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ABSTRACTS

Chemosensory Bioresponses in Man II

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Oral Presentations

1. Making Scents of Structure/Activity Correlations

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We have measured the detection thresholds of 22 odorous chemicals and cross-adaptation of selected pairs. This broad selection also included representatives of two perceptual families: analogs of androstenone and analogs of a woody odorant named Jegers' ketal. No significant cross-adaptation was observed for perceptually unrelated molecules. All cross-adapting molecules had their perception thresholds positively correlated. The existence of specific anosmia was observed for most molecules, and double anosmia occurred beyond chance level for those substances cross-adapting among normosmics. No single individual from the >80 subject panel demonstrated exquisite sensitivity for all the substances, since all had at least one specific anosmia.

The sensory data allowed the resolution of ambiguities in the odor description of these selected molecules and the construction of working models of structure–odor correlations. The definition of minimal osmophoric structure elements with the help of computer-assisted molecular modelling allowed the successful design of new analogs of androstenone and Jeger's ketal. Molecular similarity, often invoked in chemosensory research upon comparison of 2-D sketches, is a misleading preconception since molecules with widely different skeletons and even different functional groups appear to share perceptual pathways. Surprisingly, the minimal structural requirement for androstenone odor bears considerable flexibility. In contrast, Jegers' ketal analogs possess a very rigid basic common motif.

Considering the combinatorial encoding of volatile chemicals by an array of receptor proteins, the mere existence of structure–activity relationships appears puzzling, as one would rather expect a continuous space for the odor quality of the various structural homologs of a given molecule. However, the signal of the olfactory sensory neurons is tuned by lateral inhibition and modulated in the olfactory bulb according to recent reports. This processing could account for the existence of the structure–odor relationships, but also for the surprising observation that very minor changes of chemical structure can dramatically affect the odor properties.

2. The Nucleus of the Solitary Tract: A Pivotal Structure in Chemosensory Pathways

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The nucleus of the solitary tract occupies most of the dorsomedial caudal medulla. Its neuropil is interwoven with the dorsal motor nucleus of the vagus ventrally, and the area postrema dorsomedially. Laterally it blends with the parvocellular reticular formation. Primary afferents from almost the entire gastrointestinal tract, liver, pancreas, respiratory system, heart and major blood vessels and arterial chemoreceptors travelling in the Vth, VIIth, IXth and Xth cranial nerves terminate within this nucleus in a somatotopic manner. Many of these afferents carry chemosensory signals. Moreover, the area postrema, as a circumventricular organ, represents a chemosensory portion of the nucleus of the solitary tract. There is a considerable degree of convergence of chemosensory and other afferents onto second-order neurons in the nucleus of the solitary tract. The nucleus is reciprocally connected to the spinal cord and to various pontine, mesencephalic and forebrain areas which constitute the central autonomic system, e.g. the parabrachial nuclei, periaqueductal gray, paraventricular and lateral hypothalamic nuclei, central nucleus of the amygdala and insular cortex. On the output side, the nucleus of the solitary tract projects to preganglionic neurons in the medulla and spinal cord. Thus, this nucleus occupies a strategic position to integrate visceral and central afferent signals, and to control the visceral periphery via the parasympathetic and sympathetic nervous systems.

3. Immunocytochemical Identification of Cell Types and Intercellular Contacts in the Rodent Olfactory Epithelium

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The olfactory epithelium is unique among neural tissues in that it contains sensory neurons that are directly exposed to the environment and participate in the formation of an epithelial barrier. Also, the olfactory system is one of the few areas of the nervous system where neurogenesis takes place throughout the entire life-span of the individuum—olfactory neurons that have degenerated due to toxic or infectious insults are continually replaced. Thus, in the olfactory epithelium and system, various processes such as signal transduction, contact formation by neurons, neurogenesis, targeted axogenesis and synaptogenesis can be studied under physiological conditions in the adult organism. To a certain extent, the results of the investigations can be considered to provide model examples for these processes in the entire central nervous system. This is the more interesting and important since, in the course of neurodegenerative diseases, the olfactory system appears to be affected very early. Its study may thus yield results significant for clinically more important pathogenetic events in other brain areas.

One of the prerequisites for learning about these different processes is the ability to determine their anatomical localization in

the system. Proteins involved in signal transduction, neurotrophic factors and their receptors, or cell adhesion proteins have to be exactly localized to different cells types and to compartments within these cells.

In the present investigation, we have determined immunocytochemical markers which specifically label the cytoplasm and/or the plasma membranes of olfactory neurons, supporting cells, microvillar cells, or of duct cells of the Bowman's glands, respectively, in the rodent olfactory epithelium. Olfactory neurons, in addition to their intense immunoreactivity for the olfactory marker protein, display strong β-tubulin-immunoreactivity particularly in their dendrites and cilia. Neuronal plasma membranes are reactive for sodium–potassium–ATPase. Supporting cells, microvillar cells and cells of the excretory ducts of Bowman's glands show reactivity for the epithelial intermediate filament protein cytokeratin 18. Using antibodies against cytoskeletal and cytoskeleton-associated proteins it is possible to differentiate between two types (I and II) of microvillar cells. Type I microvillar cells possess ankyrin- and villin-immunoreactivity, characteristics of brush cells, which supposedly have chemoreceptive functions in the gastrointestinal tract. Type II microvillar cells display intense immunolabelling for ankyrin and sodium–potassium–ATPase in their basolateral membranes.

We have then used the immunocytochemical tools in double labellings to localize proteins of adherens junctions, which are involved in contact formation, axonal guidance and synaptogenetic processes. The results show that the different cell types use different cadherins in their zonulae adherentes and puncta adherentia. In microvillar cells and duct cells, only the epithelial E-cadherin is found. The cadherin synthesized by olfactory neurons is detected by a pancadherin-antiserum which recognizes neuronal(N)-cadherin-like cadherins. However, N-cadherin-specific antisera show only very faint labeling, indicating that the olfactory neuronal cadherin may be similar to but not identical with N-cadherin. Supporting cells synthesize both cadherins for junctions with microvillar and duct cells or with neurons, respectively.

It is now possible to localize functionally important proteins to the different cell types by light-microscopic immunocytochemistry. This can be used in the future, for instance, to further our understanding of the still elusive functions of microvillar cells, and to definitely identify the olfactory neuronal cadherin.

4. 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. Stimulation is typically performed with substances believed exclusively to excite fibers of the olfactory nerve (vanillin, hydrogen sulfide, phenyl ethyl alcohol). Our own investigations in >80 subjects revealed the following major results.

EOG amplitudes increase in relation to the subjects' intensity estimates; latencies of EOG onset decrease with increasing stimulus concentrations.

An increase of stimulus duration produces an increase of EOG amplitudes; in contrast, latencies of EOG onset remain constant.

When using repetitive stimulation, the amplitude produced by the second stimulus is as great as the first responses' amplitude, indicating that the peripheral encoding is less subject to desensitization compared to the subjective perception of odorants.

In only two of 18 trials could clear responses to two olfactory stimulants (H2S, vanillin) be recorded in the same location; this indicates that odorant receptors are not dispersed homogenously throughout the olfactory epithelium.

Endoscopical localization of EOG recording sites indicates that olfactory tissue is located more anteriorly than previously thought.

EOGs are present even when stimuli are not subjectively perceived, which may provide the basis of subconscious behavioural modifications induced by odorants.

The significance of the EOG will be discussed, both as a research tool and as a tool for the clinical assessment of patients with olfactory disorders.

This research was performed in collaboration with: G. Kobal, Erlangen, Germany; M. Knecht, Dresden, Germany; J. Mojet, Vlaardingen, The Netherlands; D.A. Leopold, S.C. Hong, Baltimore, MD, USA; and J. Schwob, Syracuse, NY, USA.

5. The Vomeronasal Organ in Man

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First described 300 years ago and forgotten for a long time, the vomeronasal organ (VNO) was 'rediscovered' in man in the last decade. Although its function in animals and in the human fetus is obvious, it is still uncertain whether it serves chemosensory functions in humans. The vomeronasal nerve and its projection area, the accessory olfactory bulb, cannot be identified after birth. Nevertheless, its presence in adults is generally accepted. Here, the VNO is a tube with a blind end in the mucosa of the nasal septum with an orifice of a diameter ranging from 0.1 to 1 cm. We carried out an MRI study in which we filled the VNOs of the subjects with a hydrophilic contrast agent that revealed an extension of the VNO tube up to 40 mm in length. In an anatomical study we were able to show that this orifice is 2.65 cm (SD 0.47 cm) from the nostril and 0.92 cm (SD 0.42 cm) from the bottom of the nose. The VNO appears more often mono- than bilaterally. The frequency of the VNO's detectability does not depend on gender or age. In histological studies, the VNO shows a typical epithelial structure with prismatic basal cells and high prismatic cells in the upper part of the epithelium, that is different from the respiratory mucosa. We saw no positive reaction to immunhistochemical markers for neuronal structures such as PGP 9.5 or OMP. However, positive immunhistochemical reactions to PCNA indicate a high cell turnover.

In conclusion, the present data indicate that the VNO in man is a distinct anatomical structure. The question of its functional significance in humans remains unanswered.

6. Chemoreceptor Excitation through Temperature-gated Ion Channels

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Discovery of heat-activated ion channels and of the vanilloid

receptors VR1 and VRL1 in sensory neurons of the spinal and trigeminal ganglion has shed essentially new light on the transduction mechanisms by which inflammatory mediators and chemical irritants excite nociceptors and contribute to pain. Prostaglandin E_2, histamine and, most potently, bradykinin and low pH as well as capsaicin, mustard oil, phorbol esters and formalin (in low concentration) induce a prominent sensitization to heat of nociceptors which includes recruitment of previously unresponsive terminals. Various membrane-bound receptors and alternative second-messenger pathways, including PKC, cAMP and calcium influx, are involved in the transduction of the sensitizing effect. Different types of temperature-gated ion channels, including the vanilloid receptors, are the target of the sensitizing action which is probably mediated by protein phosphorylation.

With bradykinin and low pH application it can be shown that the nociceptor thresholds, which normally exceed 40°C, rapidly drop into the range of room temperatures (20–25°C) which enables the actual tissue or body temperature to induce a vivid discharge with a temperature coefficient $Q_{10} < 6$ in primary afferent nerve fibers. This apparently chemically but actually thermally induced activity is then subject to classical nociceptor adaptation and to the more or less slow inactivation or desensitization of the transduction pathway. However, even with bradykinin whose apparent excitatory effect fades within minutes, nociceptor thresholds stay well below body temperature in a very sustained manner which can be shown to depend on secondary prostaglandin formation induced by bradykinin. By that nociceptor sensitization, ongoing discharge and the resulting hyperalgesia are maintained for as long as the mediators are present in the inflamed tissue.

The novel unifying theory of previously diverse and multiple nociceptive mechanisms may provide new targets for pharmaceutical development as soon as the molecular elements are identified.

7. Breathing Pattern Analysis in Human Chemoreception

Stimulation of olfactory and trigeminal afferents is most readily achieved with normal inhalation or sniffing and there has been some research to study how the rate of delivery of stimuli (due to variation in inhalation or sniff flow rate) to the nose alters perception of a fixed odorant concentration. Studies of breathing changes as candidate non-verbal correlates of perception have typically included only intense stimuli well above the trigeminal threshold, although one of us (Walker *et al.*) has shown that concentrations of environmental tobacco smoke eliciting minimal odor sensation reliably result in slower and deeper inhalations. As part of our research effort to understand all aspects of the short-term effects of environmental chemicals, we combined precision air-dilution olfactometry with equally precise measurement of breathing before and after brief odorant/irritant stimulation. In one investigation, 20 normals and four anosmics were tested with a range of propionic acid (PA) concentrations extending from peri-threshold for normals to clearly suprathreshold for anosmics. Since stimulus presentation was triggered by exhalation, odorant concentrations reached full value before the beginning of the subsequent inhalation. Two seconds after this stimulus onset, normals exhibited cumulative inhaled volume (CIV) declines of 47, 11 and 4% with presentations of 59.2, 8.2 and 1.1 ppm PA, respectively; in decreasing order of concentration; the latencies of these declines were 440, 600 and 880 ms. With anosmics, 59.2 ppm caused a 19% decline in CIV that began at 420 ms. Examination of the first inhalation after stimulus onset shows that the CIV declines in normals were achieved by a progressive decline in volume beginning with 1.1 ppm and a marked decline in duration with only the highest concentration. Anosmics exhibit declines in volume and duration with only the highest concentration, with both being much more modest than the changes seen in normals. The greater sensitivity of normals demonstrates the importance of olfactory nerve stimulation in these rapid and sensitive physiological responses and seems to parallel our finding that perceived nasal irritation sensitivity is increased by the presence of the olfactory nerve. Thus, in both normals and anosmics, nasal irritation increases are tracked reasonably well by breathing declines. With odor, however, slight increases are observed in the absence of breathing changes. Collectively these results demonstrate that breathing changes are sensitive, rapid, reliable and differentially affected by olfactory versus trigeminal stimulation. Ongoing and planned research concerns the neural basis of these breathing changes, the degree to which breathing is altered by a compound which is a much less potent trigeminal stimulus (*n*-amyl acetate), breathing responses to mixtures and the possible value of breathing changes as an endpoint in studies of ocular irritation.

