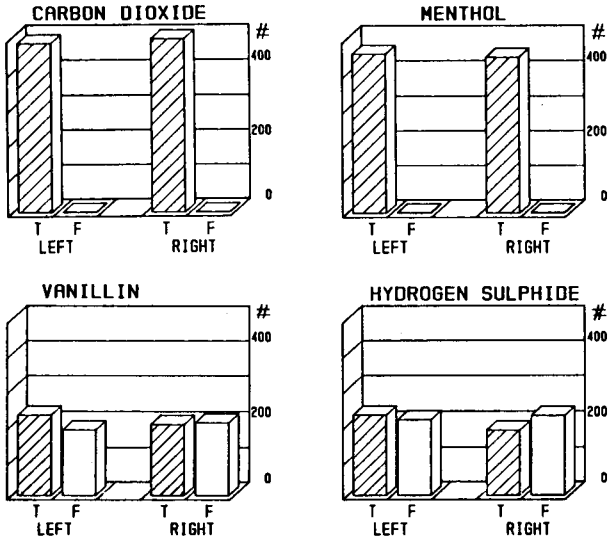
A sound source can be localized, because the emitted pressure waves reach each ear at a slightly different phase and/or with a slight difference in intensity[1-3]. One might correspondingly assume that a source of a smell could also be localized by differences in time or intensity with which the odorants reach the nostrils. Based upon this assumption v. Békésy[4] conducted an experiment in which odorants were delivered through holes in a small hollow ball. He reported that subjects were able to accurately identify positions of the odorant sources, even when they differed by only 7–10 degrees. Moreover, temporal differences of about 0.1 ms and variations in intensity of 10% of birhinally presented odorous stimuli were sufficient to create the impression that the source of the smell was located laterally to the medial plane. The various substances (benzol, eucalyptus, cloves, and lavender), which he had selected assuming them to be relatively pure odorants, were all locatable to a similar extent. This finding led v. Békésy to state that the human sense of smell possessed an equivalent to the phenomenon of directional hearing.

Contradictory to this assumption, both older and more recent publications denied odour localization mediated by the olfactory nerves. v. Skramlik[5] stated that substances, which only excite the olfactory nerve, could not be localized. Among 200 tested substances he found 50 which did not cause any 'side effects' like pain, temperature or pressure sensations (e.g. limonene, phenylethyl alcohol, vanillin, scatole, etc.). On the other hand he pointed out that it was possible to distinguish between right and left side when the substances additionally or mainly excited the trigeminal nerve, i.e. if they elicited side effects such as pain, cooling/warming, or pressure (e.g. nicotine, menthone, acetic acid, etc.). Schneider und Schmidt[6] obtained similar results. In their experiments they used coffee odour, which they regarded as being a relatively pure olfactory stimulant, as well as N-butane and ammonia, the first of which they assumed to be a moderate and the second to be a strong trigeminal stimulant. Finally, during studies reported by Van Toller and his collaborators[7], it was found that under a variety of experimental situations, subjects were only able to guess at chance levels as to which side odour strips were being presented. This finding held for both olfactory and trigeminal stimulants[8].

Material and methods. The aim of the present study was to resolve these contradictions, and to establish the crucial preconditions for neural processing of localizable olfactory information, i.e. the ability of humans to discriminate between odorous stimuli presented either to the right or to the left nostril. Since it was our opinion that the discrepancies in the previously reported findings were attributable to the manner of odorant presentation, we decided to utilize a stimulation device, by the aid of which we were able to separately present odours to each nostril in a random sequence. Subjects did not receive any additional cues that might have enabled them to guess the correct side or the onset of stimulation, i.e. the chemical stimulants were delivered without altering the mechanical or thermal conditions at the stimulated mucosa[9-12]. This was achieved by mixing pulses of the stimulants in a constantly flowing air stream with controlled temperature (36.5 °C) and humidity (80% relative humidity). Using two identical stimulators, the air stream was led into both nasal cavities by way of teflon tubing (6 cm length, 4 mm outer diameter). The total flow rate was 140 ml/s. Stimulus duration was 200 ms with a rise time below 20 ms. To avoid respiratory flow of air in the nose, subjects were trained to practise velopharyngeal closure, breathing through the mouth[10,11]. Stimuli were applied non-synchronously to breathing. The subjects were comfortably seated in an acoustically and odour-shielded chamber. White noise, of approximately 50 dB SPL, was used to mask any switching clicks of the stimulator which might have been audible.

