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ABSTRACTS

Olfactory Bioresponses in Man

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1. Clinical studies of olfaction

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The sense of smell not only warns humans of airborne toxic hazards and dangerous chemicals, such as leaking natural gas, but is the basis for flavor sensations derived from most beverages and foodstuffs. Thus, loss of this major sensory modality has a significant impact, particularly regarding issues related to safety, nutrition, and quality of life.

During the last decade and a half it has become apparent that decreased olfactory function is among the first signs of such neurodegenerative disorders as Alzheimer's disease and idiopathic Parkinson's disease. Interestingly. such loss is not found in all neurodegenerative disorders, including ones with clinical manifestations shared with AD or PD (e.g. progressive supranuclear palsy). The studies performed on this and related topics at the University of Pennsylvania are reviewed, with an emphasis on the application and interpretation of psychophysical tests.

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2. Recording of electro-olfactograms in man

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After chemical stimulation of the human olfactory epithelium it is possible to record negative responses (electro-olfactogram, EOG) which are interpreted as the summated receptor potentials of the olfactory nerve (vanillin 2.1 p.p.m.; hydrogen sulfide 0.8 p.p.m.). Stimulants were presented with stimulus durations ranging between 250 and 1000 ms (interstimulus interval 2–120 s). EOG was recorded by means of tubular electrodes (cutaneous reference contralateral bridge nose; impedance <2 kΩ; bandpass DC to 30 Hz, sampling rate 125 Hz). Investigations in >60 subjects revealed the following major results.

- EOG amplitudes increased in relation to the subjects' intensity estimates; latencies of EOG decreased with increasing stimulus concentrations.
- An increase of stimulus duration produced an increase of EOG amplitudes; in contrast, latencies of EOG onset remained constant.
- When using repetitive stimulation the amplitude produced by the second stimulus was as great as that of the first response, indicating that the peripheral encoding is less subject to desensitization than the subjective perception of odorants.
- Only in 2/18 trials could clear responses to both olfactory stimulants (H_2S, vanillin) be recorded in the same location. In line with immunohistochemical findings, the results indicate that odorant receptors are not dispersed homogeneously throughout the olfactory epithelium. In addition, endoscopic localization of EOG recording sites indicated that olfactory tissue is located more anteriorly than previously thought.

3. Chemosensory event-related potentials

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Chemosensory event-related potentials (CSERPs) have increasingly found their way into a number of fields of research. They help to establish the function of both the trigeminal and the olfactory systems. The investigation of chemosensory deficits in Parkinson or Alzheimer patients as well as pain perception in patients suffering from trigeminal neuralgia are typical ranges of application. Since the locations of the CSERPs cortical sources are known better, these methods could be helpful in the neurosurgery of epileptic loci. It can be assumed that scientists employed in industrial research will also benefit from having access to objective data concerning the hedonic and cognitive aspects of smelling. Research performed to date already indicates that CSERPs have

extended knowledge about the functions of the sense of smell so that human olfaction is better understood.

4. Olfactory transduction mechanisms in human receptor neurones

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Patch clamp recordings revealed the presence of a non-desensitizing cyclic nucleotide gated (CNG) channel on human olfactory receptor neurones (ORNs) and a fast desensitizing non-specific cation channel activated by nucleotides on human supporting cells. CNG channels showed a selective channel activation by cAMP ($K_{1/2}$ = 5 μM) and cGMP ($K_{1/2}$ = 2 mM), a unitary conductance of ~20 pS, a reversal potential of single channel currents close to 0 mV, a linear I/V relation in the range of –80 to 80 mV and a strong extracellular but only a weaker intracellular blocking effect of Ca^{2+}. The channel activity outlasted the cyclic nucleotide pulses for hundreds of milliseconds when higher agonist concentrations (>50 μM cAMP) were applied. The duration of the response was longer than in CNG channels from other species studied so far. The plateau duration and the decay remained constant for pulses with a length of 50–150 ms, whereas pulses <50 ms successively reduced the time required by shortening the plateau phase. A higher difference for the $K_{1/2}$s of cAMP ($K_{1/2}$ = 22 μM) and cGMP ($K_{1/2}$ = 2.5 μM) could be found for a small group (n = 3) of the CNG channels, pointing to the selective expression of the α-subunit in a small subgroup of ORNs.

Our immunohistochemical (OMP-staining) and electrophysiological results clearly demonstrate the occurrence of ORNs in the upper parts of the middle turbinate. The existence of more peripheral olfactory sensors as demonstrated in our studies expands the room for spatial and temporal coding. This has enabled the possibility of identifying an odorant by its characteristic spatial and temporal activation pattern in the nasal cavity.

5. Behavioral responses to chemostimulation in man

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Sensations and feelings induced by sensory stimuli occur in living organisms' most 'private domain' and are therefore inaccessible to direct measurements. Recording of stimulus-dependent bioelectric manifestations, related to receptor-, conduction-, transmission- or decoding processes, are known to indicate primarily the functional state of a particular sensory pathway. In contrast, stimulus-dependent fixed, reflectory neurobiological responses were found to reflect reliably both intensity and motivational, hedonic aspects of perceived sensations. Quantifiable psychophysical estimates are known to mirror the cognitive work-up of perceived sensations.

Our previous studies evinced that gustatory and olfactory stimuli induce in man a transient display of distinct facial features, able to be elicited at perinatal age. These reflectory responses were found to be fixed, innate and controlled by brainstem structures, reliably mirroring both the intensity and hedonics of chemosensory processes. The sets of behavioral reactions were named gustofacial- and nasofacial reflexes. These responses were found to be independent of learning, visual reinforcement or mental capabilities. Analogous stimulus-dependent reactions could be demonstrated in nonhuman primates and in many other animal species. More recent studies have shown that among autonomous responses, taste- or smell-induced changes in basal heart- and respiratory rates can also serve as sensitive indicators of intensity and hedonics of chemosensations. In studies on animals as well as humans, where verbal communication cannot be established, such behavioral indicators are of special value. While neurobiological reactions are considered to indicate chemosensations more objectively, traditional psychophysical testing methods are valid quantifiable subjective measures of man's sensory experience. Therefore, in our laboratory a testing protocol was developed based on simultaneous recording of all these indicators. This multidisciplinary methodology is applicable as a tool for consumer-evaluation as well as a clinical assessement technique of chemosensory processes.

Using this method, taste- and odor-reactivity was studied in different groups of healthy and diseased examinees of different ages. The multidisciplinary testing procedure is also routinely used as a critical clinical evaluation procedure in taste- or smell dysfunctions of innate and acquired etiologies (e.g. in cases of familial dysautonomia, partial or total loss of tasting or smelling in post-traumatic states or neurological diseases, in endocrinological evaluations such as suspicion of Kallmann's syndrome). The method yields a valid profile of chemosensory functions of the examinee.

An instructional video demonstration is included to show the easy handling of this multidisciplinary testing procedure.

