

## A New Extension to the University of Erlangen Smell Test (UEST)

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### Introduction

Over the last 15 years a unique test battery has been developed at the University of Erlangen-Nuremberg that addresses acuity in perception of chemicals in the nose. The measurement of trigeminal responses has separately evolved into a pain model, e.g. for the investigation of analgesics and to monitor therapeutic regimen, e.g. in migraine.

### Sniffin' Sticks

The first tier of the test battery is an olfactory screening test consisting of a 12-odor identification test. This test discriminates anosmia from hyposmia and normosmia. As a second tier, the Threshold-Discrimination-Identification Test offers a more extensive analysis of olfactory deficits (TDI score, Sniffin' Sticks). A growing pool of normative data (Kobal *et al.*, 2000) is now available and functional anosmia is defined as a deficit with a TDI score <15 (out of 48).

### Chemosensory event-related potentials

In case of potential malingering and because of the need for objective data for other clinical reasons, as a third tier chemosensory event-related potentials can be recorded. Without any active cooperation of the patient, a series of stimuli (usually 16 stimuli of each, CO<sub>2</sub>, vanillin, and H<sub>2</sub>S) are administered to both nostrils with an olfactometer and chemosensory event-related potentials are recorded.

An anosmic patient would typically have no responses to vanillin and H<sub>2</sub>S, but clear responses to CO<sub>2</sub>.

In general, precise stimulus control is crucial when recording event-related potentials. Event-related potentials are EEG-derived polyphasic signals reflecting activation of cortical neurons which generate electro-magnetic fields (Picton and Hillyard, 1988). The more neurons activated or synchronized, the larger the amplitude of the signal obtained at the surface of the scalp. ERPs need to be extracted from the background activity. The classical approach to this problem involves averaging of individual responses to stimuli such that random activity would cancel itself out, thereby leaving only non-random activity. Therefore, stimuli are typically presented repetitively with a steep onset (<50 ms), so that the stimulus onset synchronizes the activity of as many cortical neurons as possible.

Three prerequisites must be met to obtain clear and accurate ERPs. First, as noted above, the stimulus must have a steep onset. Although a shallow stimulus onset may lead to a sensation, this sensation may not be reflected in an ERP as the cortical activity 'drowns' in background noise. Secondly, the stimulus needs to be presented repetitively. This requires precise temporal control of stimulus onset in the range of milliseconds as fluctuations in the timing of stimulus onset will lead to differences in the peak latencies of individual ERPs ('jitter'). This jitter will lead to the modification/cancellation of peaks in the averaged response. In addition, desensitization to repeated stimuli becomes an issue. Finally, to interpret the response properly it is necessary to know whether it is derived from intranasal chemical stimulation of the trigeminal or olfactory system.

### Olfactometer

How is it possible to produce chemical stimuli that have a rectangular shape with rapid onset, that are precisely controlled in terms of timing, duration and intensity, and that do not simultaneously activate sensory systems other than chemoreceptors? Based on the principles of air-dilution olfactometry, such a system was developed in the late 1970s and 1980s (Kobal and Plattig, 1978; Kobal, 1985; Kobal and Hummel, 1988). Odorants are applied intranasally by means of a canula with an inner diameter of 2–3 mm. This canula is inserted for ~1 cm into the naris such that its opening lies beyond the nasal valve. Presentation of odorants does not simultaneously activate mechano- or thermoreceptors in the nasal mucosa, as odor pulses are embedded in a constantly flowing, humidified air stream (typically 6–8 l/min). Hence, subjects do not perceive any change in flow rate when the stimulator is switched from a no-stimulus to a stimulus condition and vice versa.