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8. Intranasal Trigeminal Chemosensitivity as a Valid Pain Model

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The trigeminal component of nasal chemosensitivity is employed in a valid experimental human pain model (Kobal, 1985, Pain, 22: 151–163). Short pulses (200 ms) of gaseous CO_2 introduced into the nasal cavity specifically stimulate nasal nociceptors. A short stinging pain is evoked that correlates with the CO_2 intensity of the applied stimulus. In response to these stimuli, pain ratings and pain-related cortical evoked potentials are recorded. The pain ratings, amplitudes and latencies of the evoked potentials correlate with the CO_2 concentrations of the applied stimulus. The evoked potentials have been shown by magnetic-source-imaging to be generated in the secondary somatosensory cortex, providing further evidence for their pain-specifity. The effects of stimulus repetition, stimulus interval, repeatability, and circadian issues have been addressed in separate studies. The combination of chemo-somatosensory evoked potentials and subjective pain ratings makes the pain model relatively insensitive to non-pain-specific drug effects. Unlike in some other acute pain models (electric tooth stimulation), the present pain model revealed no false analgesic effects of diazepam and imipramin on acute pain. The pain model was employed in many studies on analgesic effects of opioid (fentanyl, dihydrocodeine, morphine) and non-opioid

drugs (acetyl salicylic acid, ibuprofen, ketoprofen, flurbiprofen, diclofenac, azapropazon, propyphenazon). The studies focused on time- and/or dose-dependent effects, or have been conducted to investigate whether or not a specific compound has analgesic effects. The results obtained with this pain model have been clinically verified several times, and the health care authorities consider the presented model as a valid test of analgesic drug effects.

9. Psychophysical Effects of Menthol and Cold Air During Nasal Challenge

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Though menthol is well known for it's cooling properties on skin and mucous membrane, psychophysical tests examining effects in the nasal airways have been few. We investigated 19 healthy volunteers in a randomized, subject-blind, cross-over study where intensity ratings for pain, cooling and also 'sense of breathing' were graded for 10 min challenges with placebo (warm humidified air at 37C, 85% RH), three concentrations of mentholated air (1.6, 2.7 and 4.8 ppm menthol in warm humidified air) and cold dry air (CDA; 10C, 30% RH).

Cooling and pain in the nose were graded at fixed intervals during the 10 min challenge (every 15 s for the first 3 min, every 30 s thereafter), using a 0–9 numerical scale which was recorded online with a computer. Immediately post-challenge, 'sense of breathing' was graded on a 7 point pictorial rating scale. The quality of pain experienced (sudden, slowly spreading, piercing, burning, irritating, etc.) was also noted.

Menthol elicited dose-dependent increases in cooling and pain (from AUC values of response over 10 min). While the cooling response to mentholated air showed saturation above 2.5 ppm, no saturation was observed for pain even at the highest level of menthol (4.8 ppm) tested in this study. Within ~2 min of menthol stimulation, subjects adapted to pain while no adaptation was observed for cooling. CDA induced pain that increased during the 10 min challenge and did not adapt, indicating a different mechanism of pain production, probably related to chemical changes. Quality of pain was most often described as 'slowly spreading' and 'irritating' for CDA, and 'sudden', 'piercing' for menthol at 4.8 ppm. 'Sense of breathing' improved with menthol compared to warm and cold air controls, though a dose response was not observed. 'Sense of breathing' is not well understood and is probably dependent on several undefined peripheral and central factors. These results point to complex pathways and mechanisms mediating nasal sensation which need further investigation.

10. New Developments in fMRI

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Less than a decade after the first successful fMRI experiment was reported by Kwong *et al.* (Massachusetts General Hospital, Boston, MA), hundreds of publications per year demonstrate broad progress in research and development of this novel MR technique.

Data acquisition has been speeded up by scanner hardware improvements, such that full 3-D information from the human brain can be obtained with a temporal resolution of seconds, accompanied with a spatial resolution of few millimeters.

Since the signal-to-noise ratio is the limiting factor in fMRI, data have to be accumulated over minutes rather than seconds, leading to the generation of huge amounts of data. Postprocessing of the data typically took hours. Investigated subjects have left the scanner long before an interpretation of the experiment has taken place.

Improvements in computer technology, as well as the beginning of commercialization of post-processing software, now enable complex post-processing and display of the functional results in real time. A first implementation of real-time fMRI including 3-D motion correction, spatial filtering and correlation analysis including continuous updating of results at the main console of a commercial scanner will be presented in this paper.

The hardware and software developments reported in this paper allow a broad variety of users to carry out their own research without having access to a whole team of experts in data acquisition and interactive handling of post-processing packages.

On the other hand, development of stimulation paradigms as well as stimulation hardware is ongoing and it is still true that success of sophisticated fMRI experiments largely depends on the background knowledge of the researchers.

11. Functional Anatomy of the Olfactory System

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Arising from the nasal cavity, the olfactory nerves pass through the foramina of the cribriform plate to access the anterior cranial fossa and terminate at the olfactory bulb.

From the olfactory bulb the olfactory tract passes under the inferior surface of the frontal lobe to the olfactory trigone and divides into three striae (or roots). The lateral stria travels outward and leads to the hippocampal uncus, ambient gyrus and semilunar gyrus. The medial stria curves upward to reach the subcallosal and precommissural septal regions. The intermediate stria ends at the olfactory tubercle. The olfactory tract connects the olfactory bulb with the olfactory paleocortex (primary olfactory cortex).

The olfactory paleocortex is richly interconnected with the limbic system and other functionally important areas of the brain. Olfactory projections beyond the primary olfactory cortex include the orbitofrontal cortex, septal nuclei, amygdala, hippocampus, thalamic nuclei and hypothalamus.

Compression of the olfactory pathways may be caused by tumors of the central nervous system. Head injury often leads to disruption of olfactory nerves or olfactory tracts. Temporal lesions, particularly those of the hippocampal uncus, can lead to hyposmia or olfactory hallucinations.

Magnetic resonance imaging (MRI) allows the visualization of olfactory structures *in vivo*. Anatomical landmarks facilitate the identification of key structures. Studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have provided us with new insights into the functional neuroanatomy of the olfactory system. However, the specific limitations of each technique have to be considered.

12. Olfactory Brain-imaging: State of the Art

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To decipher the central processing and cognition of human olfaction is still a great challenge of modern physiology and psychology. The new imaging techniques such as fMRI (functional magnetic resonance imaging), MSI (magnetic source imaging) and PET (positron emission tomography) have begun to detail the functional neuroanatomy of the olfactory system.

The aim of this paper is to review the major strengths and weaknesses of the three techniques for studying olfaction by reviewing studies relating to olfactory functions. The number of imaging studies of olfaction still remains small when compared to studies of other sensory modalities and the use of functional neuroimaging as a diagnostic tool is even less common, but increased immensely over the past 5 years. The initial few studies in fMRI, as well as in MSI and PET, aimed primarily to identify the neuroanatomical regions active in olfactory processes in the human brain. Subsequently, studies were published that examined the role of the piriform cortex in the detection of odor. Next, studies that have attempted to characterize the regional CBF pattern associated with hedonic and semantic processes were published. Finally, some studies that have begun to explore odor memory mechanisms will be presented.

13. Exploration of Human Taste Perception with fMRI

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This study aimed at exploring taste cerebral activations in humans using functional magnetic resonance imaging (fMRI). We used a 3 Tesla MR scanner equipped with a head gradient coil for echo planar imaging. The study included ten subjects—five right-handers and five left-handers—and five gustatory stimuli (sodium chloride, aspartame, quinine hydrochloride, D-threonine and 5′-guanosine monophosphate). Data processing included extraction of gustatory activation by correlation of the NMR signal of each pixel to the individually recorded perception profile (Van de Moortele *et al.*, 1997, NMR Biomed., 10: 230–236).

Results showed that main areas activated during the gustatory perception were bilaterally located in the insula, the frontal operculum, the Rolandic operculum (base of pre- and post-central operculum) and the temporal operculum. All these areas were previously implicated in taste perception in the monkey (Ruch and Patton, 1946, Fed. Proc., 5: 89–90; Bagshaw and Pribram, 1953, J. Neurophysiol., 16: 499–508; Benjamin and Burton, 1968, Brain Res., 7: 221–231) and in the human (Bornstein, 1940, Yale J. Biol. Med., 13: 133–156; Penfield and Faulk, 1955, Brain, 78: 445–470; Motta, 1959, Bull. Sci. Med., 131: 480–493), and include the primary taste projections described in primates (Benjamin and Burton, 1968, Brain Res., 7: 221–231).

Comparing right- and left-handed subjects furthermore disclosed a lateralization of activation in the inferior part of the insula, which was reversed depending on the subject's handedness. In contrast to the superior insula, which was bilaterally activated, the inferior insula appeared predominantly unilaterally activated in the subjects' dominant hemisphere.

The specific status of left insula for right-handed subjects in taste perception is in accordance with previous PET data (Kinomura *et al.*, 1994, Brain Res., 659: 263–266) and new clinical observations (Pritchard *et al.*, 1999, Behav. Neurosci., 113: 663–671). The difference of lateralization between right- and left-handed subjects for chemoreception systems also agrees with results obtained for olfactory discrimination tasks (Hummel *et al.*, 1998, Chem. Senses, 23: 541–544). The hypothesis of some integrative processing of taste information occurring in the left insula of right-handed subjects is also supported by MEG data showing a late component localized in the left insula in response to an olfactory stimulation (Kettenmann *et al.*, 1997, Chem. Senses, 22: 493–502).

Our results show that the lateralization of some of the taste-related CNS processing depends on the handedness of the subject.

14. Human Cortical Areas Activated by Odorants: A Study by Magnetic Fields and Magnetic Potentials

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In humans, odorants activating neocortical areas have been investigated by magnetoencephalographic (MEG) recordings (Kettenmann *et al.*, 1996, Neurosci. Lett., 203: 143–145; Kettenmann *et al.*, 1997, Chem. Senses, 22: 493–502). The region between the superior temporal plane and the parainsular cortex was identified at latencies corresponding to the P1 component of the simultaneously recorded olfactory evoked potential (OEP). The anterior-central parts of the insular cortex were activated at latencies corresponding to the N1 component of the OEP and areas around the superior temporal sulcus (STS) were activated at latencies corresponding to the P2 component of the OEP.

In this study, we aimed to trace activated cortical areas by olfactory stimulation using a 64-channel whole-head SQUID system, different from that used by Kettenmann *et al.* for their recordings. During stimulation with low concentrations of phenylethyl alcohol (rose-like odor) we recorded magnetic fields (MFs) and OEPs (Cz) simultaneously. The duration of a single recording lasted for 2000 ms and a pretrigger time of 400 ms was adopted. A stimulus duration of 200 ms and an ISI of 40 s were used. Thirty stimuli were presented during one session. Five subjects participated in this experiment several times each for individual reproducibility. Clear magnetic responses started at ~300 ms after stimulus onset and peaked at ~600–700 ms. We estimated equivalent current dipoles (ECDs) by using a spheres model. ECDs were localized in the insular cortex, generally activated at latencies of the N1 component of the OEP responses and around the STS at latencies corresponding to the P2 component, bilaterally. So far, no valid results have been obtained for ECDs close to the latencies of the P1 component.

Our data confirmed parts of the results reported by Kettenmann *et al.* Ongoing data analysis estimating the ECDs by using a realistic model will give further insights into the processing of olfactory information.

15. The Use of Stimulus-dependent Behavioral Reactions as Valid and Reliable Tools in Testing Chemosensory Functions

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Feelings—sensations induced by stimulation of any of the sensory pathways—are not accessible to direct measurements. Sensory testing for both clinical and other purposes must, therefore, be based on indicators which can be evaluated either by direct quantitative or semiquantitative methods. Psychophysical testing (based on cognitive functions and verbal reports) is applicable only to fully conscious cooperative human examinees at verbal age. Testing of preverbal infants or adult examinees deprived of verbal communication, as well as testing sensory functions in animals, must be undertaken using other indicators.

Registration of event-related bioelectrical phenomena, changes in somato- or visceromotor functions, and secretory responses are somatic manifestations which can indicate aspects of the workup of a sensory message by the organism. Chemical cues are more polarized as to their hedonic (pleasure/displeasure) tone, than are stimuli of any other modality. In our earlier studies, chemical cues were found to induce innate, probably inherited, facial expressive displays. These fixed, stereotyped, distinct and differential sensory–motor coordinations are indicative of the intensity and hedonics of perceived taste or odor sensations. These were first demonstrated in normal, healthy, termborn human infants at perinatal age and were termed 'gustofacial' and 'nasofacial' responses. It was further determined that these reflexes are primarily controlled by subcortical brain structures and are independent of visual reinforcement or of higher brain functions. In later studies different animal species were also found to have analogous behavioural reactions. Video-recordings of 'gustofacial' and 'nasofacial' responses of human examinees, using a carefully designed paradigm, combined with simultaneous psychophysical examination, enabled us to develop a special, multidisciplinary testing method. The psychophysical examination was expanded with special questions to assess utilization of environmental odor cues.