6. Localization of olfactory brain areas using magnetic source imaging

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Functions of the human olfactory system are well defined up to the level of the prepiriform cortex, but very little is known about the neocortical projections. We used magnetic source imaging (MSI) with good temporal and spatial resolution to study cortical activation elicited by well-defined olfactory stimuli.

Ten healthy volunteers (five male and five female, 20–40 years old) participated in the experiments. The olfactory stimuli (vanillin and hydrogen sulfide) were delivered within a humidified and temperature-controlled constant airflow to the nasal cavity without altering the thermal conditions at the mucosa. The

stimulus sequence consisted of 200 ms pulses once every 40 s. Cortical responses were recorded with a 37-channel neuromagnetometer (Krenikon, Siemens) in a magnetically shielded chamber. Signals coinciding with eye blinks were discarded from the average. Additionally, to compare timing between magnetic and electrical responses, olfactory event-related potetials (OERPs) were recorded from the vertex (Cz/A1). The functional MSI information was combined with anatomical data from magnetic source imaging.

The peak latencies of the olfactory event-related magnetic fields corresponded to the ascending and descending slopes of the major electric deflections of the OERPs P1, N1 and P2. At these events we obtained consistent activation of parts of the insular cortex and the superior temporal sulcus. In contrast, no dipolar field pattern was identified when the OERPs peaked and no reproducible equivalent current dipole was found in other areas, including the frontal cortex. Therefore, we consider that the insular cortex and the temporal lobe play an important role in human olfactory function.

7. The effect of familiarity to odors on recognition and pleasantness; psychological studies followed by preliminary MEG and FEG studies

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Little is known about the effect of familiarity on recognition and pleasantness of odors. We investigated this effect in three different experimental set-ups.

In the first series of experiments, the perceived intensity, the recognition and the pleasantness for 22 odors concerning Japanese life were measured in children, adults and aged persons. These odors were selected based on a classification of odors of Japanese people (Saito *et al.*, 1994) and were presented to subjects as a scratch and sniff test like the UPSIT (Doty *et al.*, 1984). Children aged between 7 and 9 years showed significantly lower identification rates of odor qualities but a higher estimation rate of intensity than adults. In contrast, the aged persons showed lower ratings for both identification and intensity compared with adults. Additionally, the averaged ratings of the unpleasantness for odors were much higher than those of the adults and the aged persons. We suppose that these characteristics of olfactory preference in children originate in their lack of experience of and unfamiliarity with the presented odor, while those of the aged persons might originate in the functional decline of their olfactory organ.

In the second series of experiments we investigated the preference of odor in 2-year-old children depending on their experience to them. Phenyl ethyl alcohol was used as a pleasant odor and skatol as an unpleasant one. Two 1 m^3 boxes were odored with either one of the two odorants. In the first stage, the children had to enter each of the two boxes with their mothers to watch an animated movie. In the second stage, children participated in the same experiment after experiencing the odor for 1 month. After the first stage no difference in the selection of the two boxes could be found. After the second stage, the children with less experience of the smell of phenyl ethyl alcohol showed less preference to visit the box odored with this odorant than the children with much more experience of its smell. Therefore we suggest that experience of odor has some effect on the preference for odor in young children.

In a third experiment we aimed to localize the cortical areas activated during odor recognition and at the different states of pleasantness of different odors. Therefore we employed the technique of magnetoencephalography (MEG) with simultaneously recorded EEG. We have so far recorded olfactory event-related magnetic fields after phenyl ethyl alcohol stimulation using a Kobal's olfactometer (Kobal, 1981). For the recordings we employed a 64-channel whole head SQUID system (CTF) in a magnetically shielded room. Six subjects participated in this experiment and we were able to localize equivalent current dipoles (ECD) bilaterally in the superior temporal sulcus after separate stimulation of the two nostrils. The ECDs corresponded in latency to the P2 component of the EEG responses, which confirmed earlier results obtained by Kettenmann *et al.* (1995).

In the near future we will use MEG imaging techniques and functional magnetic resonance imaging to elucidate the brain activity related to the process of odor recognition.

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8. Effects of aging on the olfactory event-related potential

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Psychophysical studies of aging individuals reveal deficits in olfactory function at every level: threshold sensitivity, identification, differential sensitivity, identification, learning, recall, and recognition memory. We have focused on recording olfactory event-related potentials in the elderly to evaluate olfactory function, particularly because it is less susceptible to subject bias, criterion shifts and memory problems which can render psychophysical assessment less valid in the elderly.

Methods similar to those of Kobal were employed to record OERPs in young and elderly subjects. Stimuli were presented olfactometrically in a stream of air heated to 36.5°C and humidified to 80% RH. Stimulus rise time did not exceed 20 ms. EEG activity was recorded from the Fz, Cz and Pz electrode positions of the international 10/20 system, amplified and filtered

using a computer-based recording system. Electro-ocular activity was also monitored. Breathing was restricted to the mouth during trials, keeping nasal flow rate constant. Stimuli were applied randomly during breathing. Subjects produced magnitude estimates to monitor odor strength. Latency from stimulus onset as well as amplitudes of N1, P2, N2 and P3 were assessed at different ISI: 45, 60 and 90 s. Detection thresholds for odor were measured using a two-alternative, forced-choice method. All subjects had ENT examinations to rule out the presence of nasal sinus disease. A battery of neuropsychological tests were administered to screen for dementia and to provide the potential to assess the relationship between OERP measures and neuropsychological and cognitive function.

Older subjects had smaller amplitudes, slower waves and longer latencies than young subjects. Females also had larger peak amplitudes, and the difference between males and females was greatest in the elderly. Analysis of individual trials and sequential groups of five trials revealed a statistically significant difference in amplitude between young and elderly on the first trial taken alone. Varying the intertrial interval between 45, 60 and 90 s interacted with age to influence the amplitude of the OERP: older men required longer ISIs to produce maximum amplitudes. Latencies were unaffected by the ISI. Thresholds showed less robust effects of age than the OERP, but were correlated with OERP measures. Magnitude estimates of intensity, averaged over blocks of five trials, showed age differences in adaptation/habituation which were also influenced by the ISI. Requiring subjects to produce magnitude estimates of intensity appears to have elicited a P3. The latency of P3 was longer and the amplitude was decreased in the elderly.

Analysis revealed significant relationships between neuropsychological variables and the P3 measures. Amplitude was higher and latencies were shorter in subjects who showed better recall of verbal material on the CVLT. The better the performance on trails A and B, the shorter the latency and the higher the amplitude of P3. Similar relationships were revealed between neuropsychological measures and slow waves.

We have also assessed the effect of background EEG alpha activity, using three experimental conditions: eyes closed, eyes open and visual-motor tracking. OERPs were elicited using amyl acetate stimuli (200 ms duration, 60 s ISI) in young adults and elderly subjects. The amplitude of the NI, P2 and N2 OERP components was found to be more variable in the eyes closed than in the eyes open or tracking conditions, and young subjects demonstrated larger amplitudes than elderly subjects.

