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Change of Chemosensory Event–Related Potentials on Olfactory Stimulation as a Function of Odorant Concentrations

Masashi Wada, M.D.

Department of Otorhinolaryngology, National Center of Neurology and Psychiatry, Chiba, Japan

Abstract: Change of chemosensory event–related potentials on olfactory stimulation as a function of odorant concentrations is discussed. According to the ascending method, from undetectable level to clearly detectable level, the dependency of shortening of the peak latency on the concentration of odorant was recognized as a rapid decrease of amplitude from the threshold level when results of our ascending trial were averaged. However, a much higher concentration of odorant did not always evoke a positive response and a shorter peak latency.

Keywords: chemosensory event–related potential; notch; quick adaptation

INTRODUCTION

Several reports about olfactory evoked potentials have been published [1–3], although stable, constant responses evoked by odorant stimulations were not easily elicited owing to problems associated with control of the stimulus. The technique of synchronizing olfactory stimulation with a subject's inspiration than was introduced [4–6]. We then studied the effect of odorant concentrations on evoked response after olfactory stimulation.

MATERIAL AND METHODS

Each subject was instructed to lie supine on a bed. An electroencephalogram (EEG) was recorded as upper negative from the central midline by an active electrode using a monopolar recording. Other electrodes were attached to one ear auricle (indifferent electrode) and the forehead (earth electrode), according to the International 10-20 system (bandpass, 1–30 Hz; impedance, 2–6 kΩ). Odorant pulses were introduced by a new odorant stimulator at a flow rate of 1 liter/min (Fig. 1). Each bottle contained 5 ml of a given concentration of odorant.

Reprint requests: Dr. Marashi Wada, Department of Otorhinolaryngology, National Center of Neurology and Psychiatry, 7-1, 1-Chome, Kohnodai, Ichikawa-Shi, Chiba, 272, Japan.

Just prior to the onset of a subject's inspiration, the tip of the stimulator was atraumatically inserted 1 cm into the nostril (usually into the right side). After the odorant was introduced, the tip of the stimulator was removed gently from the nostril. The odorant pulse trigger was the subject's respiration. An electric valve was used to introduce the odorant stimulations and was activated for 300 msec by a trigger pulse generated by a hand-operated switch attached at the stimulator. Trigger pulses were generated just after the start of inspiration and were determined by visual inspection of the subject's abdominal movement at a rate of once in four slow, regular respirations.

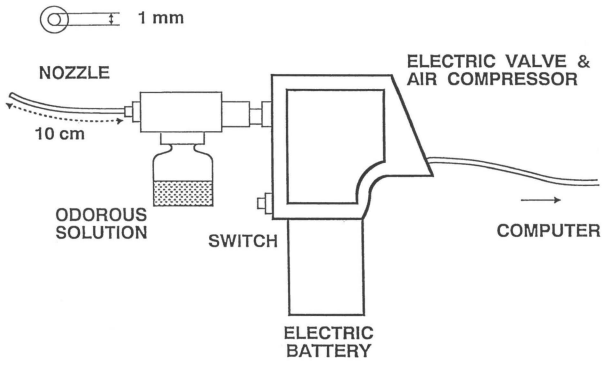

Figure 1. Schematic diagram of the stimulator (nozzle length, 10 cm; inside diameter, 1 mm).

Odorant E

Skatole

one of the standard odorants adopted by Japanese Society of Otorhinolaryngology

E5	highest concentration of "E" 9.75 % skatole
E4	0.975 % skatole
E0	9.75 ppm skatole
E − 1	0.975 ppm skatole
E − 2	lowest concentration of "E" 0.0975 ppm skatole

* Standard Odorants (adopted by Japanese Society of Otorhinolaryngology)

A	β - Phenylethyl Alcohol
B	Methyl Cyclopentenolone
C	Isovaleric acid
D	γ - undecalactone
E	Skatole

Figure 2. T & T Olfactometer [4]. *E* indicates skatole, which is one of the standard odorants adopted by the Japanese Society of Otorhinolaryngology. *E5* represents the highest concentration of skatole. (9.75%).

The subject was asked to report in each presentation whether an odor was recognized and to evaluate the intensity of odor perceived. The analysis time was 1,000 msec. After eight responses to a given concentration of odorant had been recorded, the results were averaged by a Neuropack Four Computer (Nihon Kohden Co., Japan).