From the results of testing a large sample of healthy examinees in different age groups as well as patients in different pathological conditions, the following conclusions could be drawn: (i) the verbal responses to the special questions reliably indicate to what extent testees use environmental odor cues for orientation; (ii) facial displays are most sensitive indicators for both intensity and hedonics of perceived taste and odor sensations; (iii) these facial behaviors can easily be read, interpreted and semiquantitatively rated by any naive adult observer; (iiia) inter-evaluator correlation calculated on ratings provided by an expert, somewhat experienced and completely inexperienced evaluators show high coefficients.

Examples of results will be demonstrated showing: (i) the correlation between self-estimates of taste- and odor-hedonics and ratings of their facial displays; (ii) potential use of stimulus-dependent changes in heart- or respiratory-rate as an additional, supportive parameter reflecting intensity and hedonics; (iii) taste and odor-reactivity in (a) elderly senile patients; (b) in patients with affective disorders; (c) in testees addicted to heroine; (d) in testees with excessive consumption of alcohol will be demonstrated; (e) findings of olfactory performance in patients, referred because of endocrine malfunctions (suspected Kallmann's Syndrome, delayed puberty, etc.) will also be summarized.

All these examples show that critical observation, documentation and semiquantitative evaluation of stimulus-dependent facial behaviors can be used as a reliable and valid tool in the assessment and evaluation of the functional state of chemical senses.

16. Chemosensory Evoked Potentials and Olfactory Thresholds in Normal Subjects with High Self-rated Odour Reactivity

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The symptomatology of patients suffering from 'multiple chemical sensitivity' (MCS) or 'idiopathic environmental intolerance' (IEI) is often triggered by odourous chemicals (e.g. perfumes, household chemicals, paints, lacquers, etc.). Alterations of intranasal chemoreception in carefully diagnosed MCS patients has recently been demonstrated by means of chemosensory evoked potentials (CSEPs) as well as by psychophysical tests of olfactory sensitivity and discrimination (Roscher *et al.*, 1999).

The purpose of our pilot study was to check relevant aspects of objective and subjective chemoreception in subjects outside the patient range. Students with high ($n = 15$) or normal levels ($n = 16$) of odour reactivity (questionnaire-based self-ratings) were selected in a first step. In a second step CSEPs to olfactory (H_2S) or trigeminal (CO_2) stimulation were measured according to Kobal and Hummel (1991). In addition (third step), olfactory thresholds for H_2S were determined in a subsample ($n = 16$) according to VDI 3881 (1986).

No group differences were found for CSEP amplitudes. Systematic, small, group differences of borderline significance ($P < 0.1$) occurred for CSEP latencies, however: subjects with a high level of odour reactivity exhibited shorter latencies than normal subjects, both for olfactory and for trigeminal stimulation. As for odour thresholds, marked group differences were also found, with normals exhibiting a lower median H_2S threshold (2 $\mu g/m^3$) than subjects with a high degree of odour reactivity (5 $\mu g/m^3$). Data collection is ongoing in order to determine if the observed alterations of chemoreception can be stabilized in a larger sample and, additionally, to check for the specificity of CSEP alterations.

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17. Coping with Olfactory Dysfunction and its Consequences for Life Quality and Evaluation of Treatment of Olfactory Dysfunction in Nasal Polyposis

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Considering the importance of olfaction in everyday life, we have studied consequences and effects on quality of life and coping mechanisms after olfactory loss. Among patients with olfactory dysfunction and nasal and sinus disease a majority have complete loss of olfactory function (anosmia). Several studies have showed that steroids can improve olfaction in nasal and sinus disease.

Some authors claim that sinus surgery improves olfaction, but there is a lack of randomized controlled prospective studies investigating whether surgical treatment in fact has an additive effect to medical treatment or not.

Seventy-two patients with self-reported anosmia (44%) or hyposmia (56%) participated. Reduction in smell sensitivity was verified by olfactory thresholds for 1-butanol. Coping and consequences were assessed by questionnaire.

In a randomised prospective controlled trial 32 patients with nasal polyposis and symmetrical nasal airways and no previous surgery for nasal problems were investigated. Before randomization, all patients were treated for 1 month with nasal steroids bilaterally and systemic steroids for 10 days. Thereafter the patients were randomized to FESS (functional endoscopic sinus surgery) on either left or right side and continuous nasal steroids bilaterally. Patients scored their symptoms by visual analogue scales (1–100), at start, preop and 1, 3, 6 and 12 months postop. Olfactory thresholds were assessed with butanol test at start, preop, 3 and 12 months postop.

Data from the life quality study showed that a majority considered that life quality in general had deteriorated after the onset of their dysfunction and almost everyone considered olfaction to be of more importance after than before their dysfunction started. Three-quarters experienced risks caused by their dysfunction and a majority found their daily routines to be negatively affected. One-third of the patients experienced that their dysfunction affected health and half of these latter patients reported depression. Reduced food appreciation was reported by 50%. Results from the nasal polyposis study showed that olfaction was improved by local and oral steroids in combination up to 1 month postop, but surgery had no additional effect.

Our findings suggest substantial adverse effects on life quality after olfactory dysfunction. Certain mechanisms are commonly used to cope with these problems. These findings indicate the importance of further research on diagnosis and treatment of olfactory disorders. Regarding treatment of nasal polyposis, our results indicate that if hyposmia is the dominating symptom then surgical treatment does not have an additive effect to medical treatment, as judged by olfactory thresholds and symptom scoring. However, if—after steroid treatment of nasal polyposis—nasal obstruction is the major problem additional surgical treatment is suggested.

18. Olfactory Event-related Potentials: Sensory and Cognitive Components

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Olfactory event-related potentials (OERP) have been utilized in a series of investigations probing the psychophysical and neuropsychological correlates of early and late components. Stimuli for the OERP were presented in an air-dilution olfactometer, incorporating features of Kobal's earlier designs, which delivered the test odorant with a rapid rise time (<20 ms), heated (to 35°C) and humidified (to 80% RH). EEG activity was recorded from Fz, Cz and Pz. Grand averages were computed over subject groups. Participants included both males and females, who were assessed for nasal health. Olfactory function was also assessed with psychophysical and neuropsychological measures for comparison. Results showed significant effects of both peripheral and central loss on the OERP, with greater impairment in the early components (N1/P2) when peripheral areas were involved and greater impairment reflected in later components (P3) of the OERP when central areas were involved. In the case of peripheral involvement, amplitude and latency were significant indicators of olfactory impairment and correlated with psychophysical measures of olfactory loss. In the case of central involvement, latency of the P3 was a more robust indicator of impairment than amplitude and correlated with measures of cognitive function. The results support the sensory nature of the early components and the cognitive nature of the later components of the OERP.

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19. Is the Reduced Olfactory Stimulus Processing in Patients with Major Depression a Trait-marker?

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The aim of the present study was to investigate olfactory perception in patients with major depression (MD) by means of chemosensory event-related potentials (CSERPs) analysis. Moreover, it has been examined whether olfactory perception in MD-patients can be altered by a successful therapy. ERPs in response to colors were obtained to control for modality specific effects. ERPs in response to visual emotional stimuli (pictures from the International Affective Picture System, IAPS) were recorded to find out whether a change in olfactory perception is related to the emotional aspect of odor perception.

Twenty-three patients with MD (15 male, eight female; mean age = 46.39 years, SD = 10.84) were compared with 23 control subjects (15 male, eight female; mean age = 47.52 years, SD = 12.14). All subjects participated in two sessions; for the patients the first session was held at the beginning of a therapy and the second after successful treatment. The control subjects were examined within a similar time interval. During the first part of each EEG session, two odors (isobutyraldehyde/butter acid and phenylethyl alcohol/rose) were administered using a constant flow olfactometer. In the second part, two colors (yellow and red) were presented by a slide projector and in the third part pictures from the IAPS were shown. The subjects' task was to discriminate the odors and colors by their quality and the IAPS slides by their valence. The EEG was recorded from 32 scalp locations.

In the first session the early positivities within the CSERP were reduced in MD patients. This effect was most pronounced at frontal electrode sides (P2: $P = 0.028$; P3-1: $P = 0.007$). In response to rose odor, but not to butter odor, the late positivity of CSERP was also smaller in patients than in controls (P3-2: $P = 0.022$). When colors or IAPS slides were presented the N1/P2 complex was not altered in patients. However, the amplitudes of the late positivities evoked by colors (P3: $P = 0.018$; P4: $P = 0.005$) and emotional slides (P3: $P = 0.029$; P4: $P = 0.032$) were generally smaller in MD patients.

CSERPs in MD patients did not change significantly from the

first to the second session. However, the P3 in response to colors was significantly smaller at frontal electrode sides in the second session ($P = 0.018$). The P2 in response to negative IAPS slides was significantly larger in the second than in the first session ($P = 0.039$). Furthermore, the topographic distribution of the P3 in response to the emotional slides was altered after medical treatment ($P = 0.038$).

According to these results the reduced olfactory perception in MD patients can be interpreted as a trait marker.

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20. Taste and Smell—Testing Former Workers in the Chemical Industry 10 Years after Exposure

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The respiratory tract is affected directly, continuously and intensively by harmful aerogenous substances. We have examined the influence of substances such as phosphorus, calcium–nitrogen, carbide, urea, soot, chromium and welding smoke on the degustation and the olfactory senses 10 years after exposure.

Fifty-three people were examined 10 years after exposure to the harmful substances (group II). The first examination took place 15–25 years ago (group I). All of the persons examined were smokers or had smoked in the past. We compared the results with a control group of 50 non-exposed persons of the same age (group III). This group consisted of more non-smokers. The examination of the olfactory sense was effected using sniff-bottle techniques with single olfactory substances, mixed olfactory substances and trigeminal stimulants, and additionally with 'blanks'.

We carried out the degustation test by the application of sweet, sour, salty and bitter substances to each side of the tongue.

The examination techniques were absolute identically to those used 25 year ago.

In the first examination five people were affected by degustation and olfactory disturbance, compared to seven people in the follow-up examination and six in the comparison group. Twenty-nine persons in group II were suffering from nasal obstruction, epistaxis, dry nasal mucosa and frequent sneezing.

The examination with single olfactory substances showed similar results in all three groups.

In all three groups we found more pathological results in the olfactory test, without a group-specific significant difference. The perception of mixed olfactory substances was significantly better in group III than in group I.

The sensibility of the N. trigeminus was highly significantly reduced in group I compared with groups II and III.

We found a significantly better perception of the 'salty' stimulus in group II. In group III the perception of the 'sweet' and 'bitter' stimuli was reduced to a highly significant extent.

The medical statistic group comparison showed no difference between the perception of sweet, sour, salty and bitter qualities.

In summary, the exposed persons in group I exhibited reduced perception of the mixed substances and the trigeminal stimulants, but otherwise there was no statistically significant difference in the perception of single olfactory substances and gustatory solutions.

21. Effects of Exposure to Irritants

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Chronic or repetitive exposure to an olfactory stimulus typically results in olfactory adaptation, a stimulus-specific decrease in the detectability and perceived intensity of the olfactory stimulus. In contrast, some reports have suggested that similar exposure to a chemosensory irritant leads to increases in sensitivity, although other evidence suggests that adaptation does occur for irritants. This study examined whether repetitive exposure to an irritant stimulus modulates either the psychological or the physiological response to that chemical.

Using a long-term adaptation protocol, we exposed eight men and four women to acetic acid vapor in their home environment. Before, during and after 3 weeks of exposure to acetic acid, we obtained chemosensory event-related potentials (CSERP) as a measure of central processing, nasal mucosal potentials (NMP) as a measure of peripheral response to sensory irritation, intensity ratings and lateralization thresholds to three concentrations of acetic acid (exposure odorant) and three concentrations of acetone (control odorant). Reaction times were also obtained as measures of stimulus detectability.

The CSERP and NMP showed good agreement with the psychophysical measures. Both the amplitude of the physiological measures (CSERP and NMP) and the ratings of intensity increased with stimulus intensity and, on all measures, responses to acetic acid decreased during and following exposure, indicating a long-term effect from exposure to acetic acid, both peripherally and centrally. In contrast, responses to acetone showed similar effects of stimulus intensity but little change over the course of long-term exposure to acetic acid.

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22. Irritation of Rat Nasal Mucosa and Meninges causes Peripheral Plasma Extravasation and Release of Substance P in the Trigeminal Brainstem

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Trigeminal tissues such as the nasal mucosa and the meninges are richly innervated by small diameter, presumably nociceptive afferents, a considerable proportion of which contains the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP). Stimulation of these tissues by irritants such as capsaicin or mustard oil causes release of these neuropeptides and mediates processes of neurogenic inflammation. While CGRP causes vasodilation of precapillary (arterial) vessels and increased blood flow, SP seems to be responsible for the extravasation of blood plasma from postcapillary venules. These reactions were demonstrated using laser Doppler flowmetry and a silver colloid technique, respectively. SP release occurs not only in peripheral trigeminal tissues, however, but also in the spinal trigeminal nucleus from central terminals of trigeminal afferents, which was shown by the antibody microprobe technique. Both peripheral and central release of neuropeptides are likely to contribute directly or

indirectly to sensitization of nociceptive afferents and hyperexcitability of neurons in the trigeminal nucleus. So far it is unclear under which pathophysiological conditions these mechanisms are involved in painful events at the mucosa and in headaches, and to what extent inhibition of neurogenic inflammation has therapeutic effects in these diseases.