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9. Pre- and postoperative functional results with patients after ethmoidectomy

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The flavor of foods and beverages as well as the warning of fire, toxic vapors and spoiled foodstuffs can only be detected by intact chemical senses. The most important is the sense of smell, but the intranasal trigeminal chemoreception is also a component of the so-called common chemical sense. The 'pure' odorants stimulate the CN I but most odorous chemicals also stimulate the intranasal free nerve endings of CN V. This is important for identification and localization. The odor detection threshold test using butanol and an identification test with common odors as well as a trigeminal detection threshold test using formic acid can assess intranasal chemosensory function. It is supposed that endonasal ethmoidectomy damages the free trigeminal nerve endings in the nasal mucosa. Therefore the detection threshold for formic acid should be increased. The detection threshold for butanol and the identification rate of concentrated common odors should be better after the operation than before. Fifteen patients with polyposis nasi were tested pre- and postoperatively at least 6 weeks after the operation. The tests performed were: (i) a butanol threshold test like Cain; (ii) a vanillin threshold test; (iii) a formic acid threshold test; (iv) an identification test with concentrated common odors; (v) olfactory event-related potentials (OERPs) by stimulation with vanillin; and (vi) chemosomatosensory (trigeminal) event-related potentials (CSERPs) by stimulation with CO_2.

There was a significant improvement in the ability to smell after the operation. The detection thresholds for butanol and vanillin were better postoperatively than preoperatively. Preoperatively only in one patient was an OEPR obtained from the right nasal side. This cavum nasi was normosmic for vanillin as tested by subjective olfactometry. Postoperative OERPs were recorded with six patients, all of whom were normosmic for vanillin. All other patients but one were hyposmic for vanillin. No OERPs were recorded by stimulation with vanillin. The detection threshold for formic acid decreased postoperatively. Preoperatively we could record CSERPs by stimulation with CO in 11 patients. The four other patients had an increased detection threshold for intranasal trigeminal chemosensitivity.

We recorded CSERPs in 14 patients after the operation. The only patient we could not obtain a CSERP for surprisingly had a normal subjective detection threshold for formic acid.

Endonasal ethmoidectomy improves the olfactory and trigeminal intranasal detection threshold. Improved nasal patency would be the common cause. Sniffing rates and the streaming in of stimulant molecules are increased. The postoperatively damaged free trigeminal nerve endings did not influence the functional results. OERPs and CSERPs are generated by suprathreshold stimulation. If the detection threshold for vanillin or formic acid is normal, we can record an ERP. If the subjective threshold is higher, the used stimulus concentration is too weak for recording ERPs. A perithreshold stimulus concentration is unsuitable for distinguishing ERPs from background activity.

10. The effects of selective attention on chemosensory event-related potentials

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Humans are often inattentive to chemosensory stimuli and this is especially true during task performance. This inattention is opposed to the high sensitivity of the olfactory system and suggests an active gating of olfactory and trigeminal sensory

channels. It is possible that the neural mechanisms responsible foractive gating of chemosensory attention developed contemporaneously with the neural specialization of some cognitive abilities such as language. To investigate the possibility that different tasks lead to different patterns of chemosensory response, CSERPs were recorded as subjects performed chemosensory, linguistic and spatial tasks. Fourteen subjects participated. CSERPs were recorded from 30 sites during performance of a chemosensory judgement (food odor identification), linguistic task (noun identification) or spatial judgements ('top-heavy' figure identification). Chemosensory stimuli (perfume, food extract, butyl alcohol or blank) were delivered for 0.25 s to the most patent nostril in a warmed and humidified airstream (3 l/min) during the intertrial interval (3 s) of the task-relevant stimuli. Subjects breathed through the mouth during task performance. ERP responses were recorded to both the task stimuli and the chemosensory stimuli. Results of the study indicated a large N1–P2 complex for the chemosensory condition. The temporal window of this response was used to search the CSERP responses occurring during the linguistic and spatial tasks. The N1 component of the CSERPs which occurred during attention to linguistic or spatial stimuli were analyzed separately. These analyses indicated greater frontal positivity for the N1 of the CSERP for the spatial task. Analysis of the waveforms immediately following CSERPs (1.0 s post-S1) indicated no residual differences due to task and suggests that the N1 CSERP differences were due to different cortical resource allocation in response to chemosensory stimuli when subjects perform two different types of task.

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11. The latency but not the amplitude of the olfactory event-related potential (OERP) varies with the odor concentration

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It has often been suggested by different laboratories that the amplitude as well as the latency of the OERP depends on the stimulus concentration. However, some studies propose that this relationship seems only to be valid for the negativity of the OERP while others find only the positivity to be determined by the odor concentration. These incongruous results might be due to effects of trigeminal stimulation and of the subjective stimulus significance, which have not always been clearly controlled. The aim of the present study was therefore to compare the effects of concentration of an olfactory stimulus and a trigeminal stimulus on the OERP. Moreover, we varied the task relevance during the experimental setting.

Eleven subjects (five female), ranging in age from 20 to 35 years, participated twice in the study. In each study either the olfactory (linalool) or the trigeminal (menthol) stimulus was presented. Stimuli were delivered to the subjects within a constantly flowing airstream, nonsynchronously to breathing. Each session consisted of five sets of 25 trials and the odor concentration was changed after each test-set. The concentration of linalool varied between 0.16 and 1.45 p.p.m. and the concentration of menthol between 0.10 and 4.15 p.p.m. The stimulus duration was 200 ms and the interstimulus interval was 50 s. OERP data were recorded from Fz, Cz and Pz, referred to linked mastoids. After each set of trials subjects were asked to state the perceived concentration as well as the emotional valence of the stimuli on a rating scale. Four of the subjects had to lift their index finger each time they perceived an odor, the other subjects were asked to relax and not to concentrate on the odor presentation.

The results show that the OERP can be separated into one negative (N1) and three positive components (P2, P3-1 and P3-2). The late positive components (P3-1 and P3-2) varied with the task relevance: the amplitudes were bigger when the subjects had to react to the stimuli. This effect was independent of the stimulus quality. While the amplitude of the N1 and P2 components only varied with the concentration of the trigeminal stimulus, the latency of these components also varied with the concentration of the olfactory stimulus. The subjects had difficulty determining the relative concentration of the odors. However, both stimuli were perceived to be less pleasant when the concentration was increased.

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12. Does the habituation of the olfactory event-related potential (OERP) depend on stimulus significance and complexity?

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It has been repeatedly shown that odors habituate more rapidly than tones or visual stimuli. The process of habituation is correlated with a decrease in amplitude of both the early negative (N1) and the positive components (P2/P3-1, novelty P3), even if odors are presented with long interstimulus intervals (ISIs).