The study consisted of eight apparently healthy, Japanese male nonsmokers, 26–38 years old. Their olfactory abilities were determined to be normal by testing them with a T & T Olfactometer [7]. Each subject was tested using skatole. Skatole is one of the standard odorants supplied with the T & T Olfactometer, which has been adopted by the Japanese Society of Otorhinolaryngology (Fig. 2) [7]. E5 (9.75% skatole) is the highest concentration of skatole, E4 (0.975% skatole) is a 10% dilution of E5, E3 (0.0975% skatole) is a 10% dilution of E4, and the lowest concentration of skatole is E-2 (0.0975 ppm skatole) (see Fig. 2) [7]. The odorant was introduced by the ascending method, from an undetectable level to a clearly detectable level. Eight presentations of each concentration were made. During the ascending method, much higher concentrations of odorant were introduced at an interval of 15 min [4]. The environmental temperature of the test room was maintained at between 21° and 24°C.

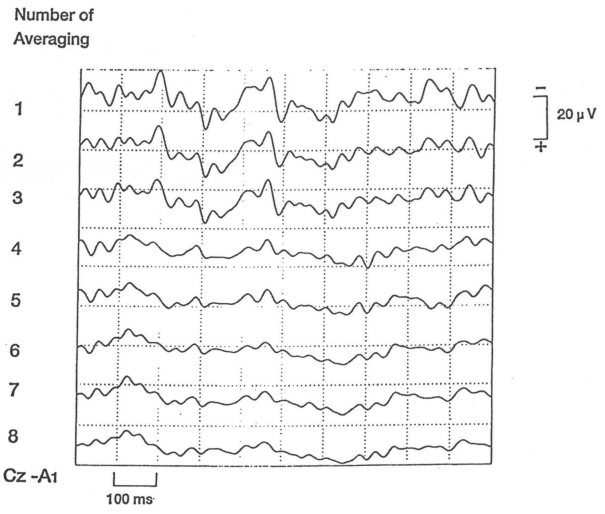

Figure 3. Unscented air produced no response.

RESULTS

There was no detectable responses in the absence of the odor, as shown in Figure 3. When the odorous stimulation was introduced at the end of inspiration or during the expiration, the evoked response was undetectable. A typical pattern of an evoked response to E5 in a normal

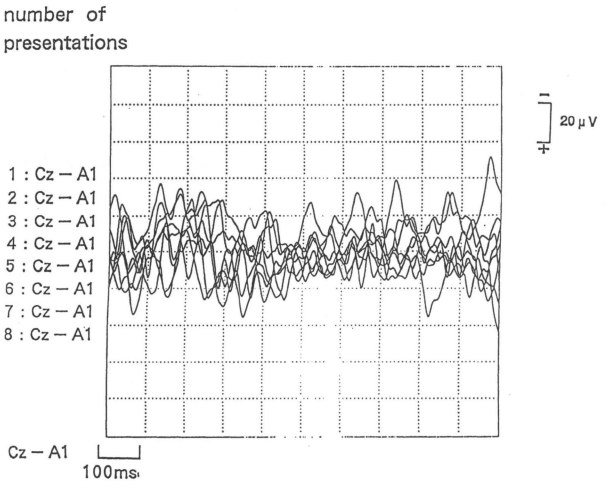

Figure 4. Evoked response to E5 (9.75% skatole) before averaging of results. Positive responses were detectable at a certain peak latency by the superimposition technique.

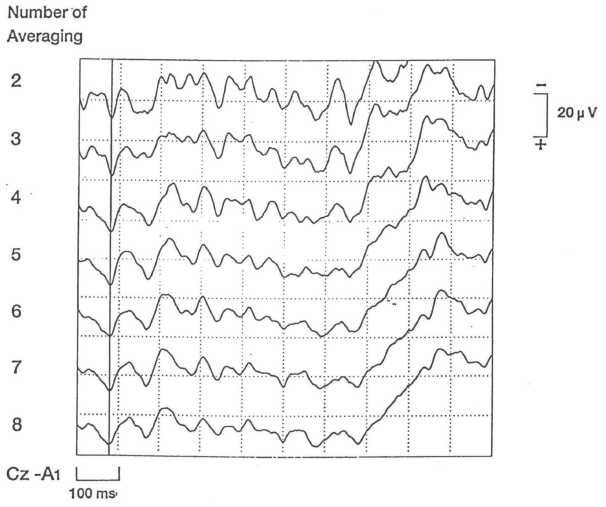

Figure 5. Averaged evoked responses to E5. Positive responses became obvious employing the averaging technique. Wave 8 represents eight times the total. Waves 4–5 show the largest amplitude.