The four substances carbon dioxide (52% v/v), menthol (21.07 ppm), hydrogen sulphide (2.06 ppm), and vanillin (0.78 ppm) were tested on eight different occasions. Our presupposition was to treat carbon dioxide as a stimulant that almost exclusively excites the trigeminal nerve[13], whereas vanillin was considered to be a pure odorant[11,14]. Menthol is generally considered to be a stimulant having mixed characteristics exciting both the olfactory and the trigeminal nerves. Encouraged by reports subjects had made in earlier studies[15], we assumed that hydrogen sulphide and vanillin were both purely olfactory stimulants. In concentrations that were used during these experiments, they were found to be imperceptible for 20 anosmics investigated in our laboratory, while the stimulants carbon dioxide and menthol were always detected. During each experimental session, which took place at always the same time of day, one of the four compounds was intranasally presented in a random sequence to both sides about 40 times. The interstimulus interval was approximately 40 s. In the second, repetitive session using the same substance, the outlets of the stimulators were swapped.

The chamber was equipped with a video monitor and a joystick. Using these, subjects were instructed to indicate after the application of each stimulus: 1. On which side had the stimulus been perceived? 2. What was the intensity of the perceived stimulus in relation to a standard stimulus given at the start of the experimental session? – Estimates of intensity were made by using a visual analogue rating scale[11]. 3. After stimulation with hydrogen sulphide the subjects were additionally asked to indicate, by once more using a visual analogue rating scale, how certain they were that they had made the correct decision in locating the stimulus.



Correct (*True*) and incorrect (*False*) localization of stimulated nostril. Four different chemical stimulants (carbon dioxide, menthol, vanillin, and hydrogen sulphide) were used. Left: stimulation of the left nostril; Right: stimulation of the right nostril. While subjects virtually never made a mistake in identifying the correct side of stimulation for the trigeminal stimulants carbon dioxide and menthol, their performance was at chance levels for the odorants vanillin and hydrogen sulphide.

Table 1. Correct and incorrect localizations

Stimulated side		Left		Right			
localization		T	F	T	F	ND	O
Carbon dioxide	N	485	1	498	1	2	31
	%	48	–	49	–	–	3
Menthol	N	457	2	447	7	1	14
	%	49	–	48	–	–	2
Hydrogen sulphide	N	228	215	186	226	8	68
	%	24	23	20	24	1	7
Vanillin	N	228	187	202	207	55	37
	%	25	20	22	23	6	4

N, Number of estimates; T, True, correct localization; F, False, incorrect localization; ND, No decision; O, not perceived; %, percentages are rounded.

Table 2. Intensity estimates

Stimulated side		Left		Right	
localization		T	F	T	F
Carbon dioxide	m	81	5	77	167
	sd	39	–	39	–
Menthol	m	81	62	82	61
	sd	26	25	33	38
Hydrogen sulphide	m	65	64	66	61
	sd	32	43	32	30
Vanillin	m	63	63	67	64
	sd	33	32	29	29

m, mean value; sd, standard deviation.

Fourteen volunteer subjects (10 male, 4 female between 25 and 45 years of age, including 2 left-handed males) participated in the experiments. The subjects received some slight recompense (14 DM/session). The experiments were conducted in accordance to the Helsinki/Tokyo/Venice declaration and the subjects were not exposed to any risks.

Results and discussion. The results showed that identification of the stimulated side was correct when carbon dioxide or menthol were presented, i.e. substances which mainly excite the trigeminal nerve. Hydrogen sulphide and vanillin, stimuli which mainly excite the olfactory nerve, were randomly assigned to either nostril (fig., tables 1 and 2).

Observed decrease in the intensity estimates during the course of the sessions was most pronounced for hydrogen sulphide and vanillin experiments. For the pure odours, estimates of intensity, relative to the very first stimulus, decreased by approximately 50%. Estimates for carbon dioxide and menthol stimuli fell to approximately 75% of the initial estimates. Analysis of variance revealed no differences in intensity estimates, for either left or right nostril stimulation. However, intensity estimates tended to be higher for correct localizations (H_2S, $p < 0.005$).