23. Odor Memory

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Until recently, research on odor memory has been dominated by methods that presuppose verbal mediation. Although some authors have critically reviewed this research and have provided strong arguments for the existence of a separate sensory memory for olfaction, even they have used methods that are based on explicit learning and explicit memory retrieval. Nevertheless, in normal everyday life most olfactory learning is implicit (incidental instead of intentional) and implicit memory and retrieval (without awareness of previous experience with the odor) are the rule rather than the exception.

Implicit learning and implicit memory for odors have been studied in three experiments using a new method. The results show not only that implicit memory for odors exists, but also that it may easily be disrupted by verbal knowledge of the odor name. The implications of this finding for psychophysical and psychological research on olfaction will be discussed.

24. Effects of some Olfactants and 'Vomeropherins'

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In a double-blind study, estradienol and methoxyestratetraene were applied to the upper lips of 240 men and women. After the application, five persons of the opposite sex (standardized whole body photographs) were assessed on 19 bipolar seven-stepped ratings scales. The session ended with a structured interview. Each subject was tested once by an experimentor of the same sex. Under estradienol the women rated the men as better, tougher and more nonchalant than under the control. Under the influence of methoxyestreatetraene the men assessed the women as better, kinder and more likeable than under the control.

In another study, an initial encounter between a man and a women was videotaped for 10 min. Estratetraenol respectively androstadienone were applied to the cheek before the recording. The videotapes were analysed with respect to body positions, movements and defined flirtation signals. At the end a questionnaire was administered. Under the influence of the substances the initiative to contact the other and the fear of rejection increased, while the attractiveness and sympathy ratings decreased. Important behaviour variables are voiced and unvoiced laughing.

25. Retronasal Olfactory Testing

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Aims of the study were (i) to develop a test kit for assessment of retronasal olfactory testing and (ii) to compare ortho- and retronasal function in patients with olfactory disorders and healthy controls.

To assess retronasal olfactory function we tested a total of 236 individuals with normosmia, hyposmia and anosmia. Orthonasal olfactory function was asessed psychophysically using the 'Sniffin' Sticks'. In addition, anosmia was confirmed electrophysiologically by means of olfactory evoked potentials. For retronasal stimulation groceries was used, all of which were available as powders, e.g. spices, instant drinks or instant soups. Thirty different substances were applied using squeezeable plastic vials with 6 cm long spouts. Although subjects were free to sample as much as needed, ~0.05 mg were placed for each single trial on the middle of the anterior portion of the tongue. After each trial subjects rinsed with tap water. Each substance was identified (forced choice) by means of a list with four verbal items. In addition, subjects indicated whether the substance was sweet, sour, salty or bitter.

From 30 items used for retronasal testing, 20 were selected according to the degree to which they were identified by normosmics and ansomics. Specifically, substances left out were those which: (i) were poorly recognised by normosmics (caraway, anise, blueberry, almond, sour cherry, bacon, mustard, coconut) and (ii) were identified to a similar degree by hyposmics, anosmics and normosmics (lemon, pepper).

In healthy subjects test–retest reliability for retronasal olfactory function was found at $r_{27} = 0.76$, which compares to other odor identification tests. Results for retronasal testing in normosmics (55 men, 63 women; mean age 33.5 years) allowed discrimination of gender-related differences with women scoring higher than men ($t = 2.77$, d.f. 118, $P = 0.007$), and the identification of a slight age-related decrease ($r_{118} = -0.20$, $P = 0.027$). The correlation between retronasal and orthonasal olfactory function for normosmics and hyposmics was found at $r_{86} = 0.74$, $P < 0.001$. However, when looking only at hyposmics this was no longer present ($r_{37} = 0.21$, $P = 0.21$). Retronasal testing allowed the discrimination between normosmics, hyposmics and anosmics [$F(2,229) = 279$, $P < 0.001$]. Interestingly, identification scores in anosmics improved slightly with duration of anosmia ($r_{60} = 0.30$, $P = 0.018$). No clear differences were found between patients with anosmia of different origin.

In conclusion, assessment of olfactory function is possible using oral stimulus presentation. Effects of age and gender are found for both retronasal and orthonasal testing. The test allows discrimination between normosmic, hyposmic and anosmic patients. In hyposmia, scores obtained by retronasal testing did not correlate with orthonasal scores. This may be due to factors such as interactions between the intraoral senses and the sense of smell. Results obtained in anosmics may also indicate adaptive changes in the processing of intraoral sensations.

26. Wiener Olfaktorische Testbatterie (WOTB)—A New Clincial Test to Evaluate Olfactory Functions

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In German-speaking countries no generally accepted clinical test for the assessment of olfactory functions is available. The goal of the present report was to study the reliability and validity of the newly developed Wiener Olfaktorischen Testbatterie (WOTB). The WOTB is a multiple-choice testing procedure to evaluate olfactory identification ability using common natural odorants. Ninety-seven healthy controls aged between 18 and 92 years were included in the study. The WOTB was shown to be highly sensitive for

age-related odor identification decline. Additionally, patients with Alzheimer's disease, Parkinsons's disease and cerebral trauma showed significantly reduced olfactory identification performance. The internal validility (= 0.71) and the test–retest reliability (r = 0.75) were satisfactory. Thus, the WOTB is a new clinical test for the assessment of olfactory functions.

27. Olfaction and Neurodegeneration

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Most neurodegenerative diseases are associated with some impairment of olfaction—particularly idiopathic Parkinson's disease (IPD) and Alzheimer's disease (AD). Despite current fashion the olfactory defect may be aetiologically more important than the associated disorders of movement or cognition

Olfaction was examined in the following neurodegenerative conditions: IPD; progressive supranuclear palsy (PSP); multiple system atrophy (MSA); corticobasal degeneration (CBD); motor neurone disease (MND); and AD. We used the University of Pennsylvania Smell Identification Test (UPSIT) and obtained olfactory-evoked potentials (OEPs) in response to H_2S gas. Pathological studies were performed on olfactory bulbs in IPD and MND.

For IPD, 126/155 (81%) patients had abnormal UPSIT scores which were significantly lower than those for controls: $P < 0.0001$; 12/37 (32%) had prolonged latency with normal amplitude measurement on OEP, but 27 had absent or unclear readings. For MND, 9/58 (16%) were abnormal on UPSIT. OEP were performed in 15 patients. In nine the responses were normal for latency and amplitude measurements. In AD, UPSIT scores were abnormal in all eight patients examined. OEP was normal in the four subjects who could be tested. In the remaining conditions—PSP, MSA and CBD—only UPSIT was performed. Olfaction was normal in all except the MSA group. All olfactory bulbs from IPD patients showed typical Lewy bodies. There was excess lipofuscin deposition in the MND olfactory bulb neurons.

Most neurodegenerative disease is associated with olfactory disorder. We have not investigated Huntington's Chorea, but patients suffering from this disease also have olfactory impairment. The significance of this association is unclear, but it might provide a clue to the underlying mechanism of disease.

28. First Clinical Experience with a New Olfactory Recognition/Threshold Test

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Olfactory testing in routine examinations is not yet a generally accepted and widely performed procedure. In contrast to otological diseases where audiology plays a major role in the diagnostic workup, olfactory testing is denied in most insitution for rhinological cases. Important disadvantages of the detailed examination of the olfactory function consisted of limited availability of special examination tools and barriers due to conceptuality in different languages.

Based on the well-established 'TDI'-score with detailed examination of threshold, discrimination and identification, we designed a new olfactory test for rhinological purposes. The main requirements consisted of limited examination time, availability for repeated examinations of the same person without learning curve, ease of applicability and evaluation, and cost-effectiveness.

The actual clinical test, the 'Random Threshold Test', consists of a 'Sniffin' Stick' row with two odourants. Phenylethyl and citronelal are presented in 16 steps of concentration besides six blanks in a randomized order. The patient has to describe the odour as lemon, rose or water. By using 38 sticks the overall correlation compared to the TDI test was found to be as high as 0.8. By limiting the number of presented concentrations we tried to shorten the required time to ~5 min per tested side (whole nose or side-different). The upper four concentrations were left out and the blanks were reduced to four in number.

Patients' acceptance of the testing was favourable. The necessary instructions for patients are minimal. Exhausting discussions with sometimes angry patients were discontinued.

Initial testing in rhinological patients has been made. The procedure requires <10 min per patient and hyposmia and anosmia can be suspected based on the limited test with 28 sticks. Detailed analysis of the results in 50 patients with respect to test–retest reliability with differing examiners and comparison to the 'TDI' test are presented.

29. Taste Function in Xerostomia before and after Saliva Replacement Therapy

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Xerostomia may affect individual dietary habits, nutritional status, oral hygiene and speech, and may lead to decreased gustatory sensitivity. The present study specifically investigated effects of saliva replacement therapy on taste function.

Whole-mouth gustatory function was assessed in 25 patients suffering from xerostomia (six male, 19 female; age range 42–82 years) before and after 4–6 weeks of saliva replacement therapy using a preparation containing carboxy methyl cellulose. Results were compared to healthy controls matched for age and gender (six male, 19 female; age range 42–82 years). Taste function was assessed quantitatively for sucrose, citric acid, sodium chloride and caffeine.

All patients easily detected the four taste qualities at the highest concentration. However, patients with xerostomia had significantly lower scores in the gustatory test compared to healthy controls ($P < 0.001$). No correlation was found between duration of xerostomia or severity of the disorder. While therapy had no effect on taste function ($P = 0.33$), saliva replacement led to a significant improvement of other xerostomia-related symptoms ($P < 0.001$).

In conclusion, the study confirms previous work indicating that xerostomia is accompanied by decreased gustatory sensitivity. Results of this pilot study also seem to indicate that the routinely

performed replacement of saliva has little or no effect on whole mouth gustatory function.

30. The Feeling of Nasal Congestion is not well Correlated to the Objective Measures of Nasal Patency

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The feeling of nasal congestion or nasal stuffiness has been correlated with objective parameters of nasal patency in numerous studies. Especially for ENT surgeons, it seems important whether they can rely on the patients perception of the nasal passage pre-operatively. No constant and convincing relation has been found. We have collected data from different studies comprising >1000 subjects examined by acoustic rhinometry, measuring the nasal area as function of distance, and a questionnaire. In a number of studies correlation between subjective and objective evaluation of nasal patency has been examined 'within subjects' during allergen or histamine challenge. Rhinomanometry, measuring the pressure–flow relationship, has been compared with acoustic rhinometry and the subjective feeling of nasal occlusion in 80 subjects. Correlation between nasal dimensions evaluated by MRI, acoustic rhinometry and questionnaire has also been examined in ten subjects.

Generally, the inter-individual correlation with subjective evaluation was similar for rhinomanometry and acoustic rhinometry ($R = 0.3$, $n = 80$). Poor intra-individual correlation ($R = –0.2$ to 0.2, $n = 5$) between acoustic rhinometry and occlusion score was found in 24 subjects undergoing repeated weekly measurements. Nasal dimensions evaluated by MRI showed results equivalent to acoustic rhinometry in correlation with subjective evaluation. The best correlation between VAS and the minimum cross-sectional area in the nasal cavity ($R > 0.9$, $n = 64$) was seen in in a few subjects during repeated allergen challenge, whereas others in the same study showed no correlation. In some subjects, 'chronic' septal deviation does not affect the feeling of nasal congestion, while in others minor structural changes induce the feeling of nasal congestion. Based on the results from all studies it appears that a large change in the dimension of the wide cavity and a minor change in the small cavity is necessary to induce the feeling of congestion. A study, using neural network for the analysis, showed that other nasal symptoms such as runny nose, itching and sneezing are influencing the feeling of congestion.

31. The Impact of a Changed Steroid Sensibility to Mucosal Reactivity in Topic Histamine Challenge Tests in Allergic Humans

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Patients with upper airway distress often describe stuffy, runny noses and itching as well as hyposmia or anosmia and pain in the frontal part of the face. The symptoms can be temporary and associated, for instance, with ongoing acute infections or with an allergic reaction. Nasal polyps or chronic rhinitis and sinusitis are also common causes. The symptoms can also be associated with environmental causes such as air pollution.

In the study of the pathology underlying nasal mucosal disease, an inflammatory cause is often suspected; mucosal swelling and micro-circulation are important as well as the content and volume of nasal secretions. Trigeminal and olfactory nerve function and response to stimuli within the nasal cavity are both probably dependent on the status of the mucosa and particularly its superficial layer.

The most common treatment for nasal disorders is steroids, often topically. The majority of patients are improved but some need higher doses or are not helped with the drug.

