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CASE REPORT

Assessment of olfactory and intranasal trigeminal function using electrophysiological and imaging tecniques

Konstantinidis I, Hummel Th

Smell & Taste Clinic, Otorhinolaryngology Dept, Carl Gustav Carus University Hospital of Dresden, Dresden, Germany

Objective: The development of olfactory screening tests for the every day clinical practice was a usefull tool for the assessment of olfactory and trigeninal function. These psychophysical tests have numerous advantages in the clinical utilization, but also important limitations. Subsequently, new techniques have been developed which rely less on the subjects' cooperation. The aim of this review is to describe the methods used to record and analyze olfactory and trigeminal event-related potentials (ERPs).
Methods: Odors are applied intranasaly by means of a special device called olfactometer. Stimulus presentation and recording of stimulus-linked EEG segments typically are under computer control. Different techniques for the recording of olfactory system response have been developed: 1.Electro-olfactograms (EOG) which are electrical potentials of the olfactory epithelium that occur in response to olfactory stimulation, collected by an electrode placed in the olfactory cleft. 2. Event-related potentials which are EEG-derived poly-phasic signals, due to the activation of cortical neurons which generate electro-magnetic fields. 3. Imaging techniques include positron emission tomography (PET), functional magnetic resonance imaging (FMRI), and magnetic source imaging (MSI).
Results: ERP olfactometry allows the investigation of subjects who have difficulties to respond properly (e.g., children, or aphasic, demented, unconscious, or inexperienced patients). It is also necessary for the diagnosis of olfactory deficits for medicolegal purposes. Olfactory dysfunction is an early symptom of some neurodegenerative diseases and the development of the techniques will be usefull for the early diagnosis of these disorders. Conclusion: Olfactory ERPs are a validated means which allows the investigation of early components of olfactory information with a special focus on high temporal resolution. This technique is a useful tool in the study of subtle alterations in olfactory perception, odor memory, or odor aversion. *Hippokratia 2005; 9 (3): 141-144*

Key words: *Olfaction, trigeminal, event-related potentials, objective olfactometry*

Corresponding author: Konstantinidis I, 3 P.Tsaldari , Neapoli, Thessaloniki, PoBox: 56727, tel: 6973229525, e-mail: jokons57@hotmail.com

The nasal cavity contains chemosensors related to the olfactory and the trigeminal systems. In fact, most odorants usually activate not only one but several of the "chemical senses". Nicotine for example, in addition to activation of the olfactory nerves, also produces activation of the intranasal chemosensory trigeminal system in a concentration-related manner.

Olfactory and intranasal trigeminal system

Olfactory perception starts at the level of the olfactory epithelium located in the roof of the nasal cavity. Olfactory receptor neurons (ORN) are embedded within the nasal mucosa and send their axons through the cribriform plate towards the olfactory bulbs. ORN carry olfactory receptors (OR) which are key element to olfactory information processing. In the olfactory bulb ORN axons synapse with second order neurons, called the mitral cells. The connection between the olfactory epithelium and the olfactory bulb is characterized by a convergence of axons of ORN. All ORN carrying the same OR converge in the same site within the bulb, called "glomerulus". In contrast to other sensory systems, no primary olfactory cortex has been identified so far. Numerous works indicate the orbitofrontal cortices to be an important relay in olfactory information processing[1].

The trigeminal nerve provides the somato-sensory innervation to the nasal mucosa. Since most odorous compounds stimulate trigeminal nerve endings, at least at higher concentrations, this system is involved in the perception of most odors. Sensations mediated by the trigeminal system include burning, stinging, tickling, or prickling. With few exceptions almost all odorants have been shown to exhibit trigeminal activation to some extent[2]. Mint for example has a somewhat fruity odor, but also the typical cooling effect which is mainly trigeminally mediated.

Similar to other sensory modalities, olfactory testing procedures will yield information which is either based on subjects' insights ("psychophysical" tests) or on more "objective" techniques less biased by the subjects' observations. Since the subjects' self ratings of olfactory function are unreliable, testing of olfactory function is necessary[3].

Objective measurements of chemoreception include the electrolfactogram (EOG), chemosensory event-related potentials (CSERP), and combination of olfactory stimulation with imaging techniques. For the initiation of all the above mentioned measurements a special

system, generator of olfactory and trigeminal stimuli is necessary.

Olfactory stimulator – the olfactometer

How is it possible to produce odorous stimuli which have a rectangular shape with rapid onset, which are precisely controlled in terms of timing, duration, and intensity, and the presentation of which does not simultaneously activate sensory systems other than the olfactory? Based on the principles of air-dilution olfactometry[4] such a system has been developed by Kobal[5,6] (Figure 1A). Odors are applied intranasaly by means of a canula which typically has an inner diameter of 2-3 mm. This canula is inserted for approximately 1 cm into the nostril in a way that its opening lies beyond the nasal valve (Figure 1B). Presentation of odor stimuli does not simultaneously activate mechano- or thermoreceptors in the nasal mucosa as odor pulses are embedded in a constantly flowing air stream (typically 6-8 L/min).

