Olfactory Evoked Potential Produced by Electrical Stimulation of the Human Olfactory Mucosa

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Abstract

Most physiological studies of the human olfactory system have concentrated on the cortical level; the olfactory bulbar level has been studied rarely. We attempted to stimulate the human olfactory mucosa by electrical pulse to detect the bulbar potentials. Electrical stimulation (2 mA, 0.5 ms) of the human olfactory mucosa evoked a change in potential recorded from the frontal sector of the head. A negative peak of the evoked potential that occurred at 19.4 ms (grand means, $n = 5$) after stimulation was the clearest. The highest amplitude of the potential was recorded from the frontal sector of the head on the stimulated side. Our findings were similar to the experimental results obtained from the olfactory bulbs of animals. This evoked potential was considered to be the human olfactory bulbar potential. When the subjects were stimulated by applying electricity to the olfactory mucosa, no sensation of smell occurred even though evoked potentials were recorded. Evoked potentials were recorded only when the stimulating electrode was located in the olfactory cleft. When the stimulating electrode was outside the olfactory cleft, the stimulation caused pain. The trigeminal nerve seemed to be stimulated by electricity. Olfactory evoked potentials produced by the electrical stimulation of the human olfactory mucosa should aid the research on human olfactory physiology, and may be applicable to clinical tests of olfactory dysfunction. **Chem. Senses 22: 77–81, 1997.**

Introduction

Data relating to evoked potentials (Finkenzeller, 1966; Allison and Goff, 1967; Kobal and Hummel, 1991), neuromagnetic fields (Kobal and Hummel, 1991; Tonoike, 1994), positron emission computer tomographies (Zatorre *et al.*, 1992) and functional magnetic resonance images (Koizuka *et al.*, 1994) are helpful in understanding the physiology of the human olfactory system. Most studies of this system have concentrated on the cortical level; few have concentrated on the olfactory bulbar level. Detection of olfactory bulb activity in humans in the absence of surgery has not been reported.

We set out to record the olfactory bulbar potentials elicited by electrical stimulation of the olfactory mucosa from the frontal sector of the human head. This is the first attempt to detect olfactory bulb activity without the neurosurgical intervention.

Materials and methods

Subjects

Five healthy Japanese subjects (four males, one female), mean (± SD) age 28 ± 8 years, range 19–39 years, without nasal disease were tested. The insertion of pieces of gauze moistened with 0.1% epinephrine into the olfactory cleft opened the cleft sufficiently to permit the insertion of a stimulating electrode.

To reduce the level of the background electroencephalograms, subjects were sedated with 10% triclofos sodium syrup (20–25 ml, p.o.). Each subject provided informed consent for participation in the study.

Stimulation

A bipolar stimulating electrode was inserted into the olfactory cleft of each subject and its tips, made of silver spheres, were applied gently to the olfactory mucosa (Figure 1). Electrical stimulation was conducted with a stimulator (SEN-3301 and SS-401J; Nihon-Khoden, Tokyo, Japan) isolated from the acquisition amplifiers. Five-milliamp fuses were inserted in both the afferent and efferent circuits to avoid electrical accidents. The polarity of electrical stimulations was reversed after every 50 trials of each averaging course to cancel direct electrical artifacts. When the stimulative polarity was reversed, although the electrical artifact was also reversed, the evoked potential was not. As a result, only the artifact was canceled after having been averaged. We confirmed that this procedure reduced the number of electrical artifacts without influencing the waveform and latency of the evoked potential (Ishimaru *et al.*, 1996). The stimulation rate was 2 Hz, except where otherwise noted.

Acquisition

Five recording electrodes were placed on both the right and left lateral and frontal sectors of the head and on the upper central frontal sector (see Figure 2a for details). Potentials from each electrode were compared with those from the neighboring electrodes and from the upper frontal central head electrode. The earlobe on the stimulated side saved as the ground. Changes in potential were amplified, band pass filtered (2–250 Hz), averaged and stored on floppy disks by the medical oscilloscope system (MEB 5500; Nihon-Khoden, Tokyo, Japan). Personal computers were used for off-line processing (PC9801; NEC, Tokyo Japan). Student's *t*-test was used for statistical analysis.