A new non-invasive technique has been developed to measure mucosal swelling, micro-circulation and tissue oedema, and content of secretion simultaneously and repeatedly. The anterior part of the nasal cavity is viewed under magnification and the equipment makes it possible to monitor swelling changes and to introduce and monitor the position of measuring instruments such as probes. Up to now we have used a laser Doppler probe to study the concentration of moving blood cells (CMBC), the velocity of moving blood cells and the product of these, called 'perfusion' or 'flow'. With a forceps small discs can be placed on the mucosa surface to collect secretion for analysis. In this way dynamic processes over time can be studied.

Twenty-seven subjects, 13 normals and 14 patients with birch pollen allergy, all free from symptoms, were studied during winter. The subjects were studied in a double-blind, cross-over procedure before and after administration of 100 l in each nostril twice a day for 1 week of either saline 0.9 mg/ml or intranasal steroid (Budesonide) 64 g/dose (one dose = 50 l). The mucosa was challenged with 0.14 ml of a 1 mg/ml solution of histamine dihydrochloride.

Eleven of the pollen-allergic patients were further studied during the pollen season. They were studied before and after treament with Budesonide and studied in the same way as during winter.

We found that the allergic non-symptomatic mucosa before the pollen season differed from the normal. The CMBC was higher, the swelling reactivity to histamine was stronger and a more pronounced and prolonged oedema development was registered. Further, the allergic mucosa had a reduced response to intranasal steroid treatment, both with respect to swelling, oedema development and albumin leakage.

During the pollen season we found, compared to in winter, an increased inflammatory activity. Pre-challenge, the perfusion was higher and a higher albumin level in the secretion was also seen. After histamine challenge a more pronounced oedema development was seen. In contrast to during winter, the allergics now showed a good response to intranasal steroid treatment, both with respect to pre-challenge perfusion, pre-challenge albumin leakage and oedema development following challenge.

The group of allergic patients changed their responsiveness to steroid treatment from non-responsiveness during winter to responsiveness during the pollen season. Therefore, steroid sensitivity in the tissue seems to change over the year. As steroids modulate the reactions of almost every cell in the body following stimulation, the tissue sensitivity to steroids should be taken into consideration in challenge studies.

32. Respiration Olfactometry—The Respiratory Reactions Evoked by Smelling of Different H_2S Concentrations

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Respiration olfactometry is an objective method for testing the ability to smell. This measuring process presents a completion to the olfactory and chemosensory event-related potentials, particularly in medicolegal cases. The question of this study was: 'Are the changes in respiration pattern evoked by lower stimulus concentrations significantly clearer than those invoked by higher stimulus concentrations?' Normosmic voluntary test persons were stimulated with different concentrations of H_2S for 2 s. The nasal flow rate was registered by a differential pressure sensor in one nostril, the other nostril was stimulated olfactorily by means of a flow olfactometer. The smell-induced respiration reactions in the respiration curve were evaluated with reference to the changes in the amplitude, frequency, area and curve form.

The respiratory reactions evoked by smelling were very different between individuals. For the evaluation one or two respiration cycles at/after the stimulus were compared with five respiration cycles immediately before the stimulus. The changes in the pattern of the respiration curve at the stimulus can be described as sniffing respiration and as changes of inspiration volume, of the amplitude or the respiratory frequency. In ~3/4 of the test persons the most intense olfactory-evoked respiratory reaction was found on the weakest olfactory stimulus.

33. Psychophysiology and Biochemistry of Nasal and Pharyngeal Irritation

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The upper respiratory tract, the nose as well as the pharynx, is a site of numerous diseases. Many of them are accompanied by inflammation or irritation of the respiratory mucosa. In order to investigate underlying mechanisms and therapy of irritation, controlled experimental models are required.

We have used constantly applied cold dry air (CDA) in numerous previous studies to demonstrate the effects of NSAIDs (non-steroidal anti-inflammatory drugs—ibuprofen, ketoprofen, azapropazon) in volunteers. This experimental irritation model has been extended to the pharyngeal mucosa. In this model, psychophysical parameters (e.g. estimates of intensity and aversity) were acquired. Since psychophysical measurements in general are quite susceptible, objective parameters are useful to provide additional information on irritation. Quantification of experimental nasal irritation by CDA was demonstrated by determining inflammatory mediators in nasal lavage fluid (Mohammadian *et al.*, 1998). An investigation was made to determine if an objective indicator of inflammation could be found in pharyngeal lavage fluid.

Inflammatory mediators (PGE2, TXB2 and SP) were significantly elevated compared to baseline values (PGE2: $P < 0.05$, $F = 3.54$, d.f. = 2; TXB2: $P < 0.05$, $F = 5.193$, d.f. = 1.539; SP: $P < 0.01$, $F = 16.3$, d.f. = 1.539). This demonstrated that biochemical parameters can be used to provide evidence of irritation in the pharynx.

In conclusion, this additional objective information on irritation applied to the nasal and pharyngeal cavity contributes to a further validation of the model. It enhances the suitability of the model to investigate systemic or local drug effects in man.

34. Volatile Compounds in Human Breath

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The detection of volatile compounds in the human breath could be a helpful non-invasive tool in the diagnosis of several diseases in the clinical routine. Used in conjunction with established analysis systems, the abnormal presence of some molecules, for instance hydrogen, hydrogen peroxide or acetone, in the exhaled air gives additional information on the nature and intensity of, for example, fructose intolerance, inflammations, blood sugar level or liver diseases. In the present study a system will be presented which allows on-line detection of some relevant compounds. The system enables sensitive detection in the human breath in the presence of water, oxygen and the other common compounds in the range of parts per billion (Bischoff *et al.*, 1998, Biomed. Techn., 43: 266–267).

35. The Role of Neuronal Nicotinic Acetylcholine Receptors in Nicotine Perception

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Nicotine applied to the nasal cavity can evoke 'odorous' sensations in the concentration range near the detection threshold by the activation of the olfactory sensory system and at higher concentrations 'burning' and 'stinging' sensations by the dose-dependent recruitment of C- and A-fibers of the trigeminal sensory system. Neuronal nicotinic acetylcholine receptors (nnAchR) subunits are expressed in trigeminal primary afferents and could constitute the receptors involved in nicotine perception.

In order to test the hypothesis of stereoselective activation of nnAchR by nicotine enantiomers we conducted two studies.

In humans we dose-dependently investigated the stereoselective effects of R(+)- and S(–)-nicotine on the trigeminal and olfactory sensory system by determining trigeminal and olfactory detection thresholds, recording summated electrical responses from the respiratory nasal mucosa during stimulation with (R(+)- and S(–)-nicotine vapor (40, 80, 120, 160 ng/ml; stimulus duration = 250 ms) and registering intensity ratings of 'odorous', 'burning' and 'stinging' sensations.

In rats we dose-dependently investigated the stereoselective effects of R(+)- and S(–)-nicotine and the effects of hexamethonium during stimulation with R(+)- and S(–)-nicotine and CO_2 on responses of primary trigeminal afferents recorded from the ipsilateral Gasserian ganglion.

We found significant stereoselective differences in activating the trigeminal sensory system in humans [higher summated responses, higher trigeminal intensity estimates and lower trigeminal detection thresholds for S(–)- compared to R(+)-nicotine] and in rats

[significant lower spike-responses for R(+)- compared to S(–)-nicotine]. In rats we could demonstrate that hexamethonium blocks responses to nicotine but not to CO_2.

Results of both studies clearly show the different stereoselective activation of the trigeminal sensory system by R(+)- and S(–)-nicotine. These results and the blocking of nicotine responses by hexamethonium in rats demonstrate the presence of specific stereoselective nnAchR on trigeminal nociceptive A- and C-fibers.

In a recent study we investigated in smokers and non-smokers discrimination ability and hedonic estimation of nicotine enantiomers in olfactory and trigeminal concentrations in humans (n = 30). A randomized sequence of R(+)- and S(–)-nicotine stimuli [seven R(+)- and seven S(–)-nicotine stimuli] were presented to the right nostril of the subjects. The subjects were instructed to group the stimuli into two categories (A and B). Nicotine enantiomers were presented at the individual 'olfactory' and 'trigeminal' concentration levels. The subjects estimated the hedonic properties using a bipolar visual analog scale. Statistical evaluation (t-test) revealed that subjects were able to identify R(+)- and S(–)-nicotine at olfactory and trigeminal concentrations ($P < 0.01$, $P < 0.001$). At concentrations near the detection thresholds, i.e. clearly below subjective pain thresholds, smokers rated both nicotine stereoisomers to be significantly more pleasant than non-smokers [t-test: R(+)-nicotine, $P < 0.05$; S(-)-nicotine, $P < 0.01$). Increasing the concentrations to above trigeminal thresholds resulted in a difference in hedonic estimates. Smokers perceived the S(–)-isomer as being less unpleasant than non-smokers at trigeminal concentrations (t-test: $P < 0.001$). This difference might be explained by smokers' experience with S(–)-nicotine, which is the natural nicotine enantiomer in tobacco.

36. Effect of the NMDA Antagonist Caroverine on Non-conductive Olfactory Disorders

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Treatment of non-conductive olfactory disorders still is an unsolved problem. Many different therapies have been tried over the years, but none has been proven to be effective.

Some disturbances of the olfactory system may be related to transmitter activities. Glutamate is the main neurotransmitter between the olfactory neurones and the mitral cells of the olfactory bulb. It is well-known that this neurotransmitter exerts a neurotoxic action in conditions of excessive stimulation. This excitotoxicity is mediated by NMDA, as well as the two non-NMDA receptors, which are involved at every excitatory synapse in the olfactory system. Calcium influx plays an important role in the aetiology of the glutamate-induced cell damage. This glutamate-receptor-linked neurotoxicity has been implicated in conditions such as ischaemia, hypoglycaemia, anoxia and trauma. In non-conductive olfactory disorders similar pathological conditions are hypothesized.

Caroverine, a quinoxaline-dione, acts as a specific but reversible antagonist of glutamate receptor subtypes and acts additionally as a potent free-radical scavenger.

Therefore, caroverine fulfills crucial preconditions for successful neuroprotective action. More importantly, it may be hypothesized that administration of NMDA receptor antagonists may lead to reduced feedback inhibition in the olfactory bulb.

Based upon these thoughts, caroverine was tried in patients with non-conductive olfactory disorders under the conditions of a feasibility and a clinical phase II trial approved by the ethics committee.

A total of 47 patients with non-conductive olfactory disorders received caroverine for 1 month (dosage = 120 mg per day), and a reference population received zinc sulfate over the same period of time (dosage = 400 mg per day).

Psychophysical testing was performed by means of 'Sniffin' Sticks' before and 1 month after treatment, i.e. testing included assessment of odor thresholds, odor discrimination and odor identification.

When compared to baseline results, treatment with caroverine significantly improved both odor thresholds [$F(1,42) = 8.22$, $P = 0.006$] and odor identification ability [$F(1,14) = 5.03$, $P = 0.042$]. In contrast, zinc administration had no significant effect on the ability to smell.

The demonstrated statistical outcome justifies the design of a subsequent clinical double-blinded, randomized, multicenter phase III study, in order to confirm the presented effect of caroverine in the treatment of non-conductive olfactory disorders.

Poster Presentations

1. Trigeminal Impact of Odorants Assessed with Lateralized Stimulation

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When lateralized intranasal stimulation is performed, identification of the stimulated nostril is believed to be due to trigeminal chemoreception (Kobal *et al*., 1989, Experientia, 45: 130). It is, however, unclear as to whether the degree of nostril localization reflects graded trigeminal activation. The present study aimed to compare the ability of healthy subjects to localize odors with data obtained in a previous study; there, anosmics and a 'trigeminal focus group', comprised of healthy subjects, rated the presence of trigeminal activation (Doty, 1978, Physiol. Behav., 20: 175). In addition, we investigated odor localization in relation to stimulus intensity and gender.

Forty healthy volunteers [20 women, 20 men; mean age 24 (range 18–44) years] participated in four sessions separated by at least 1 day. Using an odor identification test (UPSIT), normal olfactory function was established in all participants. During each session two of eight odorants were tested (vanillin, phenylethyl alcohol, geraniol, limonene, methyl salicylate, anethole, linalool and menthol). The sequence of testing the eight odorants was randomized across subjects. Each odor was presented 40 times at intervals of 30 s (20 times to the left and right, randomized sequence). Mounted in a hand-held squeezer, air from two polyethylene bottles was applied to the left and right nostrils of the blindfolded subjects. One bottle contained 20 ml of undiluted odorant, the other was empty. In 20 of the subjects (ten women, ten men) 11.1 ml of odorized air were presented to each nostril; the other half of the participants always received 21.1 ml.

Except for vanillin, geraniol and methyl salicylate, all odors could be localized. The subjects' overall ability to localize odorants correlated with odor detection in anosmics ($r_8 = 0.74$, $P = 0.035$)

and data from the 'trigeminal focus group' ($r_8 = 0.85$, $P = 0.007$). A higher degree of localization was found when larger volumes of odorized air were presented (which also produced higher intensity ratings). In addition, the experiment revealed that women localize odors to a higher degree than men.

The results suggest that the degree of trigeminal activation can be quantified by odor localization of the stimulated nostril.