In commercially available olfactometers valves and air-flows (using mass-flow controllers) are typically under computer control, and recording of stimulus-linked EEG segments is integrated in the same software which controls the olfactometer, and thus, stimulus presentation. This equipment also allows the setup of sequences of stimuli with different quality, intensity, or duration, presented at variable interstimulus intervals.

Electrolfactogram (EOG)

Electro-olfactograms (EOG) are electrical potentials of the olfactory epithelium that occur in response to olfactory stimulation, collected by an electrode placed in the olfactory cleft over the middle turbinate (Figure 2). The EOG represents the sum of generator potentials of ORN. While this response has been used extensively in olfactory research in animals[7], there are only a handful of reports describing the properties of the human EOG. Among other results, EOGs have been used to provide evidence for the dominant role of the central nervous system in olfactory desensitisation[8], for the functional characterisation of the olfactory epithelium[9], the specific topographical distribution of ORN, the expression of ORN in response to exposure to odorants[10], and the characterisation of certain odorants as OR antagonists[11]. However, the EOG so far has not been systematically used in patients with olfactory dysfunction. This is due to the topographical specificity of EOG responses, meaning that EOGs to certain odorants may be recorded only at certain epithelial sites. Thus, the subjects' odorous impressions may not always be reflected through the presence of an EOG response[12,13]. Despite of these drawbacks EOGs may be extremely helpful in terms of the elucidation of pathological processes at the mucosal level.

Chemosensory event-related potentials (CSERP)

Event-related potentials are EEG-derived poly-phasic signals. They are due to the activation of cortical neurons which generate electro-magnetic fields[14]. As the EEG is a noisy signal which contains activity from many cortical neurons, ERP need to be extracted from this background activity. The classical approach to this problem involves averaging of individual responses to olfactory stimuli such that random activity would cancel itself out while all non-random activation would still be left. In addition, stimuli are typically presented with a steep onset (<20 ms) in an extremely well-controlled, monotonous environment in order to synchronize the activity of as many cortical neurons as possible.

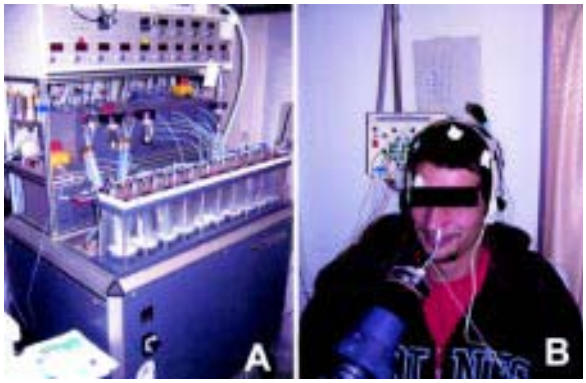

Figure 1: *A. Computer-controlled olfactometer suited for separate/combined application of 6 different odorous stimuli (OM6, Burghart, Wedel, Germany). B. Intranasal application of odorous stimulation and EEG recording.*

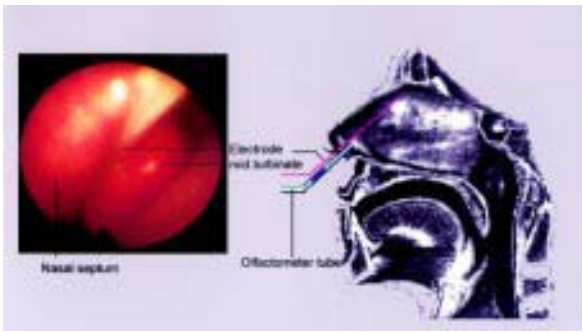

Figure 2: *Endoscopic and schematic representation of the electrode positioning above the middle turbinate in the olfactory cleft, for recordings of the electrolfactogram.*

Olfactory ERP are direct correlates of neuronal activation, unlike the responses that are seen, for example, in functional MR imaging. They have an extremely high temporal resolution in the range of micro-seconds. They allow the investigation of the sequential processing of olfactory information, and this can be obtained independently of the subject's response bias. Thus they allow the investigation of subjects who have difficulties to respond properly such as children, aphasic patients etc. In contrast to hearing and vision, to date no early ERP have been recorded in response to olfactory stimuli but only late near-field ERP, which are responses from cortical neurons. Earlier peaks like N1 (Figure 3) encode exogenous stimulus characteristics to a larger extent than

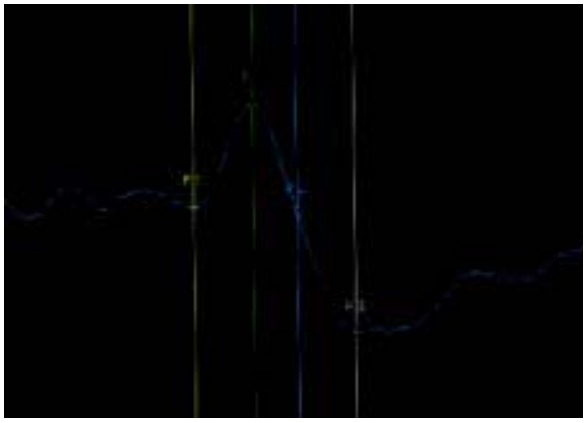

Figure 3: *Typical appearance of an olfactory event-related potential following stimulation with phenyl ethyl alcohol.*

later peaks, so-called endogenous components. That is, earlier components encode stimulus intensity or stimulus quality, whereas later components are more related to the frequency, or the salience of the stimulus[15,16].