young subject before averaging is shown in Figure 4. Positive responses were detectable at a certain peak latency by using a superimposition technique and, after results averaging, positive responses became obvious (Fig. 5). In the graph shown, wave 8 represents eight times the total; thus, wave 4 or 5 shows the largest amplitude and wave 7 or 8 represents the clearest wave. E5 evoked a positive response with a peak latency of between 68 and 84 msec in eight normal young subjects.

According to our previous study, the sufficient interval for producing a good response to a subsequent different odorant was 15 min [4]. Figure 6 depicts the evoked response to E-2 in a normal subject. The detection threshold measured by the T & T Olfactometer was E-1 (0.975 ppm skatole) in this case. As shown here, positive response to E-2 was not found and, 5 minutes later, a much higher concentration of odorant, E-1 evoked a positive response with a peak latency of 118 msec (Fig. 7). In this response, quick adaptation was noted: that is, a rapid decrease of amplitude in averaged responses was recognized, and the main positive response was undetectable after six averagings. Responses to E-1 presented again 15 min later had a shorter peak latency as compared with a first E-1 presentation (Fig. 8). Fifteen minutes later, a higher concentration of odorant, E0 (9.75 ppm skatole), evoked a positive response with quick adaptation (Fig. 9). Another 15 min later, E1 (90.75 ppm skatole) evoked a positive response (Fig. 10).

Hence, in all eight subjects tested, the olfactory threshold measured by the chemosensory event–related potentials on olfactory stimulations according to our ascending method was E-1 or E-2. Detection of an olfactory threshold according to each subject's report was equal to a threshold measured by evoked response in all of the eight subjects tested. In all subjects, responses to E-1 had a peak latency of 72–120 msec, and responses to E1 had a peak latency of 73–108 msec.

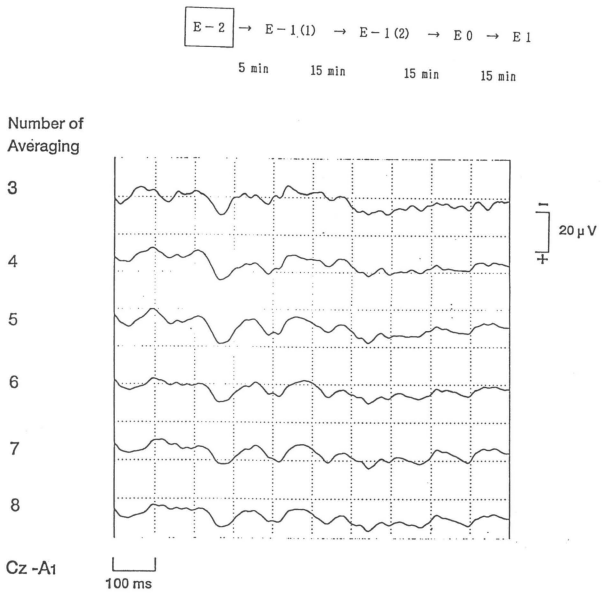

Figure 6. Evoked response to E-2 (0.0975 ppm skatole). The detection threshold measured by the T & T Olfactometer was E-1 in this case. No positive response was found.

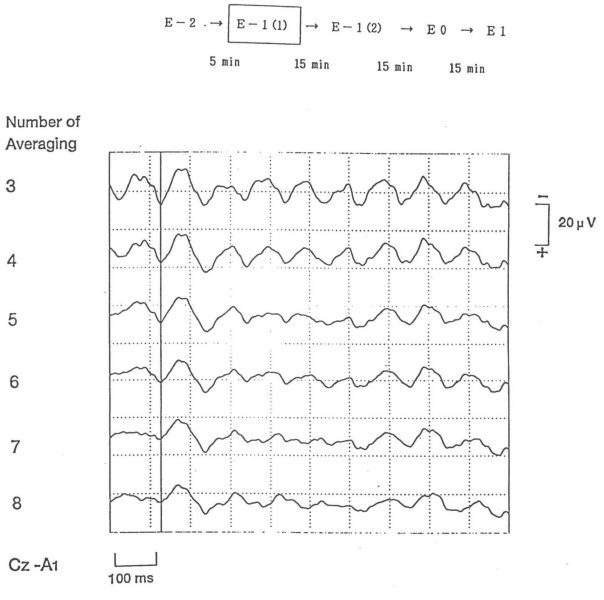

Figure 7. Evoked response to E-1 (0.975 ppm skatole). Five minutes later, E-1 evoked a positive response with a peak latency of 118 msec. In this response, rapid adaptation was found.