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2. Olfactory Learning and Piriform Cortex Plasticity: A Study Based on Optical Monitoring of Piriform Activity with a Voltage-sensitive Dye

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Piriform cortex (PCx), the main olfactory cortical area, receives the largest amount of olfactory bulb (OB) projections via the lateral olfactory tract (LOT). According to its anatomic and functional organization, the PCx is considered as a good model for content-addressable memory. Moreover, long-term potentiation has been described in this structure in *in vivo* and *in vitro* experiments. Here we report an experiment which was performed *in vivo* in the rat in order to study the influence of olfactory learning on PCx responses to electrical stimulation of either the OB or the LOT. Three groups of animals were considered: trained (T), control (C) i.e. pseudo-trained, and naive (N) rats. The N group was only water-deprived. The T rats were trained in a four-arm maze. They had to learn to discriminate between two odors delivered in two different arms of the maze. Only one of the odor was always rewarded by a small volume of water. In the C group, the water reward was randomly associated with one or the other of the two odors. The criterion for learning was 80% of correct responses in the T group during 20 successive trials. The C group reached a score of ~50% only. The criteria being reached, the PCx responses to electrical stimulation of OB or LOT were recorded in anaesthetized and curarized rats using optical recording with a voltage-sensitive dye, RH 795. Responses were mapped in real time with a 256 photodiode array coupled with 256 electronic channels. The first data obtained showed that the amplitude of the responses was slightly larger in the C group than in the T or N groups. Data analysis is still in progress.

3. Olfactory Event-related Potentials in Patients with Brain Tumors

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Olfactory performance in patients with unilateral brain tumors was investigated by evaluation of olfactory event-related potentials (OERP). Ten patients who suffered from tumors of the temporal or frontal lobe were examined. Four of them had reported changes in their olfactory sense. For olfactory stimulation, linalool and allylcaproate were presented monorhinally by a constant-flow olfactometer. EEG was recorded from three midline electrodes (Fz, Cz, Pz) and four lateral positions (F3/F4, P3/P4). While sniffing, the patients were asked to discriminate the two odors by responding to the odors with a specific reaction. Sensory olfactory functions were checked by an odor detection threshold test.

In comparison to a control group with healthy subjects, the patients showed distinct deficits in the discrimination task. Moreover, three patients with right-sided lesions had an increased odor detection threshold. Patients with right-sided cerebral lesions showed a decreased number of correct reactions after right- and left-sided stimulation, while patients with left-sided lesions had an attenuation of the correct reactions only after ipsilateral stimulation. According to these results the P2- and P3-amplitudes in patients with right-sided lesions were altered after right- and left-sided stimulation: The amplitudes were decreased at parietal sides and the maximum amplitude showed a frontal shift over the right hemisphere. In patients with left-sided lesions a frontal shift of P2-amplitudes was seen after left-sided stimulation. These patients also showed an increase of the P3-amplitude after right-sided stimulation. To control for effects of non-modality-specific cognitive impairment on the olfactory components, acoustic event-related potentials (AERPs) were registered by use of a classical oddball paradigm. After right-sided stimulation a correlation between the P3-amplitudes of the OERP and the AERP was seen in patients with right-sided lesions.

The decrease and the frontal shift of the P2-amplitude might reflect the patients' deficits in the olfactory discrimination task. The meaning of the results concerning the P3-amplitude will be discussed.

5. Examining the Existence of Odour Hierarchies

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This study revolves around the use of odour primes with congruent and incongruent visual images, the amplitude of the N400 waveform, and the oddball paradigm. Earlier studies (Grigor *et al.*, 1999, Chem. Senses, 24: 137–144; Sarfarazi *et al.*, 1999, Chem. Senses, 24: 145–154; Castle *et al.*, 1999, submitted for publication) have shown that the amplitude of the N400 waveform reflects perceptions of contextual incongruity. Using the methodology of Castle *et al.* (1999), pleasant 'simple' and 'complex' odour primes were presented with congruent and incongruent visual images. The prediction was that there would be a difference in the processing of 'simple' and 'complex' incongruent stimuli. Such a difference in the processing of simple and complex odours is believed to suggest the existence of an odour hierarchy based on odour complexity.

In the results, the mean N400 amplitude for the 'simple' incongruent stimuli was greater than the mean N400 amplitude for the 'simple' congruent stimuli, but considerably smaller than the mean amplitude for incongruent 'complex' stimuli. The incongruent complex stimuli were therefore associated with the greatest mean N400 amplitude.

At the current level of analysis, these results offer support for the hypothesis that odour representations may be arranged hierarchically. It has been suggested (Lawless, 1997, Tasting and Smelling: Handbook of Perception and Cognition, 2nd edn, Academic Press, San Diego, CA) that 'simple' odours may be processed pre-cognitively, with a greater focus on sensory features, whereas complex odours are processed more cognitively, with an emphasis on evaluative analysis. The greater N400 amplitude observed for 'complex' odours may be associated with more

cognitive/evaluative analysis of the odour. However, this assertion requires much further exploration.

6. An Effort to Record Contingent Negative Variation with Pheromone Stimuli in Humans

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Pheromone detection by the vomeronasal system is, in all vertebrates, an important condition for reproduction. In humans, the function of the vomeronasal organ (VNO) is uncertain. Stensaas *et al.* (Stensaas *et al.*, 1991, J. Steroid Biochem. Mol. Biol., 39: 553–560) observed the VNO in all of >400 subjects, except those with pathological conditions. Monti-Bloch *et al.* (Monti-Bloch *et al.*, 1994, Psychoneuroendocrinology, 19: 673–686) found sexually specific receptor potentials and autonomic responses (increased alpha activity, electrodermal activity, skin temperature) after administration of vomeropherins. Axillary compounds of women can change the menstrual cycle of recipient women (Stern and McClintock, 1998, Nature, 392: 177–179).

In animals, VNO neurons synapse with mitral cells in the accessory olfactory bulb. Connections between the VNO and the brain have not so far been demonstrated in humans. The recording of cortical responses evoked by pheromones is problematic on account of attributed odorants. So we tried to record the contingent negative variation (CNV) after discrimination of two smells, one of them containing supposed pheromones, while the proband is expecting a second acoustical stimulus. Axillary compounds were collected from ten women at ovulation, following the method of Stern and McClintock. Sweat odor was masked by perfume, which was also used as an alternative stimulus. All subjects showed a CNV discriminating perfume and camphor.

In a group of 10 female subjects, only two showed CNV responses after the stimuli of five respectively two donors. This result can be caused by methodological problems: preparation and storing of axillary compounds, or the interaction with perfume. The time needed for pheromone effectiveness may be longer than the 1.7 s interval between smell and tone stimuli, there may exist a strong adaptation, or the pheromone effect may not be cortically represented. The positive responses of the two probands may be evoked by an exceptional sensitivity to the masked sweat odor. Our investigation will be continued with records of male probands, and using synthetic pheromones.

7. Smell and Emotion: Emotional Conditioning of Smell

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It is well-known that olfactory information is directed to the limbic system, which is thought to be the location of emotional processes. The aim of the present study was to investigate the influence of odor effects on emotional and cognitive situations, such as watching movies.

Twenty healthy volunteers (age 18–33 years; ten male, ten female) participated in the study. In pilot experiments, subjects were divided in four groups based on their preferences towards movies (action, science/documentary, historical, nature). For the main experiment two sets of five videoclips lasting 3 min were prepared for each preference group. In two consecutive sessions the clips were presented within five odor conditions. Two pleasant odorants (jasmine and phenylethyl alcohol), two unpleasant odorants [indole and 3-methyl-2 hexenoic acid (MHA)] and non-odorous air were applied using an olfactometer. After every clip subjects estimated their actual mood and how entertaining they found the clips using visual analog scales. At the end of each session they estimated the intensity of each odorant.

Estimates of mood and estimates of entertainment significantly improved in the second session compared to the first one (F:2,64; $P < 0.05$), particularly in the MHA condition. This was independent of preference group and sex. Female subjects, in general, rated the clips as more pleasant than their male counterparts (F: 4,26; $P < 0.05$).

In summary, we found an improvement of mood estimates when unpleasant odours were presented and when this presentation was repeated. This was particularly true in the MHA condition. However, MHA by itself was estimated as unpleasant. This discrepancy of the hedonic value of an odour and its effect on the mood of a subject needs further validation. We hypothesize that MHA carries a primary biological message.

8. Comparison of Stereospecific Action of Nicotine on Nasal Trigeminal Receptors in Man and Rat

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The responses of the olfactory and the trigeminal systems in the nose of humans and rats to nicotine vapor were investigated. An olfactometer was used to stimulate the nasal mucosa with R- and S-nicotine vapor applied randomly at different concentrations.

In anaesthetized Wistar rats, extracellular trigeminal single cell units were recorded from the cranial ipsilateral Gasserian ganglion. Antagonists and local anaesthetics were topically applied to the nasal mucosa. Neuronal activity induced by S-nicotine was significantly greater than that induced by R-nicotine (ANOVA, $\alpha = 0.05$), indicating stereospecificity of the receptor. Hexamethonium and mecamylamine inhibited responses to both R- and S-nicotine, whereas α-bungarotoxine did not. Local anaesthetics were able to block responses to both enantiomers and the positive control CO_2. These results indicate the involvement of nicotinic acetylcholine receptors.

For humans, individual thresholds for odorous, burning and stinging sensations were determined. Enantiomeric differences were seen only for the burning threshold (factor 3) and the stinging threshold (factor 2). Trigeminal activation in the nose was quantified by recording negative mucosa potentials (NMPs) and by estimating the intensity of burning and stinging sensations. NMP amplitudes were twice as high and intensity estimates 2–6 times higher for S-nicotine ($P < 0.001$). These observations indicate that the nasal olfactory and the nasal trigeminal systems are both involved in the sensory detection of nicotine and they are similar in man and rats. In humans, receptors in the olfactory epithelium respond equally to R- and S-nicotine. Their threshold concentration is 2 μg/l. Trigeminal receptors are able to discriminate between R- and S-nicotine at concentrations >35 μg/l

and involve a stereospecific nicotinic acetylcholine receptor. Furthermore, thresholds and discriminability in humans are not influenced by smoking history. Comparison of results indicates that the same receptor is involved in both rats and humans.

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9. Human Gustatory Areas Studied by fMRI and MEG

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We have located the primary gustatory area (area G) at the transition between the inner face of the parietal operculum and the insula in the human cerebral cortex, by means of MEG. The cortical region at around the central sulcus was activated by NaCl in almost the same latency as area G, but less frequently. Following the activation in area G, we found activation in several cortical regions, e.g. the frontal operculum, the anterior part of the insula, the hippocampus, the parahippocampal gyrus and the superior temporal sulcus.

MEG has a good temporal resolution and can give a good estimation of the location of the activity because the magnetic field generated from the living brain is free from distortion by the skull. But when the number of activated regions increases to more than two, the authenticity of estimation of the equivalent current dipoles might decrease. fMRI, on the other hand, has good authenticity for detecting many activated regions at a time, but very poor temporal resolution. In the present study, we tried to measure changes in the regional cerebral blood flow (rCBF) induced by gustatory stimulation, using the fMRI technique to find activated areas, and compared the results with the findings by MEG.

Multislice fMRI data ware acquired on a 1 T Siemens Expert Magnetom Impact with a RF whole-head coil. A gradient EPI (echo planar imager) with ten slices was used for imaging. Target–reference subtraction, which is a pairwise comparison between a target and a reference, was used. The process of interest is identified by subtracting the pattern of brain activity in the reference condition from that in the target condition. The duration of the target was 30 s, and that of the reference was 18 s. In the target condition, gustatory stimuli with short duration (500 ms) were given, followed by 1 s of deionized water. The gustatory stimulus used was 1 M NaCl, applied to the tongue of the subject by using a taste stimulator with rapid-rise time (Kobayakawa *et al.*, 1999, Chem. Senses, 24: 201–209). The gustatory stimulus and deionized water were separated by air. The short-duration stimulus was repeated 15 times. In the reference condition, the gustatory stimulus was replaced with deionized water. Five pairs of target condition and reference one were repeated. Two subjects were used, and each of them participated in the experiment three times.

Changed rCBF was observed at the central sulcus as well as at the transition between the parietal operculum and the insular cortex. They are the regions which our MEG study estimated to be activated in a short latency. The activations were also observed in the pre-central sulcus, post-central sulcus, the frontal operculum, the anterior part of the insula, the angular gyrus and intraparietal sulcus. The MEG study located activity on some of the latter regions in a long latency after the stimulation.

The present study showed that MEG and fMRI used together compensate for each other's shortcomings to increase the validity of the method.

10. Does Early Olfactory Stimulus Processing depend on Antidepressive Medication in Patients with Major Depression?

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The present study examined whether there is a specific effect of antidepressants on early olfactory stimulus processing in patients with major depression (MD). Olfactory performance was measured by means of chemosensory event-related potential (CSERP) analysis.

Fourteen patients with MD, divided into three groups by antidepressive medication, have been investigated. Each subject participated in two sessions (M1, at the beginning of their stay in hospital, without chronic effect of medication; M2, at discharge, after successful treatment, with chronic effect of antidepressants).