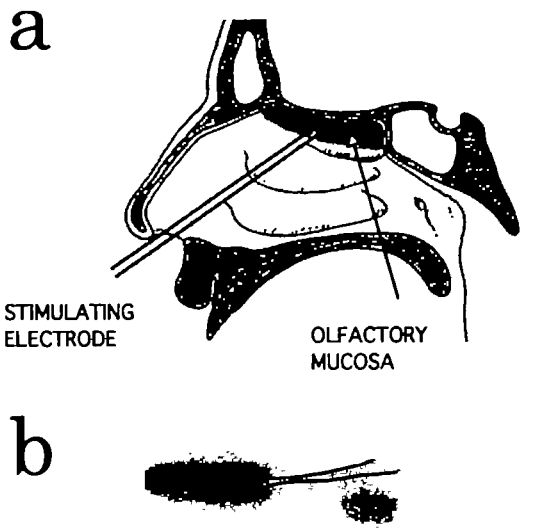

Figure 1 Stimulating electrode. **(a)** A bipolar stimulating electrode was inserted into the human olfactory cleft via a nostril and used to stimulate the olfactory mucosa. **(b)** Enlarged view of the bipolar tips of the stimulating electrode, made of silver spheres.

Results

Evoked potentials and their localization

The olfactory epithelium of one side was stimulated with an electrical current of 2 mA for 0.5 ms. The results of 300 trials were averaged.

The olfactory evoked potential produced by electrical stimulation of the olfactory mucosa was termed 'electrical olfactory evoked potential' (EOEP).

The EOEP of a 27-year-old male with the lowest background level in cortical electroencephalograms is shown in Figure 2b. This EOEP included two peaks, the first a negative peak 25.6 ms after stimulation (N1) and the second a positive peak 55.6 ms after stimulation (P1). N1 appeared clearly in the EOEPs of all subjects, but P1 was rather unclear except in the subject in Figure 2.

The amplitude of N1 was maximal in the frontal sector of the head on the stimulated side (Figure 2b, record B). Records A–B and B–C represent the potential difference between records A and B and the difference between records B and C. N1 was reversed in the frontal sector of the head on the stimulated side between record A–B and B–C (Figure 2b).

The amplitude of the potential difference between N1 and P1 was largest in the frontal sector of the head of the stimulated side (Figure 3, $n = 5$, t-test, $P < 0.005$).

When the stimulating electrode was located outside the olfactory cleft, the subject developed somatosensory pain and the EOEPs could not be detected ($n = 5$).