When hydrogen sulphide was used as the stimulant, the subjects' confidence estimates produced no differences between the left or the right side, and also no differences between correct or incorrect localization. Confidence estimates showed a tendency to decrease during the sessions.

To summarize, it can be stated that the subjects were virtually always correct in localizing the stimulated nostril when carbon dioxide and menthol were used. These substances undoubtedly excited chemoreceptors of the somatosensory system which here is represented by the trigeminal nerve. The remaining two stimulants vanillin and hydrogen sulphide, which excite mainly the olfactory receptors, were not localizable. The probability of the subjects' correct decision for the odours was at chance levels.

From these results we conclude that for single momentary odorous sensations, directional olfactory orientation can only be assumed, when the olfactory stimulants simultaneously excite the trigeminal somatosensory system. In other words, directional smelling exclusively mediated by the olfactory nerve does not exist. On the other hand it was shown without a doubt that the trigeminal nerve conveys information about the location of a source of smell. It would be expedient to support these findings by clinical investigations in patients with olfactory or trigeminial deficits.

This study did not concern itself with establishing just noticeable intensity differences between nostrils. It is conceivable that differences in concentration at the frontal line of a slowly spreading odour that also excites free nerve endings of the trigeminal nerve, are better detected when moving the head, thus allowing the nostrils to pick up and to discern differences in intensity. Schneider and Schmidt [6] also ascertained an enhanced ability to localize the source of a smell when the head was rotated from side to side. This kind of sniffing behaviour is a well-observed fact in perception of odours in natural surroundings.

The act of sniffing in combination with movements of the head might not only lead to a detection of differences in intensity but also to a detection of temporal differences between the intensity patterns of the stimulants that reach the nostrils. As mentioned in the introduction, v. Békésy [4] determined that subjects were able to detect temporal differences between stimuli presented to the left and the right nostril in the range of 0.1 ms. Leveteau and MacLeod [16] showed that in rabbits, temporal differences of approximately 10 ms between stimuli, with an optimum at 3 ms, caused an inhibition of the olfactory bulb of the side which had been stimulated secondly. Future experiments in humans must show to what extent differences in time and flow-rates are evaluated in the process of locating an odour source.

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1 v. Hornbostel, E. M., Psychol. Forsch. *4* (1923) 64.
2 Masterton, R. B., and Imig, Th. J., A. Rev. Physiol. *46* (1984) 275.
3 Rayleigh, L., Phil. Mag. *13* (1907) 214.

4 v. Békésy, G., J. appl. Physiol. *19* (1964) 369.
5 v. Skramlik, E., Z. Sinnesphysiol. *56* (1925) 69.
6 Schneider, R. A., and Schmidt, Ch. E., Physiol. Behav. *2* (1967) 305.
7 Van Toller, C., Kirk-Smith, M., Sleight, D., and Wood, N., Biol Psych. *11* (1980) 262.
8 Youngetob, S. L., Kurtz, D. B., Leopold, D. A., Mozell, M. M., and Hornung, D. E., Chem. Senses *7* (1982) 11.
9 Kobal, G., Elektrophysiologische Untersuchungen des menschlichen Geruchssinns. Thieme Verlag, Stuttgart/New York 1981.
10 Kobal, G., Pain *22* (1985) 151.
11 Kobal, G., and Hummel, C., Electroenceph. clin. Neurophysiol. *71* (1988) 241.
12 Kobal G., and Plattig, K.-H., Z. EEG-EMG *9* (1978) 135.
13 Cain, W. S., and Murphy, C., Nature *284* (1980) 255.
14 Doty, R. L., Brugger, W. P. E., Jurs, P. C., Orndorff, M. A., Snyder, P. J., and Lowry, L. D., Physiol. Behav. *20* (1978) 175.
15 Kobal, G., and Thiele, V., Proceedings of the VI. World Congress on Air Quality Paris *2* (1983) 435.
16 Leveteau, J., and MacLeod, P., Reciprocal inhibition at glomerular level during bilateral olfactory stimulation, in: Olfaction and Taste, pp. 212–215. Ed. C. Pfaffmann. Rockefeller University Press, New York 1969.