Two odors (isobutyraldehyde/butter and phenylethyl alcohol/rose) were presented by a computer-controlled constant-flow olfactometer.The subject task was to discriminate the stimuli by their quality. The EEG was recorded from 32 scalp positions and the CSERP was evaluated by ANOVA analysis. Groups of medication were: (1) medication concerning the serotonin (5-HT) system; (2) carbamazepin; and (3) tricyclic antidepressant (TCA).

In the TCA group no effect of medication on the CSERP was observed. With chronic effects on the 5-HT system, group 1 demonstrated a significantly smaller P3 (650–900 ms after stimulus onset) for butter odor but not for rose odor. The carbamazepin group showed at discharge a smaller P3 for butter odor at left frontal electrodes. A tendency for the same effect was obtained when rose odor was presented. The early peaks (N1, 300–550 ms and P2, 400–650 ms after stimulus onset) became more negative at left electrode positions than at middle and right positions at M2, independent of the medical treatment (group 1 or 2).

The P3 effect in group 1 could indicate a decreased subjective importance of the aversive odor at M2. The changed topographic distribution of the early components in groups 1 and 2 could be interpreted in terms of a normalization of frontal EEG activity.

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11. Olfactory Assessment in Patients with Temporal Lobe Epilepsy (TLE)

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Medial temporal lobe structures play a prominent part in olfactory

information processing and lesions in this region have resulted in olfactory dysfunction.

In our study the extent to which TLE patients show unilateral olfactory deficits was addressed and, furthermore, whether this postulated impairment is a primary sensory event (e.g. loss in absolute sensitivity) or a higher olfactory system malfunction (e.g. odor-naming and odor-memory impairment). Unilateral olfactory evaluation was performed in patients who were considered as candidates for epilepsy surgery. All patients had continuous video-EEG monitoring, magnetic resonance imaging (MRI), single photon emission CT (SPECT) and neuropsychological testing. The patients also underwent a standard sodium amobarbital procedure (WADA test) for lateralization of language and memory functions. Only patients with (i) left-sided speech dominance and (ii) normal MRI or hippocampal atrophy/sclerosis were included. Twenty-five patients with left-sided TLE, 18 with right-sided TLE and 20 controls were tested. The olfactory test battery assessed monorhinically the following functions: olfactory threshold, odor identification and odor memory. No difference concerning olfactory threshold was found, indicating normal olfactory sensitivity. However, right-sided TLE patients showed significantly lower right-sided odor-identification and right-sided odor-memory scores. In addition, left-sided TLE patients were significantly impaired in left-sided odor naming. Our study showed that olfactory sensitivity is not impaired in patients with TLE. However, right-sided TLE patients exhibited a greater magnitude of olfactory loss, indicating the superiority of the right hemisphere for olfaction.

12. Does Hunger Affect the Rating of Food Odors?

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It has been shown that the time-point to start or terminate a meal and thereby the amount of food ingested is affected by its taste and odor. The present study was conducted to investigate whether the perception and rating of food odors depend on the amount of perceived hunger.

Twenty-two different food odors were presented individually to 25 women aged between 19 and 49 years (mean = 24.92 years, SD = 7.25). They rated the odors on the following dimensions: valence, intensity, palatability and 'desire to eat the food at the moment'. The subjects did not know that all odorants were food flavours. To guarantee that the food-associated questions would not affect reactions to other questions, ratings were divided into two subsets. In the first block, subjects described the odors by listing all associations and rated their valence and intensity. When odors were presented a second time, answers to food-associated questions were recorded, while subjects were instructed to imagine that they were smelling food. At the beginning of the experiment, subjects rated their degree of hunger on a 10-point scale (0—not at all hungry; 9—very hungry).

First, a cluster analysis was used to divide subjects into three groups according to their hunger ratings. In a second step, odors with similar ratings were grouped by factor-analysing all odors on every dimension separately. Odors loading high on the first factor within each dimension were combined to form groups. The scores on every dimension for these four odor groups were averaged and entered into separate discriminant analyses to differentiate between hungry and satiated subjects. None of the discriminant functions reached significance. Mean differences between groups were investigated and a tendency for significance for the mean valence of odors loading high on the first intensity factor was found. These odors were located at the upper end of the intensity scale and obtained either very high or low valence scores. These findings suggest that odors that discriminate best between hungry and satiated subjects have high intensities and extreme positive or negative valence scores.

Separate correlation matrices between the four dependent variables for hungry and for satiated persons showed some interesting differences: while there was no significant correlation between intensity and 'desire to eat the food' for hungry subjects, this correlation reached high significance for satiated persons. This finding indicates that in satiated persons a desire to eat some kind of food only emerged with a high intensity of the food odor, whereas hungry subjects had a desire to eat the food independent of the perceived intensity. The variables 'palatability' and 'desire to eat the food' seemed to be strongly connected in hungry but not in satiated subjects. This result suggests that satiated subjects developed only a weak desire to eat the food, despite the fact that it smelled very appetising to them.

13. Are there Gender-related Differences of Responses to Repetitive Intranasal Chemosensory Stimuli?

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Sex differences in olfactory sensitivity have been reported since the late 1800s. Women often outperform men on tests of odor identification, detection and discrimination. Whether women adapt differently to odorous stimuli than men is not known.

Seventeen healthy volunteers participated (nine female, eight male, mean age 22 years). As established by an odor identification test (UPSIT, score 38), all subjects had normal olfactory function. ERPs were recorded in response to olfactory (25% v/v phenylethyl alcohol, PEA) and trigeminal (44% v/v CO_2) stimuli presented to the left or right side (flow 8 l/min; stimulus duration 200 ms). Stimuli were applied at four different intervals (5, 10, 20 and 60 s). Amplitudes and latencies of ERP peaks P1, N1, P2 and P3 were measured. Using visual analogue scales, subjects also rated stimulus intensity.

When compared to PEA, the slightly more intense CO_2 produced larger amplitudes [P1, N1, P3, N1P2 and N1P3: $F(1,15) > 4.93$, $P < 0.043$] and shorter latencies [P1, N1, P2, P3: $F(1,15) > 5.66$, $P < 0.032$]. Responses to the trigeminal and olfactory stimuli changed similarly in relation to repetitive stimulation [inter-action 'stimulant' by 'interval'; amplitudes: $F(3,45) < 1.14$, $P > 0.344$; latencies: $F(3,45) < 2.48$, $P > 0.073$; ratings: $F(3,36) = 0.86$, $P = 0.47$). Both ratings [$F(3,45) = 5.76$, $P = 0.003$] and ERP amplitudes P3 and N1P3 [$F(3,45) > 7.71$, $P < 0.001$] decreased with the interval between stimuli. Women had larger ERP amplitudes P3, N1P2 and N1P3 [$F(1,15) > 5.39$, $P < 0.036$]; however, they tended to rate intensities lower than men [$F(1,12) = 4.03$, $P = 0.068$]. No gender-related difference in relation to repeated stimulation was observed [interaction 'sex' by 'interval': $F(3,45) < 2.38$, $P > 0.08$].

These data indicate on a psychophysical and an electrophysiological level that there is no difference between young, healthy men and women in relation to short-term adaptation to suprathreshold chemosensory stimulation.

14. Evidence for Multiple Transduction Pathways in Human Olfactory Neurons

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A large body of data has accumulated describing the signal transduction pathways involved in translating the information that an odorant has bound to its receptor protein into an electrical signal traversing the neuron's axon to the olfactory bulb. The vast majority of these data have arisen from studies in a wide variety of invertebrate and vertebrate species other than the human. While a substantial amount of conservation has been observed in a broad range of species, significant species diversity also exists in various aspects of the cellular response to odorant stimulation. One goal of our laboratory has been to investigate the extent to which models of olfactory transduction, developed largely on the basis of studies of other species, applies to human olfactory receptor neurons (ORNs). Our work over the past 6 years has identified both similarities and differences compared with similar studies with rodent species.

The accumulated results of our studies of imaging intracellular calcium in >500 individual human ORNs will be presented; these demonstrate basic conservation of some aspects of olfactory transduction as well as certain features that are distinct from those seen in other species studied to date. These results suggest that a single transduction pathway involving activation of cAMP-gated channels fails to account for all types of calcium responses in human ORNs, and implicate involvement of a phospholipase-C-dependent pathway in human olfactory transduction.

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15. Magnetic Responses of Primary Gustatory Area (Area G) in Human Cortex Evoked by various Concentrations of NaCl

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By using magnetoencephalography (MEG), we have located area G at the transition between the inner face of the parietal operculum and the insula in the human cerebral cortex (Kobayakawa *et al.*, 1996, Neurosci. Lett., 212: 155–158; Kobayakawa *et al.*, 1999, Chem. Senses, 24: 201–209). The location is in contrast to that in macaque monkeys (Ogawa, 1994, Neurosci. Res, 20: 1–13), which situates at a similar position but anterior to the central sulcus. In the present study, we examined the responses of area G to various concentrations of NaCl solutions to investigate whether or not the onset latency and the magnitude of the gustatory-evoked magnetic fields (GEMs) vary depending upon the concentration.

Taste solutions of 100 mM, 300 mM and 1 M NaCl, in which range NaCl is known to induce different reaction times in humans, were used as stimuli and applied to the tongue of the subject by using a taste stimulator with a rapid rise time (Ogawa, 1994, Neurosci. Res, 20: 1–13; Kobayakawa *et al.*, 1996, Electroencephalogr. Clin. Neurophysiol. Suppl. 47: 133–141; Kobayakawa *et al.*, 1996, Neurosci. Lett., 212: 155–158; Kobayakawa *et al.*, 1999, Chem. Senses, 24: 201–209). The duration of each stimulus was 400 ms in one trial and the inter-stimulus interval was ~30 s, during which the tongue was continuously rinsed with deionized water. The taste stimuli and rinsing water were maintained at the same temperature as that of the tongue to avoid thermal stimulation. Five neurologically normal adults (two females and three males), aged 22–35 years, participated in the experiment. The 64-channel whole-head SQUID system (CTF Systems Inc., Canada) was used to measure the GEMs. The sampling rate was 250 Hz and the low-pass filter was 40 Hz.

In each subject, GEMs to a given concentration of NaCl were measured separately by applying 40 trials of stimulation. The trials contaminated with eye movement were rejected from signal averaging. Averaged GEMs in all 64 channels were superimposed on the same sheet to measure the GEM onset latency from the stimulus onset. We confirmed, in most sessions, that the first peak of the GEMs was yielded in area G, by locating equivalent current dipoles on the subject's anatomical MRI. After each trial of measurements, subjects were asked to show the perceived intensity by using their fingers. The magnitude of the dipole in area G increased with increased concentrations ($F = 17.67$, $n = 4$, $P < 0.01$, variance analysis), but the onset latency remained unchanged (as reported in Saito *et al.*. 1998, Ann. N.Y. Acad. Sci., 855: 493–497). The results indicated that the onset latency of the GEMs to NaCl was independent of concentrations used in the present experiment. One of the reasons might be that the range of concentration used was too high to affect the onset latency. We are now studying on the onset latency of GEMs of lower concentrations.

16. Computer-assisted Questioning and Assessing Patients with Smell and Taste Disorders

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Eighty-four patients were interviewed by a computer-assisted questionnaire before smell-examination. All questions were subtitled in German, with touch-screen entry and automatic data capture and storage. The programme comprises 72 questions.

The acceptance for this kind of interviewing was well accepted by all age-groups (14–84 years). Only persons above 60 years of age need a short instructions.

The computer-assisted questioning and assessing by touch-screen technology makes it possible for patients to process a large number of questions in an efficient way and may be useful in routine clinical assessment.

17. Responses to Olfactory and Intranasal Trigeminal Stimuli: Relation to the Respiratory Cycle

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The aim of the study was to investigate whether the perception

of intranasal chemosensory stimuli changes in relation to the respiratory cycle. We investigated 40 healthy subjects with normal olfactory function who participated in four sessions (20 women, 20 men, age range 19–39 years, mean age 23 years). The first session was used to adapt subjects to the experimental conditions, and specifically to train a certain breathing technique (velopharyngeal closure) which prevents intranasal respiratory airflow. In each of the following three sessions one of three stimulants was tested, namely phenyl ethyl alcohol (25% v/v), hydrogen sulfide (2 ppm) or the trigeminal stimulant carbon dioxide (50% v/v). The sequence of testing the three stimulants was randomized across all participants. Sessions were separated by at least 1 day. Chemosensory event-related potentials (ERP) were recorded in response to 80 stimuli each (interstimulus interval ~30 s, stimulus duration 200 ms) from F3, F4, C3, C4, P3, P4 and Fp2. Following each stimulus subjects rated its intensity using a computerized visual analogue scale. Respiration was recorded using a probe in front of the subject's mouth. While presentation of chemosensory stimuli was performed independent of the respiratory cycle, responses were averaged off-line according to the subject's respiratory phase when the stimuli had been presented. On average, the trigeminal stimulant CO_2 was perceived as most intense [$F(2,50) = 1.28$, $P = 0.29$)]. Perceived intensity of olfactory or trigeminal stimuli did not differ significantly in relation to the respiratory cycle [$F(2,68) > 2.61$, $P > 0.097$). Olfactory ERP to PEA were larger when stimuli were presented during inspiration [amplitudes N1: $F(1,38) = 4.96$, $P = 0.032$; amplitude P1N1: $F(1,38) = 4.40$, $P = 0.043$; amplitude N1P3: $F(1,38) = 10.1$, $P = 0.003$]. Similarly, responses to H2S tended to be larger when stimuli were presented during inspiratory phases [amplitudes N1: $F(1,35) = 3.97$, $P = 0.054$]. In addition, responses to CO_2 were larger when stimuli were presented during inspiration [amplitude N1: $F(1,37) = 6.94$, $P = 0.012$]. In general, differences in relation to the respiratory cycle were found for early ERP components, while they were less pronounced for late components. ERP latencies were not affected by the respiratory cycle. These data indicate on an electrophysiological level that there is priming of both olfactory and trigeminally mediated sensations in relation to the respiratory cycle.