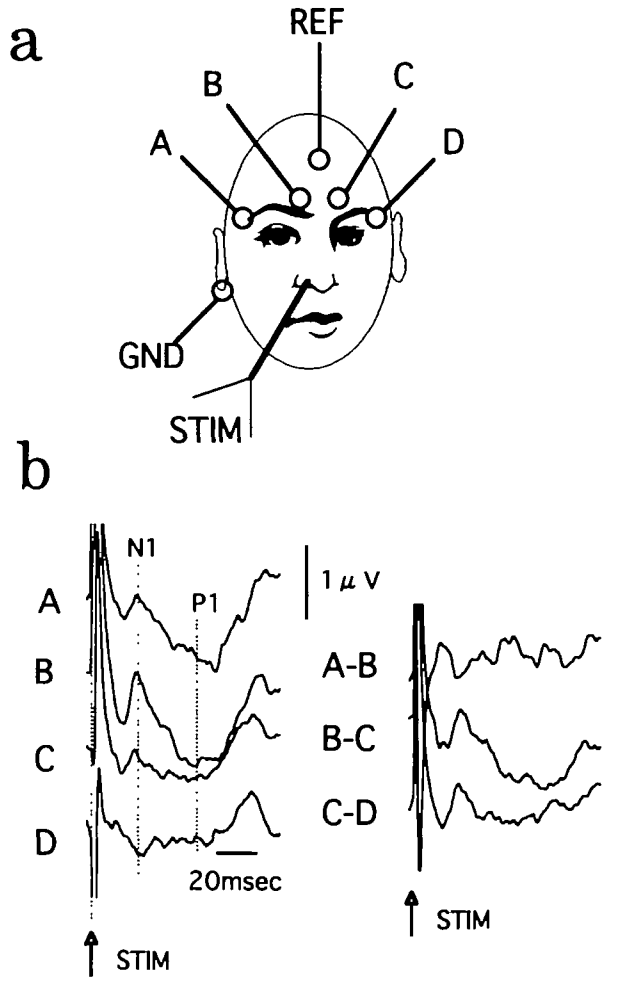

Figure 2 Evoked potentials and their localization. **(a)** Locations of recording electrodes are illustrated. Potentials from frontal and lateral electrodes of both sides (A–D) were compared with those from to the neighboring electrodes and from an upper frontal electrode (REF). The earlobe on the ipsi-stimulated side served as ground (GND). STIM indicates an inserted stimulating electrode. **(b)** Olfactory evoked potentials produced by electrical stimulation of the olfactory mucosa of a normal 27-year-old male. The evoked potentials were recorded from a subject sedated with 25 ml of 10% triclofos sodium. The acquisition electrodes were located as follows: stimulated side lateral sector of the head (A), stimulated side frontal sector of the head (B), non-stimulated side frontal sector of the head (C) and non-stimulated side lateral sector of the head (D). Records A, B, C and D are the differences in potential between each recording electrode (A–D) and REF. Records A–B, B–C and C–D indicate the difference in potential differences between successive records (A–D). The clearest negative peak, 25.6 ms after stimulation, was observed at the highest voltage in record B. This peak was reversed between records A–B and B–C. STIM indicates stimulation, and upward deflection is negative.

Grand means of the evoked potentials

EOEPs from the frontal sector of the head of the stimulated side were averaged (n = 5). The stimulated side was the right side in four subjects and the left side in one subject. An N1 of 19.4 ms after stimulation was observed, but P1 was unclear (Figure 4).

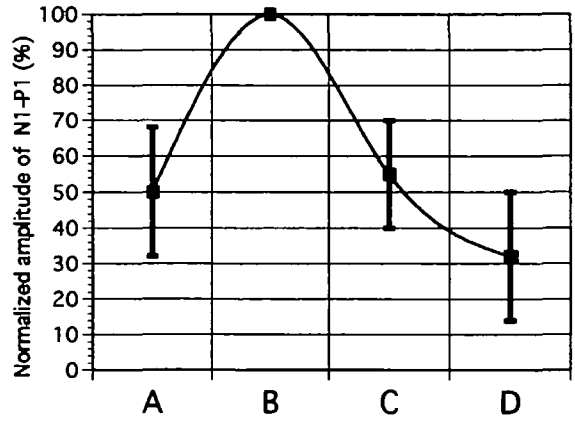

Figure 3 The relationship between the differential amplitude of N1–P1 (peak-to-peak) and the location of the active electrode was plotted (mean ± SD, n = 5). A, B, C and D show the locations of the active electrodes (see legend to Figure 2). The every amplitude was explained as the percentage of the N1–P1 amplitude of record B.

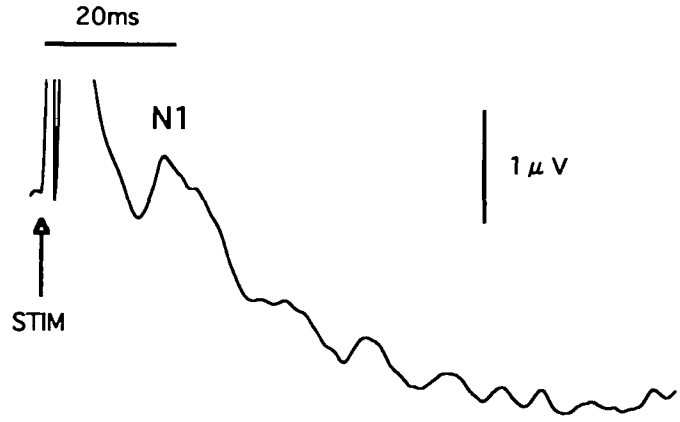

Figure 4 Grand means of five normal subjects. Evoked potentials of five normal subjects recorded from the frontal sector of the head on the stimulated side (see Figure 2a, B and b, B) were averaged. Four subjects were male and one was female. The mean age of these subjects was 28 ± 8 years (mean ± SD), with a range of 19–39 years. A negative peak 19.4 ms after stimulation (N1) was observed.

Subjective sensations experienced during electrical stimulation of the olfactory mucosa

Before sedation, we questioned the subjects about subjective sensations experienced during the electrical stimulation of the olfactory mucosa. No smell sensation occurred during the electrical stimulation of the olfactory mucosa with a 2 mA current. Subjects sometimes complained of somatic sensations, but by shifting the stimulating electrode a little, we found it easy to position it so as to avoid them. These somatic sensations were tactile-like senses and differed from those of pain. Pain occurred when the stimulating electrode was outside of the olfactory cleft and touching the respiratory mucosa. Somatic sensations did not affected the

EOEPs, which were evoked with or without such sensations. One subject complained of a feeling of dryness of the nose after the study.