Olfactory ERP are recorded all over the scalp. In terms of the topographic distribution of olfactory ERP amplitudes exhibit characteristic patterns with a centro-parietal maximum for both amplitudes N1 and P2[17] (Figure 3). Using magneto-encephalographic techniques Kobal and co-workers conducted a series of experiments which addressed the question of the generation of olfactory ERP. Cortical generators of the responses to trigeminal stimulation with CO_2 were localized in the secondary somato-sensory cortex[18]. Other work[19,20] indicated that olfactory stimuli activate anterior-central parts of the insula, the para-insular cortex, and the superior temporal sulcus[21].

Clinical testing with chemosensory ERP typically includes the recording of responses to olfactory (e.g., hydrogen sulfide, and phenyl ethyl alcohol) and trigeminal (e.g., CO_2) stimuli[22]. So far, in all investigated anosmic patients intranasal trigeminal ERP could be obtained after stimulation with CO_2 - although with significantly smaller amplitudes than in healthy controls[23]. In contrast, no olfactory ERP could be detected in anosmic patients after stimulation with the odorants hydrogen sulfide and vanillin[24]. Results form ERP investigations provide significant information in the testing of malingering patients.

Functional Magnetic resonance Imaging (FMRI), Positron Emission Tomography (PET), and Magnetic Source Imaging (MSI)

Recent progress in the field of imaging opened the opportunity to study the functional topography of the human olfactory system in detail[25-27]. There are three major techniques being used: positron emission tomography (PET)[28,29], functional magnetic resonance imaging (FMRI)[30,31], and magnetic source imaging (MSI) based on magneto-encephalography[32]. While bio-magnetic fields directly reflect electrophysiological events, PET

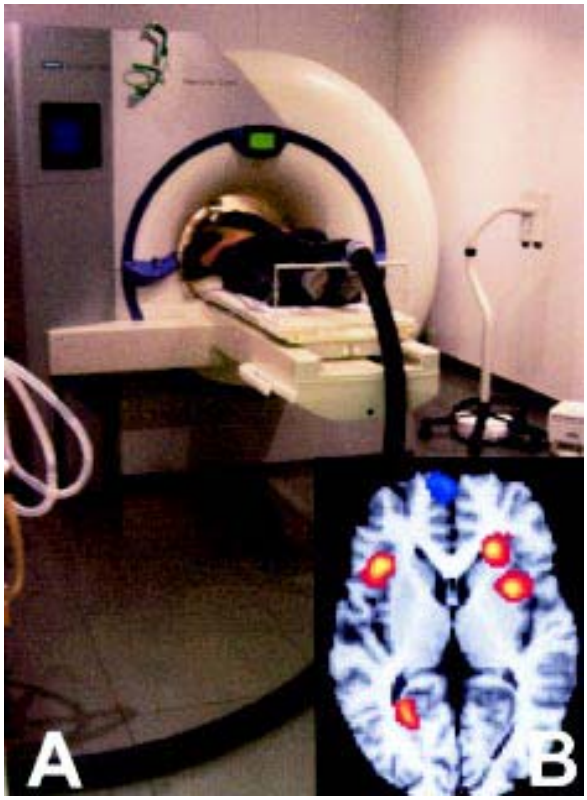

Figure 4: *A. Position of tubings of an olfactometer in the MRI room. B. Activated (yellow) and dectivated (blue) areas of the brain following olfactory stimulation.*

and FMRI (figure 4A) reflect either changes in blood flow or changes in metabolism which are epiphenomena of neuronal activity. Thus the influence of an odorant in brain function can be seen as activated and deactivated areas in FMRI slices[33] (figure 4B). Other major differences between these techniques relate to the temporal and spatial resolution. All three techniques have been used extensively to perform basic research, on olfactory induced emotions, odor memory, mechanisms of sniffing, and age- and sex-related differences in terms of olfactory function[34]. However, in order to become relevant for routine clinical investigations[35], these intriguing techniques await further standardization.

Applications

Apart from the solid body of literature and their clinical convenience, psychophysical tests have one major limitation: as soon as the patients' collaboration is not guaranteed, interpretation of test results becomes difficult or even impossible. The use of less biased olfactometric techniques such as olfactory ERP accounts mainly for willful non-collaboration in cases of malingering, children, or for demented, unconscious or inexperienced patients. The standardized test procedure[36] includes the recording of responses to olfactory (e.g., hydrogen sulfide, and vanillin) and trigeminal (e.g., CO_2) stimuli. All of the methods described above are also extensively used in research on human chemoreception.

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