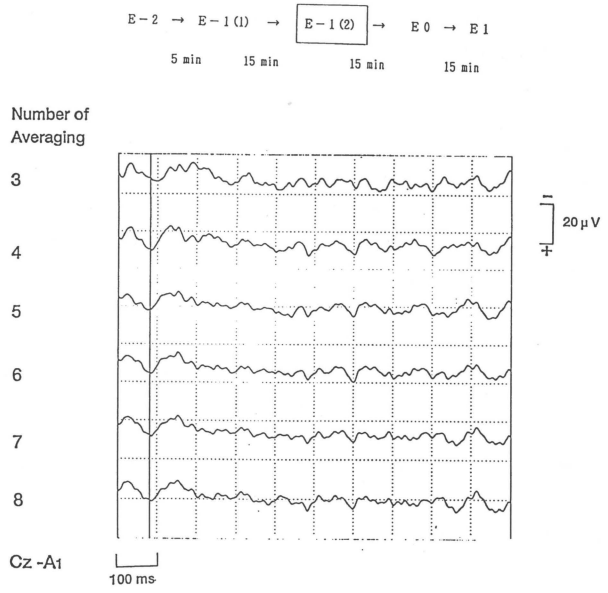

Figure 8. Evoked response to another presentation of E-1 (0.975 ppm skatole). Fifteen minutes later, another presentation of E-1 evoked a positive response with a shorter peak latency as compared with the first E-1 presentation.

The dependence of a shortening of the peak latency on the concentration of odorant was recognized as a rapid decrease of amplitude from the threshold level when results of our ascending trial were averaged. However, a much higher concentration of odorant did not always evoke a positive response and a shorter peak latency.

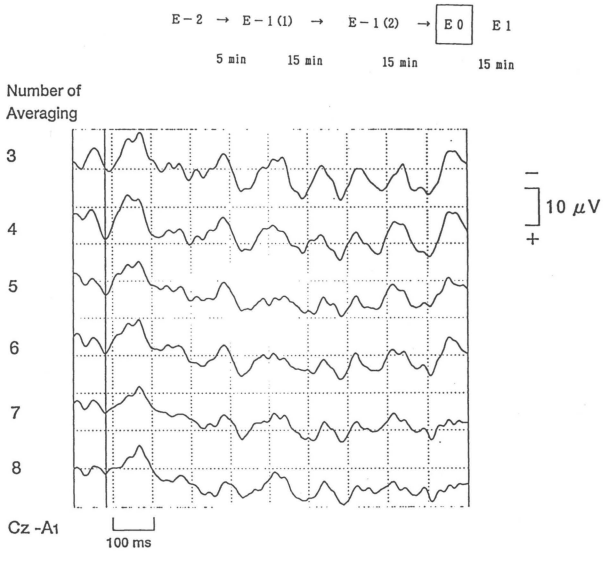

Figure 9. Evoked response to EO (9.75 ppm skatole). Fifteen minutes later, a much higher concentration of EO showed a positive response with rapid adaptation.

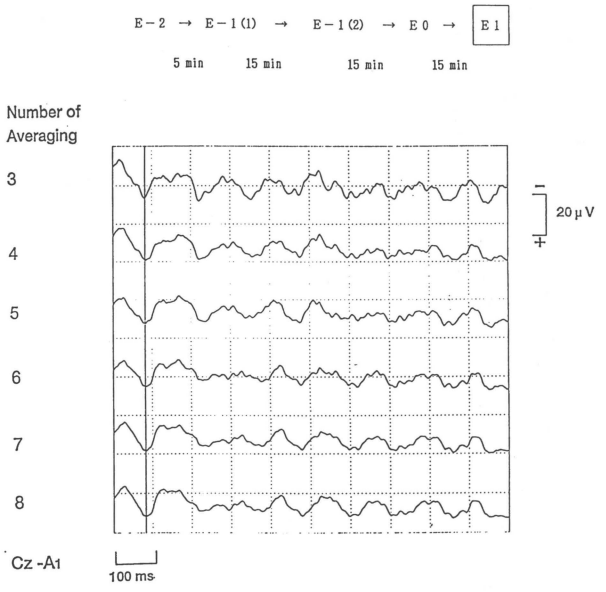

Figure 10. Evoked response to E1 (90.75 ppmm skatole). Fifteen minutes later, E1 evoked a positive response.