To investigate if stimulus quality, complexity or significance influence habituation, OERPs were recorded in response to different odors. Linalool (single odor), a mixture of isoamyl-acetate, citral, linalool, eugenol and menthol (artificial complex odor), freshly ground coffee (daily significance) and axillary hair (biological significance) were used as odorants in each session. The intensities of the different odors were matched beforehand. Each odor was presented within a constantly flowing airstream in six blocks of ten stimuli. Stimulus duration was 600 ms, with an ISI of 8 s. Between blocks subjects had a 1 min break. The sequence in which the odors were presented during the session was varied across subjects. In the first block of the session the subjects always received odorless air as a control for somatosensory artifacts which could arise through the switching of the solenoid valve. After each block the subjects were asked to give a hedonic evaluation. They were further asked if and how the intensity of the odor had changed within and between blocks.

Ten male students, aged 20–40 years, all right-handed and non-smokers, participated in the study. They were instructed to wash themselves only with clear water the day before the session

and to cut the hair of both axillae on the morning of the experiment.

BEG data were collected from Fz, Cz and Pz, referred to linked mastoids. The heart rate and the electrodermal response were also recorded. Averages were calculated for trial position 1, trial positions 2/3/4, trial positions 5/6/7 and trial positions 8/9/10 for each odor.

First results indicate that the OERP in response to axillary hair declines less in amplitude than the OERPs for linalool, the mixture and coffee. This finding suggests that at least the personal body odor seems to have a certain inherent significance that leads to a consecutive update. Further features of the OERPs, for example, elicited by the mixture are discussed.

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13. Human respiratory responses to odorants

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A variety of non-verbal experimental techniques has been used to quantify the human response to odors, ranging from the relatively simple (galvanic skin response) to the very elaborate (magneto-encephalography). This paper assesses the use of respiration as a psychophysiological index of response in human subjects. We are investigating the hypothesis that relative activation of the olfactory and nasal trigeminal nerves, in response to varying concentrations of an odorant, is reflected in respiratory changes. A brief history of the method will precede a description of the techniques employed in our laboratory, followed by a discussion of recent experiments.

Using a computerized air-dilution olfactometer, we repeatedly presented both normal and anosmic subjects with four verified concentrations of propionic acid (0.2–85 p.p.m., vol/vol), randomly interspersed with clean air trials. Subjects' respiration patterns were measured before and during exposure, following which they made ratings of sensory aspects of the stimulus. Each subject underwent 200 testing trials, spread over four identical weekly sessions.

Results indicated surprisingly little variability in threshold estimations based on odor magnitude ratings from individuals, with most subjects contained within a range of ~0.5 log units. Nasal irritation ratings, however, showed much more intra-subject variation.

Our technique permitted the examination of several respiratory parameters, including tidal volume, peak flow and breath duration. The percentage decline in inhaled volume from before stimulation to the first breath of the stimulation period appeared to provide a sensitive measure of a respiratory effect of propionic acid, and reasonably good inverse relationships between this measure and nasal irritation magnitude were seen.

Results from normosmics are discussed in comparison with those from anosmics in an effort to elucidate the complex interactions between the olfactory and nasal trigeminal nerves in both the perception of nasal irritation and breathing responses to chemical stimuli.

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14. Asymmetrical interaction between noise and odour: experimental evidence of one-way bimodal interference

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Previous efforts to demonstrate perceptual interactions between noise and odour have not proved successful. Such studies have typically followed a psychophysical approach preventing adaptation/habituation from occurring. In order to allow adaptation to become influential we have recently continuously exposed ~40 subjects for 1 h to either traffic noise (70 dB) or H_2S (300 p.p.b.), or to the combination of both. A nonexposed control group (n = 20) served as the reference. Intensity ratings as well as annoyance responses were recorded at the beginning of exposure and three times thereafter at 20 min intervals.

Both odour annoyance responses ($F = 10.7$; $P < 0.01$) and odour intensity ratings ($F = 7.5$; $P < 0.05$) were significantly reduced during odour exposure alone relative to the combined exposure condition. Psychological/perceptual responses to noise were not differentially affected, however. It is therefore concluded that central adaptation ('habituation') to odours is reduced in the presence of noise but not vice versa, thus giving rise to asymmetrical bimodal interaction.

15. Assessment of olfaction in multiple sclerosis

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There remains controversy about whether the olfactory pathways are affected in multiple sclerosis (MS). To resolve this, olfaction was assessed by: (i) the University of Pennsylvania Smell Identification Test (UPSIT, which uses microencapsulated odours which are released when scratched with a pencil) in 72 MS patients and 90 controls; and (ii) olfactory evoked potentials (OEPs) to 20 p.p.m. H_2S by volume and 50% CO_2 in air in 45 MS patients and 47 controls. The abnormality rate in MS patients for both tests was compared with that for visual evoked responses measured using a standard checkerboard technique. In comparison with controls, patients exhibited significantly lower scores on the smell identification test, with 15% of patients scoring outside the 95%

confidence limits for controls. The UPSIT was occasionally abnormal when the pattern evoked visual response (VER) was normal. In general, UPSIT scores correlated well with the H_2S-evoked response in controls and patients. For H_2S, there was a statistically significant increase of latency and decrease of amplitude for patients compared with controls. Increased H_2S latency and reduced UPSIT score correlated with greater disability on conventional rating scales. Overall, H_2S responses were abnormal in about one-quarter of MS subjects. The sensitivity of UPSIT and OEP was similar, although disorder on one test did not necessarily indicate abnormality in the other. The VER was found to be a more sensitive indicator of disease than OEP or UPSIT. Nonetheless, our findings confirm the existence of olfactory dysfunction in MS and validate a new evoked potential technique.

16. Objective olfactometry by recording simultaneously olfactory evoked potentials and contingent negative variation

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Objective diagnosis of olfaction can be performed by registration of cortical olfactory evoked potentials (OEP) and of contingent negative variation (CNV). The CNV is a negative voltage developing at the vertex after discrimination of one of two smells while the proband is expecting a second (auditory) stimulus. Using a relatively long time window (2.5 s) and an appropriate high-pass filter, the two tests can be performed simultaneously. The CNV does not need a steep stimulus onset and can be recorded also from less calm patients, as long as they are attentive. In cases of anosmia neither an OEP nor a CNV can be measured. With parosmia, OEPs are present for every stimulus, but no CNV develops when the patient is unable to differentiate the applied two odors. In hyposmia, OEP amplitude is reduced in correspondence with the smelling loss, but CNV amplitude can even be enhanced just above the discrimination threshold as a consequence of an increase in the patient's attention.