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Hemoglobin causes both endothelium-dependent and endothelium-independent contraction of the pig coronary arteries, independently of an inhibition of EDRF effects

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Summary. Hemoglobin is widely used as an inhibitor of EDRF effects. Hemoglobin contracts pig coronary arteries in vitro. However, during this contraction, effects of substance P and bradykinin which act via the EDRF are not inhibited. This means that the hemoglobin contraction is not caused by inhibition of the EDRF. This contraction is caused by a substance released from the endothelium, and by eicosanoïds released from the smooth muscles.
Key words. Coronary artery; oxyhemoglobin; endothelium; EDRF; electrophysiology; eicosanoïds.

Oxyhemoglobin has been shown to be a vasoconstrictor for cerebral arteries [1, 2]. Consequently, oxyhemoglobin could be the cause of cerebral vasospasms which follow subarachnoid hemorrhage. The contraction caused by oxyhemoglobin is more pronounced for cerebral arteries, yet it also contracts other arteries, among them the coronary arteries [1]. These observations were made before the discovery of the role played by the endothelium in vasodilation [3]. This implies that the studies undertaken previously were done without considering the possible role of a functional endothelium. Since the discovery of the endothelial-derived relaxing factor (EDRF), oxyhemoglobin has been extensively used as an inhibitor of the vasodilation caused by EDRF [4]. In this context, vasoconstriction caused by oxyhemoglobin on many arteries has been interpreted as a result of suppression of the relaxation caused by the EDRF [5]. Yet destruction of the endothelium in cerebral arteries does not inhibit the vasoconstriction caused by oxyhemoglobin [6]. This demonstrates that oxyhemoglobin may induce arterial constriction by at least two distinct mechanisms: an inhibition of the EDRF and a direct action on smooth muscles.

In the present study we investigate whether oxyhemoglobin contracts pig coronary arteries by inhibiting EDRF or by a direct action on smooth muscle, or both together. The endothelium-dependent relaxation in pig coronary arteries is characterized by a concomitant hyperpolarization [7, 8]. We used this hyperpolarization as an indication of endothelium-dependent relaxation. Thus measurement of smooth muscle membrane potential together with isometric tension were performed in this study.

We report here that oxyhemoglobin contracts pig coronary arterial strips in vitro in two ways: by an action on smooth muscles via eicosanoïds and by the release from the endothelium of a contracting substance, and not by inhibiting the EDRF. The existence of both an endothelium-dependent and an endothelium-independent vasoconstriction of coronary arteries caused by oxyhemoglobin could be important in cardiac physiopathology.

Materials and methods. Preparation of tissues. The anterior descending branches of coronary arteries were obtained from freshly killed pigs. The coronary lumen was rinsed by injection of cold oxygenated Krebs solution (mM: NaCl 118.7, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 24.8, $MgSO_4$ 1.2, glucose 10.1; 4 °C; pH 7.3). Segments of the coronary artery were cleaned of all adherent fat and connective tissue. Then they were cut into 2-mm-wide rings which were cut to give strips of about 5 mm in length. For some experiments, the endothelium was removed by gently rubbing the internal face of the strip with a cotton-tip. To check that the endothelium was removed by this procedure, a 0.5% w/v solution of Evans blue in Krebs solution was applied for 10 s to the strip, which was then washed with Krebs solution [9]. The luminal face of a strip with intact endothelium remained white whereas a de-endothelialized strip was colored blue. The response of the strip to substance P produces a marked endothelial-dependent relaxation of precontracted smooth muscle [10]. The absence of such a response, plus the positive Evans blue staining, was taken as evidence for the complete removal of the endothelium. When intact and de-endothelialized strips were compared as in figure 1, they were from adjacent coronary rings.

Two types of in vitro experiment were performed. In each type of experiment, changes in tension were measured isometrically (Grass force displacement transducer FT03C) and amplified (Lectromed 3559). Contractile responses were recorded on chart paper with polygraphs (W + W Electronics).

In each experiment, Krebs solution was pumped to the preparation from a siliconized glass or plastic beaker with a peristaltic pump. Oxyhemoglobin and peptides were applied to the preparations by diluting them directly in the beaker containing the superfusion solution. To avoid the production of mechanical artifacts during the experiment all changes in perfusate were achieved without the introduction of bubbles into the tissue chamber [7].

Pharmacological experiments. To measure the tension only, strips were suspended in a 85-µl bath [11] using two silk threads attached to the edges of the strips in parallel with the circular smooth muscles. Strips were continuously superfused with oxygenated Krebs solution (1.250 µl/min) main-