DISCUSSION

In our study, the positive waves were distinguishable as the evoked response by using the technique of superimposition before averaging, and the positive response became obvious after averaging [4–6]. A saturation of responses was found after four to five averagings. The saturation phenomenon was related to the amplitude of the evoked potentials for repeated pulse stimuli and was found to be related to olfactory fatigue. Olfaction must be stimulated precisely at a certain time in order to calculate the responses for discussing olfactory evoked response. For this reason, we opted to deliver the aerosolized odorant to the olfactory fissure via pressurized air synchronized with a subject's inspiration. Introduction of an odorant at the end of inspiration or during expiration resulted in undetectability of an evoked response.

A reproducible and stable response was recorded by our technique for the following reasons: Respiration is constant in the same subject. If slow and regular respiration is achieved, respiration will be repeated at a constant speed and, consequently, the odorant air will be delivered to the olfactory fissure at a certain time in the same subject. There was no detectable response in the absence of the odor. In addition, when the odorous stimulation was introduced at the end of inspiration or during the expiration, the evoked response was undetectable. In our previous study, glacial acetic acid, which is believed to be a trigeminal nerve–sensitive substance, evoked a negative response without detection of odor in an anosmic patient. Accordingly, the evoked response to skatole re-

ported in this article was believed to be elicited mainly by odorant stimulation to the olfactory nerve.

A difference in peak latency as reported in many articles is considered to be due to variations in the odorant transmission system, including the length and inside diameter of the tube used [1–4,6]. Kobal and Hummel [2] reported that the change in peak latency depends on the concentration of the odorant. In their study, the shortening of peak latency was recorded on stimulation by the much higher concentrations of odorant. The dependence of shortening of peak latency on the odorant concentration during our ascending trial was recognized as a change from threshold level to a level showing a rapid decrease of amplitude with averagings, which were considered to correspond with "notches" marked by Doty [8]. However, a much higher concentration of odorant did not always evoke a positive response and a shorter peak latency.

In our ascending method, an odorant's concentration is increased incrementally from an initially undetectable level to a level at which it is reported as being clearly detected by the subject. Subjects were asked to report in each presentation whether an odor was recognized. In some cases, a positive response may be influenced by the previous odorant stimulation when the interstimulus interval is insufficient, even if the intensity of odorant presented first is subthreshold. According to the influence of the previous presentations of lower concentrations of odorant, a change of peak latency (shortening or delay) and quick adaptation were seen. In the ascending method, detection of olfactory threshold according to each subject's report equalled the threshold measured by evoked response.

The lowest concentration of odor that can be perceived is commonly termed the *odor detection threshold.* This concentration is not a fixed entity. Rarely it is appreciated that among so-called normal individuals, threshold measures fluctuate from moment to moment and that average threshold values differ by several orders of magnitude [8]. We noted a very interesting result using suprathreshold odorant stimulation—that is, rapid adaptation in averaged responses with a quick decrease in intensity of odor perceived. Much higher concentrations of odorant evoked clearly detectable positive responses again. This phenomenon is considered to correspond with "notches" marked by Doty [8] in the stimulus-response functions found for many odorants. This notch was found in all of our eight subjects tested. Not all odorants show reversals in the stimulus-response function, and a number of odorants exhibit only minor alterations [8]. At least in some species, the location of the reversal on the stimulus-response curve varies as a function of a molecule's position within a homologous series [8]. According to a result by Doty [8] for methyl ethyl ketone, a nonmonotonic relationship between odor detection performance and odorant concentration was recognized. When we measure the olfactory threshold, we pay attention to this notch.

Our findings suggest that the evoked potential technique and olfactory electroencephalographic response as measured by our method could be beneficial in a clinical setting for assessing abnormal olfaction.

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