17. Chemosensory event-related potentials in patients with temporal lobe epilepsy

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The aim of the study was to investigate chemosensory functions in patients with temporal lobe epilepsy in order to find out whether both olfactory and trigeminal stimuli applied either ipsilaterally or contralaterally to the epileptic focus are processed differently. Twenty-two patients were investigated, 12 of whom suffered from epilepsy with a focus located in the left temporal lobe. The remaining 10 patients had a right-sided temporal lobe focus. Input from the trigeminal system was examined by the use of carbon dioxide; input from the olfactory system was evaluated using vanillin and hydrogen sulphide as stimuli. Chemosensory function was assessed by evaluating chemosensory event-related potentials (CSERP) and the patients' verbal reports in an odor identification test. In both groups of patients, prolonged CSERP latencies were found after stimulation of the left nostril with carbon dioxide when compared with stimulation of the right nostril. In contrast, a different pattern emerged for olfactory stimuli. After left-sided olfactory stimulation, latencies were prolonged in patients with left-sided epileptical focus. Similarly, when the right nostril was stimulated in patients with a right-sided focus, CSERP latencies were prolonged. Thus, it is suggested that the neocortical processing of olfactory- but not trigeminally mediated information is affected by functional lesions of the temporal lobe. After olfactory stimulation in patients with a right-sided focus, the distribution of amplitudes was different from normal. Moreover, analyses revealed non-overlapping 95% confidence intervals for latency N1 when vanillin was applied to the right nostril. These results indicate that the right temporal lobe may play a different role in the processing of olfactory information than the left temporal lobe.

18. Presentation of odor stimuli during an amobarbital procedure (Wada test)

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The amobarbital procedure or Wada test is a diagnostic tool to determine the lateralization of speech and memory functions. During this test each hemisphere of the brain is briefly and sequentially anesthetized and the awake hemisphere tested neuropsychologically. In the neurological clinic of the University of Munich this procedure is used in the pre-operative diagnosis of epilepsy and tumor patients. Here, in addition to visual and verbal testing material, olfactory stimuli were for the first time applied to examine more closely non-verbal memory functions. First results from an ongoing study with everyday odors and artificial substances are reported, in which patients were tested with a recognition paradigm. During anesthesia patients were monorhinally stimulated on the side contralateral to the injection with two natural and two artificial odors, which they had to recognize among a set with four other distractors as soon as the effect of the barbiturate had vanished. For both hemispheres different odor stimuli were used. The performance of the olfactory memory was compared with results from an equivalent test without anesthesia as well as with the performance of a healthy control group.

19. Time-course of olfactory function at the beginning of a grass-pollen season

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Olfactory dysfunction is one of the major complaints in patients suffering from allergic rhinitis. Little is known about the onset of hyposmia in seasonal allergy.

We examined the time-course of olfactory dysfunction in 17 patients (eight females, nine males, 32.7 + 6.9 years) with a history of at least 2 years of allergic rhinitis requiring medical treatment, positive skin prick test and RAST (>class II) to grass pollen. All had undergone a rhinological evaluation including nasal endoscopy and A-scan ultrasonography of the paranasal sinuses. Exclusion criteria were the coexistence of nonallergic nasal or paranasal sinus disease, nasal allergy to perennial allergens, chronic disease other than atopic allergy, pregnancy, immunotherapy for allergy during the last 3 years, antiallergic therapy at study entry, refused consent, and age under 20 or over 45 years.

Allergic symptoms of rhinorrhea, nasal obstruction, itching and sneezing were recorded daily in a diary. Olfactory function was evaluated birhinally using a modified CCCRC-testing (CAIN) procedure for threshold, identification and discrimination. The result of the better smelling side of the nose was used for statistical analyses. Patients were tested preseasonally and on days 3, 7, 14 and 21 of the season.

Preseasonally, all patients were normosmics. There was a significant decrease in threshold and identification testing between days 3 and 14 of the season, but there were no further changes up to day 21 of the season and no changes in discrimination over the entire period.

Olfactory dysfunctions were not chronologically correlated to the time course of nasal obstruction.

20. Blink responses and eye movement-evoked olfactory stimuli

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We investigated the influences to the olfactory evoked potentials of blink responses and eye movements. Forty normal subjects participated in this study. Four odorants—phenyl ethyl alcohol, isoamyl acetate, isovalic acid and methyl cyclopentenelone—were presented using a new apparatus developed by our department. The eye movement was observed from ~100 ms after the olfactory stimuli and the blink response was observed in an earlier phase than that of the eye movement. The blink response for each odorant was found in 25–35% more subjects than that for air, while the response was 18% for air. Although the incidence of the eye movement for each odorant was less than that of the blink response, there were significant differences between the incidence of the eye movement for each odorant and for air. We suggest that those responses may be useful for a mean of the objective olfactory function test.

21. Effects of normal variation in nasal patency and thermal and cardiac function on odor and pain sensitivity in healthy young adults

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Effects of normal variation in nasal patency, body temperature, blood pressure and heart rate on odor and pain sensitivity (nasal mucosa), as well as effects of body temperature, blood pressure and heart rate on nasal patency, were studied extensively in five young, healthy men across the diurnal cycle. Odor (H_2S) and pain (CO_2) thresholds were assessed with a dynamic olfactometer, applying the staircase procedure. Nasal patency was measured with acoustic rhinometry. The results showed a weak but significant negative correlation between nasal patency and odor threshold (a tendency for such a relation also for pain). The range of normal variation in both nasal patency and body temperature were estimated to affect the odor-threshold concentration by a factor of 1.7. No other significant correspondences were observed. These findings suggest that nasal patency should be monitored when highly accurate evaluation of odor sensitivity is required. The findings also encourage further research on the relation between chemosensitivity and normal variation in body temperature.

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22. Olfactory event-related potentials in middle-aged persons

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There has been substantial research of event-related potentials of the human senses of vision and audition, yet very little has focused on the chemical sense of smell. In the past, difficulty in eliciting and reproducing non-trigeminal OERPs has significantly curtailed research on them. With the continuing perfection of recording techniques, researchers are now able to record reproducible, purely olfactory OERPs. The purpose of this study was to determine if the components of the OERP are affected differentially by age. OERPs were recorded monopolarly at the Fz, Cz and Pz electrode sites on eight middle-aged adults (41–56 years old), 16 young adults (19–27 years old) and 16 older adults (60–78 years old). Based on the results of a previous study a 60 s inter-stimulus interval was used with amyl acetate selected as the odorant. Latency and amplitudes of N1, P2, N2 and N1/P2 peak-to-peak amplitudes were measured. Analysis consisted of an ANOVA on each of the dependent variables, with age group as the between-subject independent variable and electrode site as the

within-subject variable. Post-hoc analysis consisted of main effect analysis and Fisher's post-hoc analysis. Results demonstrated that middle-aged participants had longer P2 latencies at all recorded electrode sites than young adults. No significant differences in latencies were demonstrated between middle-aged participants and older adults. There was no significant change in amplitude for N1, P2, N2 or N1/P2 peak-to-peak amplitudes when middle-aged participants were compared to either young or older participants. Previous studies have found age-related changes in elderly adults in the OERP, specifically increased latency and decreased amplitudes. Results from this study suggest these age-related olfactory changes may begin earlier than previously thought. Since P2 latency was significantly affected in middle-aged adults, this would suggest that latency measures may be more sensitive to age-related changes in the olfactory system than amplitude measures.