Discussion

EOEPs were elicited by 2 mA, 0.5 ms current pulses. These stimulating parameters were sufficient to evoke changes in the potentials of the rabbit olfactory bulb that were detectable from the skull (Ishimaru *et al.*, 1996). Rabbit EOEPs are thought to represent changes in potentials of bulbar neurons (Yamamoto, 1961; Martinez and Freeman, 1984). When rats were stimulated with odors, a similar change in potential was recorded from the olfactory bulb and it was thought that findings were equivalent to those of rabbit EOEPs (Evans and Starr, 1992).

The first negative peak, N1, occurred at 25.6 (one subject) or 19.4 ms (grand means). The peak latency was between 23 and 28 ms in rabbits (Yamamoto, 1961; Martinetz and Freeman, 1984; Ishimaru *et al.*, 1996).

Olfactory axons have been reported to be ~0.2 μm in diameter both in the rabbit (Lorenzo, 1957) and in the human (Moran *et al.*, 1982). If the thickness of axons is the same, they appear to have a similar transduction velocity. Because the human olfactory mucosa along the antero-posterior axis is narrow, axons of sensory neurons are shorter in the human than in the rabbit, and as a result, latency should also be shorter.

The amplitude of EOEPs was the highest in the frontal sector of the head on the stimulated side (Figure 2, record B). Differential EOEPs reversed the polarity between records A–B and B–C in Figure 2, a result that indicates the origin of human EOEPs located in the frontal sector of the head of the stimulated side. It is possible, therefore, that the olfactory bulb is the origin of EOEPs.

The amplitude of human EOEPs was only one-tenth that of rabbit EOEPs (Ishimaru *et al.*, 1996). Because the human cerebral cortex develops and covers the olfactory bulb, the origin of the current is located farther from the active electrodes than the rabbit's, so the amplitude of EOEPs was attenuated.

P1 was unclear in the grand means. P1 was thought to be more easily masked by background noise than was N1. Background noise increased during light sleep. Subjects seemed to find it difficult to achieve deep sleep with the nasal electrode inserted, as was the case with the subject in Figure 2.

It has been suggested that the EOEP is really an olfactory potential or trigeminal somatosensory evoked potential (TSSEP). However, the fact that TSSEP originates in the brainstem means that the greatest change in potential should not be recorded from the frontal sector of the head and should lie superior contralateral rather than ipsilateral (Bennett and Jannetta, 1980; Ishiko *et al.*, 1980; Hashimoto, 1988). It is thus unlikely that the EOEP is a TSSEP.

An important result of our study was that the EOEPs were recorded even though the subjects did not experience any sensation of smell when their olfactory mucosa was electrically stimulated. This electrical stimulation on the human olfactory mucosa not provoking a sensation of smell has also been seen in the literature (Uziel, 1973; Straschill *et al.*, 1983). We propose two hypotheses to explain this phenomenon.

(i) While the selected olfactory cells are excited when the stimulus is a given odor (Mathews, 1972; Buck and Axel, 1991; Hirono et al., 1992), all types of olfactory cells are excited when the stimulation is electrical. When all kinds of odorants stimulate the olfactory mucosa, it is not well known what kind of smell occurs. We call such a smell 'white smell', akin to white noise or white light, but do not know whether it can be recognized. It seems that a 'white smell' is ignored by the cortex higher than the olfactory bulb. The fact that no one perceives a sensation of smell even though olfactory cells fire spike discharges in a non-stimulated stage (Frings and Lindemann, 1991) supports this hypothesis.

(ii) Whereas odorous stimulation excites a wide area of the olfactory mucosa, resulting in a characteristic map of the odor (Kent and Mozell, 1992; Mackay-Sim and Scott, 1994; Mori and Yoshihara, 1995), electrical stimulation is concentrated in the very small part of the olfactory mucosa that the electrode touches. Such stimulation may be enough to excite the olfactory bulb but not the olfactory cortex.

Human olfactory evoked cortical potentials (OECP) are applied not only in basic research but also clinically (Ohba, 1985; Auffermann *et al.*, 1993; Ito, 1993). The cortical potential is strongly affected by the consciousness of an experimental subject. EOEP seems better for studying objective olfactometry than OECP, because EOEP can be recorded without concatenation. Clinically, the relationship between olfactory disturbance and dysfunction of the olfactory bulb is unclear, except in conditions such as Kallman's syndrome. EOEP should help us to acquire more information about diseases of the olfactory bulb.

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