18. Development of Subjective Methods for the Assessment of Odor Quality in Occupational Settings. Experiments with Ethylbenzene and 2-Butanone

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Within neurotoxicology the early perception of exposure to organic solvents is usually attributed to the olfactory system. However, there is a lack of knowledge about the perceived quality of the odorous stimuli and its possible effect on further steps of perceptional processing (e.g. development of acute symptoms). It can be assumed that the perceived odor quality might affect subjective signs of solvent exposure. Research of chemosensory responses in man has provided information about adjectives used to evaluate perceptional qualities of odorous chemicals. Some of them correspond to pleasant odors (i.e. flowery), while others are associated with trigeminal aspects of odors (i.e. pungent).

The aim of the present study is the development of two different methods for the assessment of odorous/perceptional qualities of organic solvents.

Twenty-four male volunteers were experimentally exposed to four conditions which differ with respect to type of solvent (2-butanone versus ethylbenzene) and level of exposure (high versus low). During the 'high' conditions five peaks of exposure were used. Average exposure did not exceed the German MAC's of 200 and 100 ppm, respectively. The experiments were carried out in an exposure laboratory (28 m^3) which allowed to expose four volunteers simultaneously and to perform computerised psychological tests. Approximately 2 h after the onset of exposure subjects evaluated the quality of the solvent by (a) two versions of 21 paired comparisons and (b) a profile scale of 69 odor-/irritant-related adjectives. The paired comparisons were carried out for adjectives labeled as 'olfactory perception' (i.e. soft, fresh, aromatic, rotten, caustic, pungent, strong) as well as for seven adjectives classified as 'perception at skin and mucous membranes' (i.e. soft, caustic, acrid, searing, scratchy, tickling, irritant). The subjects had to mark the preferred adjective. The profile scale required to estimate the extend of qualities on rating scales with seven categories.

The scaling for 'olfactory perception' has a stable rank order of adjectives despite of exposure level only during exposure to ethylbenzene. The dominant characteristic is 'pungent'. The same adjective was preferred for the high exposure to 2-butanone, but during low exposure to 2-butanone no dominant characteristics could be found. The scaling for 'perception at skin and mucous membranes' showed no remarkable difference of qualities between substances.

Non-linear principal components analysis (PRINCALS) of the profile scale revealed three dimensions that accounted 67.51% of the variance. The dimensions were named 'irritant adjectives', 'negative odorous adjectives' and 'positive odorous adjectives', respectively. Subsequently, the five adjectives with the highest component loading on these dimensions were aggregated to a sumscore. Based on these sumscores, 'quality profiles' of the four exposure conditions were compared. Under high exposure conditions both substances had comparable profiles. They showed high scores on the dimension 'irritant adjectives' and simultaneously low scores for the two other dimensions. At low exposure conditions the 'quality profiles' of the two substances differ with respect to all dimensions. While ethylbenzene yielded moderate scores on the dimension 'irritant adjectives', 2-butanone showed the highest scores on the dimension 'negative odorous stimuli'.

The method of the paired comparisons seemed to be a promising way to evaluate the perceptional quality of organic solvents on a phenomenological level. Additionally, the 'profile scale' yielded information about the configuration of the perceptional qualities on different dimensions. Thus, a broader perceptual impression could be assessed by this method. The perceptional quality of substances can be used to understand the succeeding steps of processing and evaluating solvent exposure. Impairments of well-being or acute symptoms might be caused or moderated by such qualities. The toxicological classification of substances as 'irritant' or 'strong odorous' needs a reappraisal which reflects reliable judgements bearing on real perceptions.

19. Olfactory Function in Patients with Olfactory Meningioma

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Olfactory meningiomas are benign tumors. They represent ~11% of all basal meningiomas. Unilateral/bilateral reduction of olfactory sensitivity is among the first clinical symptoms. Eventually, the tumor may lead to complete anosmia. However, normal olfactory function does not rule out the presence of an olfactory meningioma.

We examined eight patients (four male, four women) with olfactory meningiomas. Mean age was 48.5 years (range 21–71 years). In five cases the meningioma was located on the left side, and in one case on the right side. In two cases radiographical investigations revealed a bilateral meningioma. All patients received a physical examination by an ENT specialist. Olfactory examination was performed using the 'Sniffin' Sticks'. Except for the cases with bilateral meningioma lateralized testing was performed. MRT imaging was done in all patients.

Based on preoperative olfactory testing, five patients were found to be anosmic on the side of the tumor; lateralized hyposmia was observed in one patient. Two patients were normosmic. Postoperative investigations indicated lateralized anosmia in two patients on the operated side. The remaining six patients were completely anosmic.

Anosmia or hyposmia is believed to be a characteristic sign in patients with olfactory meningioma. Interestingly, we found two preoperative patients with normal olfactory function. Overall, preservation of olfactory function on the side of the tumor was impossible. However, postoperatively two of the eight patients exhibited unilateral normosmia. Since in both of these cases the tumor was relatively small, the size of the tumor seems to be a major determinant of outcome in terms of olfactory function.

20. Release of Calcitonin Gene-related Peptide and Prostaglandin E_2 from Rat Dura Mater Following Stimulation with ATP and low pH

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Functional expression of purinoreceptors for ATP on primary afferent nociceptors is controversial but of particular interest in the dura where theories of migraine headache start from. A recent *ex vivo* preparation was used to determine trigeminal nociceptor activation indirectly by measuring stimulated release of calcitonin gene-related peptide (CGRP) using an enzyme immunoassay, and less specific tissue responses were monitored measuring release of prostaglandin E_2 (PGE_2) (Ebersberger *et al.*, 1999).

Hemisected skulls of adult Wistar rats were mounted in a shaking bath at 38°C, following removal of the cerebral hemispheres, and used as an organ bath of their own lining, the dura mater. The cavities were first washed for 30 min with oxygenated synthetic interstitial fluid (SIF) and then filled with up to 1 ml of SIF which remained *in situ* for 5 min. The eluates were then recovered and processed immediately; this procedure was repeated four times. During the third incubation period the putative stimulant ATP was contained in the eluate and/or the pH of the solution was lowered to values known to excite nociceptors.

ATP at 10^{-4} M slightly and 10^{-3} M significantly augmented PGE_2 release from the dura by 72% whereas both concentrations did not alter the constant basal release of CGRP (42 ± 8 pg/ml). Preparations (n = 8) were pretreated with hexokinase during the second incubation in order to remove endogenous extracellular ATP and to overcome the possible desensitization of the purinoreceptors. This allowed for a slight enhancement of the CGRP release during the subsequent stimulation with 10^{-4} M ATP (n.s.). Acid buffers at pH 5.9 and 5.4 caused a pH-dependent increase in dural CGRP release by a factor of 2 and 6, respectively; pH 6.8 was ineffective. On the contrary, PGE_2 release was profoundly suppressed by any low pH level, and this effect was partly counteracted by combining pH 5.9 with 10^{-3} M ATP. This combination, however, significantly exceeded the effect of plain pH stimulation, increasing the CGRP release by a factor of 3 over baseline.

At present, the poor effect of hexokinase, the stimulation of PGE_2 formation and the limited effect of combination with low pH suggests that P_2Y rather than P_2X receptors to be involved in the weak effects of ATP in meningeal nociception.

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21. Effects of Myrtol Standardized on Experimental Rhinitis in Healthy Volunteers

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The inflammatory nature of the diseases on which myrtol standardized, a mixture of 1,8-cineol, D-limonene and α-pinene, has clinical effects raises the question about a direct anti-inflammatory mechanism of action.

The effects of oral administration of myrtol standardized (Gelomyrtol®) on experimental nasal inflammation were investigated in 31 healthy volunteers (16 males, 15 females, aged 19–43 years, body wt 67.9 ± 10.6 kg). In a placebo-controlled cross-over study, subjects received 2 × 300 mg Gelomyrtol® per day (treatment period 23 days), separated by a wash-out period of at least 6 days. Anti-inflammatory effects were assessed at baseline, 2.5 h after administration of the first capsule, and at days 2, 5, 9, 16 and 23. For this purpose the nasal mucosa was challenged with cold, dry air (flow 8 l/min, 18°C, 18% relative humidity) introduced into the nasal cavity for 20 min. Inflammatory effects were assessed by determining inflammatory mediators (prostaglandin E_2 and peptide leukotrienes) in nasal lavage fluid sampled immediately before and after stimulation. In addition, nasal patency was measured by means of acoustic rhinometry and subjects estimated the intensity of the pain perceived during stimulation.

Cold, dry air introduced into the nasal cavity induced a reproducible local inflammation, as indicated by the significant increase of inflammatory mediators in the nasal secretions produced by the stimulation, and by the significant increase of pain intensity during stimulation. Consequently a swelling of the nasal mucosa could be verified by a significant decrease of the minimal cross-sectional area and the volume of the anterior

part of the nasal cavity. Myrtol standardized had no effect on inflammation compared with placebo.

It is unlikely that myrtol standardized acts through anti-inflammatory mechanisms. Thus, possible mechanisms of action of myrtol standardized have to be sought elsewhere, eventually in the processing of afferent nerve activity in the respiratory system. Nevertheless the study demonstrated the suitability of the present model for assessment of anti-inflammatory effects in the upper airways.

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22. Peripheral Electrophysiological Responses Decrease in Response to Repetitive Painful Stimulation of the Human Nasal Mucosa

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The study aimed to investigate the relation of both negative mucosal potentials (NMP; obtained from respiratory epithelium) and pain ratings to repetitive stimulation with CO_2 (21 subjects). Trains of four stimuli of identical intensity (70% v/v) were applied at a constant interseries interval (~60 s) but different interstimulus intervals (ISI of 2 or 6 s). At an ISI of 6 s ratings decreased while they increased at an interval of 2 s ($P < 0.01$). This change was accompanied by the build-up of burning pain, probably relating to the 'wind-up' of spinal neurons. In contrast, the decrease of NMP amplitudes was the stronger the shorter the ISI ($P < 0.01$). These findings are in line with the view that the NMP reflects activation of epithelial nociceptors (C-fibers and/or Ad-fibers).

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23. Intranasal Chemoreception in Patients with Multiple Chemical Sensitivities and Healthy Controls

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Multiple chemical sensitivity (MCS) has become an increasingly frequent diagnosis assigned to patients with symptoms associated with exposures to environmental chemicals. Since the characteristic symptoms of MCS are triggered by very low concentrations of chemicals, i.e. in the range of olfactory threshold, it is widely believed that intranasal chemoreceptive senses are involved in the pathophysiology. In most interviews it was reported that patients believed they had an increased ability in perceiving odorants. Also the quality of odorants perception seemed to be changed.

Having more sophisticated olfactometric techniques available today, we wanted to test the hypothesis that in MCS the sense of smell is affected. Twenty-three MCS patients (mean age 47 years; 13 female, 10 male; range 29–63 years) participated in the experiments. Based on their medical history, patients fulfilled the criteria published by Cullen (1987). A control group of 23 healthy volunteers matched for sex and age was additionally investigated. The testing included two sessions that were separated by an interval of ~15 min during which patients were exposed to either 2-isopropyl alcohol (2-prop) or room air. Acoustic rhinometry, psychophysical tests and recording of chemosensory evoked potentials (CSERPs) were performed before and after provocation.

Acoustic rhinometry demonstrated that both groups exhibited a decreased mean volume of the anterior nasal cavity after provocation regardless of the type of challenge. On the other hand, without reaching the level of statistical significance, patients tended to have a pronounced decrease of nasal volume after provocation (see also Doty *et al.*, 1988). Psychophysical investigations showed that controls had a significantly better ability in olfactory identification and discrimination. In the threshold test there was a tendency of lower thresholds in healthy controls. CSERPs amplitudes P1N1 and N1P2 in healthy controls were significantly larger after stimulation with H_2S regardless of the type of challenge. In contrast, healthy controls' N1P2 amplitudes but not P1N1 were increased after CO_2 stimulation. The experiments clearly demonstrated that MCS patients are not more sensitive to odors at the afferent sensory level; they may even be less sensitive. However, when further processing is involved, MCS patients react differently to olfactory stimuli. Based on our data, we hypothesize that MCS patients seem to have a decreased habituation to repeated stimuli. Also, the different pattern of mucosal swelling after provocation might indicate that neurogenic inflammation is involved in the pathophysiology of this disease. These factors might explain MSC patients' complaints when they are exposed to odorants.