The results demonstrated that several OERP components change with age and are associated with changes in neuropsychological performance. Specifically, an increase in P3 amplitude and decrease in P3 latency was associated with an increase in the number of items recalled on the CVLT. Furthermore, faster completion of the TMT was correlated with increased P3 amplitudes and decreased P3 latencies. Similar relationships were found between cognitive performance and slow wave components. Analysis of variance revealed that young and old subjects differentially rated odor stimulus magnitudes, and this effect was most pronounced for geraniol and phenylethal alcohol.

These findings suggest that OERPs in conjunction with neuropsychological measures should prove useful in clinical and applied settings to assess and diagnose olfactory dysfunction and cognitive dementia associated with aging.

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23. Olfactory event-related potentials are related to neuropsychological performance and estimates of stimulus magnitude in young and elderly patients

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The objective was to determine which neuropsychological measures are related to olfactory event-related potentials (OERPs) and to assess whether subjects' estimates of stimulus magnitude are related to OERPs in young and elderly patients. Studies relating neuropsychological measures with event-related potential recordings in the visual and auditory systems are often used in clinical and research settings. However, relatively few studies have related neuropsychology with olfactory processing. The recent advances in OERP recording techniques allow for greater control of stimulus parameters, leading to significant improvement in the reliability and stability of OERP recordings. These improvements now allow for cognitive olfactory processing to be measured electrophysiologically and its relationship to neuropsychological performance to be assessed.

OERPs were recorded monopolarly at the Fz, Cz and Pz electrode sites in 16 young adults (eight males, eight females) and 16 older adults (eight males, eight females) using inter-stimulus intervals of 45, 60 and 90 s with amyl acetate, geraniol and phenylethyl alcohol as odorants. N1, P2, N2 and P3 peak amplitudes and latencies, N1/P2, P2/N2 and N2/P3 peak-to-peak amplitudes, and a slow wave component were measured. Neuropsychological performance was assessed using the California verbal learning test (CVLT), the trail making test (TMT), the dementia rating scale, the mini-mental status exam, and the Blessed and the Ravens progressive matrices. Using the method of magnitude matching, participants were asked to estimate the perceived magnitude of the odor stimulus on a scale of 1–10 and intermittently asked to rate (on the same 1–10 scale) the perceived heaviness of several randomly presented weights, which allowed for anchoring of odor estimates.

24. Optimal number of trials to obtain a reliable olfactory event-related potential

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The objective was to determine the number of trials necessary to obtain a robust olfactory event-related potential (OERP). Compared with auditory and visual event-related potentials, relatively few studies have been performed on OERPs because of the difficulties involved in their recording. Recent developments have led to recording techniques which address the problem of stimulus control and concurrent stimulation of the auditory, somatosensory and trigeminal systems. However, recording parameters needed to acquire reliable waveforms continue to be explored.

Using the data from two previous studies (study 1: alpha blocking; study 2: interstimulus interval), this study considered the N1–P2 interpeak amplitude in the first artifact-free trial as well as the average of trials 1–5, 1–10, 1–15 and 1–20. Monopolar recordings at the Fz, Cz and Pz electrode sites of eight young adults (four males, four females) and eight older adults (four males, four females) were used. All the trials in this study were recorded with the stimulus odor isoamyl acetate at a 60 s interstimulus interval as the participant engaged in a computer tracking task. Only data from those participants who completed study 1 and study 2 was included.

Analysis consisted of a MANOVA on the dependent variable with age group as the between-subject independent variable and study, electrode site and trials as the within-subject independent variables. No difference in interpeak amplitude was found between study 1 and study 2. Both studies demonstrated a larger N1–P2 amplitude for the first trial than for the averaged trials. Collapsing over trials on both studies, young adults have a larger interpeak amplitude than older adults. Study 1 alone revealed an interaction between age and trials such that the first trial for the young adults was larger than for the older adults. Furthermore, the electrode

sites in study 1 demonstrated the usual scalp distribution, with the N1–P2 amplitude increasing from the Fz to the Pz scalp location. These findings suggest that <20 averaged trials may be sufficient to produce a reliable OERP.

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25. Age-related changes in the olfactory event-related potential: older males benefit from longer inter-stimulus intervals

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Event-related potential (ERP) recordings of the visual and auditory systems are widely used today in clinical and research settings. Relatively few studies have been done on olfactory ERPs (OERPs) because techniques for recording them have only recently been developed due to problems with control of the stimulus and simultaneous stimulation of the auditory, somatosensory or trigeminal systems. In this study OERPs were recorded monopolarly at the Fz, Cz and Pz electrode sites in 16 young adults (eight males, eight females) and 16 older adults (eight males, eight females) using inter-stimulus intervals (ISIs) of 45, 60 and 90 s, with amyl acetate, geraniol and phenylethyl alcohol as odorants. N1, P2, N2 and P3 peak amplitudes and latencies, N1/P2, P2/N2 and N2/P3 peak-to-peak amplitudes, and a slow wave were measured. Analysis consisted of a MANOVA on each of the dependent variables, with age group and gender as the between-subject independent variables and electrode site, ISI and odorants as the within-subject independent variables.

Post-hoc analysis consisted of simple main effect analysis and the Student–Newman–Keuls procedure. Results showed that age affected the OERP: peak amplitudes decreased and peak latencies increased in older participants. This may be due to degenerative changes in the olfactory bulb, hippocampus and amygdala associated with aging. Cognitive changes and slowing of the central nervous system may also have affected elements of the OERP. Gender was also significant, with females having larger peak amplitudes than males, which may have been a result of head size and skull thickness. More likely, however, it may be a combination of the effects of age on gender, in that olfactory deficits appear to be more severe in older males than older females. Older males had the smallest peak amplitudes of any of the four age/gender groups, and benefited most from longer ISIs. At the 45 and 60 s ISIs older males had significantly smaller amplitudes than older females, young males and young females, while at the 90 s ISI, although amplitudes tended to be smaller, the difference was not significant. The largest peak amplitudes were recorded from the Pz electrode site (and at times both the Cz and Pz sites), as has been shown in previous studies.

This may indicate that a dipole which processes olfactory information is directed toward the parietal cortex. Studies using magneto-encephalography have shown this to be the case. This study also showed an olfactory P3, which is assumed to reflect post-stimulus cognitive processing, which was smaller in the older adults than in the young adults, and may be a reflection of the cognitive changes associated with the aging process. The P3 was elicited by having the participants judge odorant intensity after each trial. Comparisons of OERP measures to traditional psychophysical testing suggest that OERPs may be more sensitive to changes in the olfactory system than commonly used odor threshold tests. OERPs also promise to be useful in the clinical setting to assess and diagnose olfactory dysfunction.

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26. Measurement of gustatory-evoked magnetic fields

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No study on the gustatory-evoked magnetic fields (GEMs) has been reported to date. Therefore, we developed a new gustatory stimulation apparatus with short rise time. Using this apparatus, we measured the GEMs and estimated the source of gustatory current dipole in the human cerebral cortex.