In this system, two air streams are directed towards the outlet of the olfactometer. Both have the same flow-rate, temperature and humidity. One contains an odorant at a defined concentration, whereas the other contains odorless air. Different odorant concentrations are generated by means of air dilution; hence, a pre-established, fully odorant-saturated air stream is mixed with an odorless air stream. While the sum of the two air streams is always constant, different ratios produce different stimulus concentrations. A separate system of finely tuned pressure and vacuum is applied such that, similar to an air-curtain, a small current of odorless air prevents molecules from odorant-containing tubings from being drawn into other odorless tubes. This crosscurrent allows for the attachment of several different odor lines to the same dilution line. During the interstimulus interval, a precisely tuned vacuum draws the odorant-containing air stream from the vicinity of the flowing air, ensuring that only odorless air enters the subject's nose during this time. Employing this device, it is possible to switch between an odorized air stream and control air in <20 ms. Depending on the physico-chemical properties of the odorants employed, a switch from one odorant to another can be made in <5 s without contamination from the previous stimulus.

The constant airflow directed into a subject's nose requires humidification (~80% relative humidity) and a stable temperature (36°C), since dry cool air produces nasal congestion, mucus discharge and pain which can interfere with the olfactory process (Mohammadian *et al.*, 1997, 1999; Lotsch *et al.*, 1998). The warmed and humidified air stream employed in our studies becomes undetectable within a few seconds of its introduction.

In the commercially available olfactometers based upon our designs (Burghart Germany), air flow rates are determined by mass-flow controllers that, along with switching valves, are computer-controlled. This equipment also allows the setup of sequences of stimuli with different quality, intensity, duration, or interstimulus interval. Thus, the recording of the olfactory event-related potential (OERP) becomes a routine procedure that can be carried out by any technician—a fact of particular importance in clinical applications.

## Olfactory stimulation using air puffs

In contrast to the stimulus presentation procedures described above, some laboratories record OERPs in relation to stimuli that are puffed into the nasal cavity, a procedure that we do not recommend. What happens when odorants are puffed into the nose? Under these conditions, it is not only possible to obtain ERPs in anosmic subjects (Herberhold, 1976; Cianfrone and Subiaco, 1978; Bauer and Mott, 1996; Sakuma *et al.*, 1996), but ERPs in normosmics that reflect the mixed activation of both the trigeminal and the olfactory systems. Such combined activity leads to numerous interactions at various levels of neuronal processing (for a review, see Hummel and Livermore, 2001) which cannot be remedied by simple mathematical procedures. For example, the average of responses to individual stimulation with CO<sub>2</sub> and vanillin or H<sub>2</sub>S is significantly different from the response obtained after stimulation with the binary mixture of CO<sub>2</sub> and H<sub>2</sub>S (Kobal and Hummel, 1988; Livermore *et al.*, 1992). Thus, it is difficult to interpret responses to olfactory stimuli contaminated by mechanical stimulation in patients with olfactory disorders. In addition, since the interactions between the trigeminal and olfactory systems are difficult to predict, it is misleading to interpret responses to mixed olfactory or trigeminal stimuli to reflect predominantly (and, implicitly, more or less exclusively) olfactory or trigeminal activation (Geisler and Murphy, 2000).

## Sniff' Table

In order to further increase diagnostic efficiency the olfactometer has been equipped with a new device (Sniff' Table) that offers the possibility of measuring thresholds in a triple forced stair case procedure in a fully computer-controlled fashion, thus extending the spectrum of possible test odors to match specific clinical needs. Here, instead of having an outlet of the olfactometer that delivers the odorant intranasally, the olfactometer is connected to a sophisticated switching device in the form of a table in front of the subject. This table delivers the odorant for a determined period of time through one of three sniff-ports at any concentration in the range the olfactometer is enabled to produce. The sniff-port delivering the odorant can be randomly selected, while the other two sniff-ports provide clean non-odorous control air. Based on the response of the subject, i.e. correct or incorrect identification of the odorant-delivering sniff-port, the olfactometer program delivers a higher or lower concentration at the same or another randomly selected port. Hence, it is possible to conduct a threshold measurement following the triple forced choice stair case procedure. An additional variation of this technique can also be used for an odor discrimination task. In this situation, one odorant would be directed to one port and another odorant would be directed to the two remaining ports. However, the number of different odorants is limited to a total of six, because of

the olfactometer specifications. The advantage of this new system is that thresholds and discrimination abilities can be measured automatically without any interference of an experimenter. Hence, this new system is allowing for high-throughput measurements in a large number of subjects providing precise concentrations of odorants for precise durations.

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