The tastants and rinsing water were separated by air. There were four phases in one trial, air–tastant–air–water. The air was randomly mixed in the rinsing water so that the subject could not guess the stimulus onset time by the air appearing. The air, the tastant and the water were transduced by a Teflon tube onto the subject's tongue. There was a hole in the tube, and the subject put his tongue on the hole. The air, the tastant and the water flowed onto the tongue.

In order to ensure strict trigger timing for averaging, the signal data for trigger should be acquired from a point as near to the tongue as possible. However, electric circuits could not be placed in the magnetically shielded room; therefore we developed a new optical sensor which could detect the timing of the stimulus timing without electric circuits in the magnetic shielded room.

The rise time to 80% of the level of the signal was 16.5 ms (SD = 1.49 ms). The distance between the sensor and the edge of a subject's tongue was ~6.5 cm. It took 300 ms (SD = 2.9 ms) for the liquid to pass through this distance. In this way, the rise time was 16.5 ms and the variance of reaching time from the sensor to the tongue 2.9 ms. This performance was enough to ensure strict triggering.

We used the 64-channel whole-cortex SQUID system (CTF Systems Inc., Canada). The subject sat on a wooden chair, and his jaw was supported by a wooden chin rest. The tube in which the liquids flow was introduced to the subject's tongue by a guide attached to the chair. The subject did not feel excessive fatigue during the experiment because of this apparatus.

The subjects were two males and two females (aged 19–32 years). We used a 0.003 M saccharin solution and a 1 M NaCl solution as tastants. Forty trials were presented to each subject. The duration of each stimulus was 400 ms, then deionized water was presented for rinsing for ~30 s. The tastant and the rinsing water were maintained at the same temperature. The taste stimulus was applied onto the front center of the tongue. The subjects were

instructed to watch a fixation point. The sampling rate was 250 Hz, and a 40 Hz low pass filter was used.

We measured the reaction times in separate experiments using the same stimuluation method. In this experiment, we requested subjects to push a button as soon as they perceived any taste.

The averaged GEMs of 64 channels were superimposed on the same graph, aligned according to the time after the raising of the stimulus. We found a difference in the delay of the starting point of GEMs between the saccharin solution and the NaCl solution. This was consistent with the difference of reaction times between saccharin and NaCl. From this result, it was confirmed that the acquired magnetic fields are caused by the gustatory stimulus.

Two dipoles were estimated in both cases of saccharin and NaCl. They seemed to be located near the inner face of the frontal operculum and insula. This was consistent with the result of Ogawa (1994) of the gustatory area of the macaque monkey using inplanted electrodes.

27. How the discrimination of body odors is represented in the olfactoly event related potential (OERP)

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Various studies using visual, auditory and recently also olfactory stimuli have shown that the presentation of a rare and deviant stimulus within a series of standard stimuli elicits distinctive components (N2, P3) within the ERP, whose parameters vary with the amount of attention invested by the subject.

The aim of this study was to investigate whether body odors (axillary hair) from different donors could be discriminated and in what way this process is centrally represented.

The stimulus material (axillary hair) for each subject and session was collected from different women. Within 10 trials (=one block) one of the odors was interspersed with a probability of 0.2. The subjects received four sets of six blocks, with the inter-stimulus interval being 8 s and the inter-block interval 60 s. Stimulus duration was 600 ms.

Stimuli were presented within a constantly flowing airstream under both 'attend' and 'ignore' conditions. In the 'attend' condition subjects were introduced to both odors before thc EEG-recording. The subjects were instructed to which odor they had to respond. They were further allowed to practice the discrimination of both odors beforehand. In the 'ignore' condition subjects were told to ignore the odors and listen to a tape of classical music.

Six subjects, aged 20–35 years, participated in the study. They were all non-smokers and their ability to smell was not restricted by any acute or chronic sickness of the respiratory system. EEG data were collected from Fz, Cz and Pz, referred to linked mastoids.

Preliminary data show that the target effect associated with the P3 in various modalities does not appear in the 'attend' condition when body odors are employed: both odors (standard and target) elicited an equally large P3 amplitude. No similar finding has been reported for odors or visual and auditory stimuli. OERP data from the 'ignore' condition are presented and discussed in comparison with OERP elicited by the discrimination of single odors.

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28. Circadian variations of nociceptive and olfactory thresholds in healthy young adults

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Circadian changes of nociceptive (CO_2) and olfactory (H_2S) thresholds were studied in five healthy young male volunteers. Four measurements each were taken at 0:00, 4:00, 8:00, 12:00, 16:00 and 20:00 h on different days, separately for the two nostrils. Odor and pain thresholds were assessed separately using a dynamic olfactometer (staircase method). Concentrations ranged between 30 and 60% vol/vol for CO_2 and between 0.1 and 2 p.p.m. for H_2S. A total of 12 logarithmic concentration steps were used; threshold measurements always started from the lowest concentrations.

Absolute values of neither nociceptive nor olfactory threshold showed a circadian rhythm. However, circadian variation was observed with regard to the interindividual variability of olfactory thresholds. This was smallest at 04:00 h, with thresholds between 0.4 and 1.2 p.p.m. The variability increased continually until 16:00 h, when thresholds ranged between 0.1 and 2 p.p.m. From this time, the interindividual variability of odor thresholds steadily decreased. In contrast, the interindividual variability of pain thresholds remained relatively stable, ranging between 33 and 60% vol/vol CO_2.

Based on these results, it is hypothesized that nociceptive thresholds are less subject to environmental influences than olfactory thresholds. At 04:00 h, before starting the daily activity, environmental influences are small. In contrast, at 16:00 h subjects might have experienced various environmental factors during their daily activity, resulting in a peak of interindividual variability of olfactory thresholds. These findings encourage further research on environmental effects on the sense of smell.

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29. Age-related changes of chemosensory event-related potentials after trigeminal and olfactory stimulation

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The aim of this study was to investigate whether it is possible to trace age-related changes in the perception of olfactory and

trigeminal chemical stimuli by means of chemosensory event-related potentials (CSERP).

Three groups of healthy volunteers participated in the experiments [age 15–34 (group A), 35–54 (B) and 55–74 years (C)]. Each group comprised eight female and eight male subjects. Stimulants were chosen to stimulate fibers of either the trigeminal (CO_2) or the olfactory nerve (vanillin, H_2S). Within one session each of the three stimuli was applied 16 times to the left nostril (200 ms, interval 40 s). In an additional three short sessions, olfactory (session 1: standard H_2S, target vanillin; session 2: standard vanillin, target H_2S) and acoustical stimuli (standard 1.5 kHz, target 2 kHz) were presented in an odd-ball paradigm. The probability of the target stimuli was $P = 0.16$. For olfactory stimuli the interstimulus interval was 6 s; for acoustical stimuli it was 2 s. Moreover, the subjects' ability to discriminate (eight pairs of odorants) and detect odorants (pyridine, phenyl ethylalcohol) was tested by means of a triple-forced-choice paradigm. Odor identification was investigated by means of eight odors.

CSERP amplitudes in response to CO_2 decreased in an age-related manner, whereas responses to olfactory stimuli decreased sharply in group C. The decrease of P3 amplitudes to olfactory stimuli was also most pronounced in group C. This finding was accompanied by a similar change of the subjects' ability to discriminate odors. Measurements of both odor identification and thresholds revealed that there was virtually no difference between groups A and B, whereas performance declined in group C. The results indicate that the age-related perception of olfactory stimuli may change in a different manner when compared with the perception of trigeminal stimuli.

30. 'Sniffin' Sticks': new ways to test olfactory performance

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'Sniffin' Sticks' is a new test of nasal chemosensory performance based on pen-like odor dispensing devices. Thus, it is possible to create a portable screening test of olfactory performance which is easy to administer. Since the test may be used over a period of 4 months, costs are kept low. It consists of two major parts: a basic screening test and a more sophisticated test suitable for forensic and scientific questions. The screening test includes an identification task for both smell (seven odorants) and taste (four stimulants stored in pen-shaped flasks) which is performed by means of a list of four items (forced choice). The advanced test is composed of three subtests: odor identification (16 odorants, multiple choice from a list of four items), odor discrimination (triple forced choice, 16 triplets) and a threshold test for butanol (triple forced choice, staircase method).

When investigating the test the following results were established.

(i) In 98 subjects the basic screening test was compared with a down-scaled version of the UPSIT (MODSIT, 'scratch and sniff' technique); 'Sniffin' Sticks' exhibited a relatively higher coefficient of correlation with the subjects' age. In addition, in contrast to the MODSIT, the 'sticks' demonstrated a superior olfactory sensitivity in women than in men.

(ii) Threshold testing by means of 'Sniffin' Sticks' was compared with the CCCRC (squeeze technique; method of ascending limits). There was a significant correlation between the two bottle tests ($r26 = 0.49$, $p < 0.05$); with regard to test–retest reliability, the 'sticks' reached a coefficient of correlation of $r26 = 0.68$ ($p < 0.001$).

(iii) The three subtests of the advanced part of the 'Sniffin' Sticks' test were investigated in 104 subjects on two different days, and a good test–retest reliability was exhibited (thresholds: $r104 = 0.61$, $p < 0.001$; discrimination: $r104 = 0.54$, $p < 0.001$; identification: $r104 = 0.73$, $p < 0.001$). In addition, a significant lifespan decrease of olfactory performance was observed.

31. Experimentally induced pain in man

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The aim of the present study was to assess the secretion of inflammatory mediators after challenging the nasal mucosa with tonic (long-term) painful stimulation.

To induce tonic pain, cold, dry air (8 l/min, 22°C, 20% RH) was applied to the nasal cavity. Sixteen healthy subjects took part in one experiment. In this experiment subjects were stimulated with tonic pain. To assess inflammatory mediators [prostaglandin E2 (PGE2), thromboxane B2 (TXB2), peptide leukotriene (pLT)], the nasal cavity was washed with 6 ml of lactate solution before, immediately after and 1 h after the stimulation period. Concentrations of inflammatory mediators were established by ELISA.

A significant release of mediator pLT was found after tonic painful stimulation. PGE2 and TXB2 release was also increased after tonic pain but this did not reach the significance level. The concentration of inflammatory mediators returned to baseline after 1 h, indicating the reversibility of inflammation.

This finding confirmed results of our previous studies in volunteers which also showed that NSAIDs (ibuprofen, ketoprofen, azapropazone) inhibited responses to tonic pain.

In conclusion, cold, dry air applied to the nasal cavity can be used as an experimental rhinitis model in man.

32. An easy method for separating olfactory from trigeminal stimulation

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When chemical stimuli are presented to the left or to the right nostril, the subject's ability to localize the stimulated side is an

important psychophysical indicator for estimating the degree of olfactory or trigeminal stimulation. Considering single phasic odorous stimulants, this ability depends on the excitation of the trigeminal somatosensory system. Trigeminal stimuli (e.g. menthol) can be localized with identification rates of >96%. In contrast, localization is random when pure odorants are used (e.g. vanillin). These findings were established in a previous study by means of a highly sophisticated chemical stimulator.

The aim of the present study was to develop a more economical approach which would allow the differentiation between the degrees of trigeminal and olfactory excitation in the perception of chemical stimulants. Twenty healthy subjects (10 male, mean age: 22,3; 10 female, mean age 24,6) participated in the study. For stimulation a pair of squeeze-bottles was used of which one contained the stimulant while the other contained solvent only. This pair of bottles was mounted in a holder which allowed the simultaneous application of the substances to both nostrils. The investigated substances were 2-phenylethyl acetate, patchoulol, menthol and vanillin. Menthol and vanillin served as gold standard with established identification rates.

The results indicated the following: (i) localization rates of menthol and vanillin were almost replicated with 87% and 37% respectively; and (ii) by means of the percentage of correct localizations, 2-phenylethyl acetate and patchoulol could be identified as clearly localizable odors with rates of 87% each.

Compared with the results of the previous study, the findings of the present study demonstrate that the estimation of olfactory and trigeminal excitation is possible by means of a very simple and convenient method.

33. Olfactory coding in the honeybee's antennal lobes revealed by optical imaging of Ca^{2+} and voltage-sensitive dyes

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In insects the antennal lobes (ALs) are the primary olfactory neuropiles which process sensory inputs from ~60 000 olfactory receptors and project to higher brain neuropiles via hundreds of output neurones. The glomerular subunits of the AL form a network, connected by local excitatory and inhibitory interneurones. Using optical imaging of voltage as well as calcium-sensitive dyes, we investigated the honeybee's olfactory code.

Voltage-sensitive dyes gave a signal in the range of 0.1% dF/F, with specific patterns for four tested odours (citral, carnation, hexanal and hexanol) and their binary mixtures. The measured activity patterns could reliably be related to the morphological image of the olfactory glomeruli. Glomeruli with distinct properties were distinguished: 'odour selective glomeruli' responded selectively and with constant amplitude for a given odour irrespective of additional compound odours; 'linear integration glomeruli' responded to compound odours with an activity level equal to the linear summation of its components' responses; and 'complex glomeruli' showed a reduced response to some compound odours and enhanced responses to others.

Calcium imaging also yielded odor-specific activity maps, but with a better signal to noise ratio (signals up to 3%). This was important because signals could be measured without averaging. The Ca^{2+} response could be measured within one frame after stimulus onset (300 ms after stimulation) and decays within 1–3 s.

Our results indicate that the code for odours in the AL is a distributed pattern of glomerular activity. The overlap between the patterns shows that there is no labelled line coding. The activity induced by a binary mixture of odours is not a mere superposition of the components' activities but results in a new pattern.

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