◈ Application Papers

Development of An Apparatus to Control Odorous Stimuli for Olfactory Evoked Responses

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Abstract

We developed an apparatus for odorous stimuli control to record olfactory evoked potentials from human scalp. The characteristics of the apparatus were as follows. 1. Translating the subjects respiration into electric signals with a sensor attached to the nose. The period and timing of odorous stimuli could be adjusted, so that stimuli could be synchronous with respiration. 2. The respirations translated into electric signals were made constant in amplitude by using an auto gain control circuit. 3. The interstimulus interval of odorous could be arbitrarily selected once every 1 to 9 respirations so that adaptation could be prevented. We obtained olfactory - evoked potentials (OEPs) to odorous stimuli using this apparatus from the site of Cz, whose positive peak latencies were approximately 180 ±23ms. Such response were not recorded if oxygen stimuli were used instead of odorous or with click sounds produced by the switching electromagnetic valve.

Key Words: Olfactory evoked potential, odorous stimuli, apparatus, human

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1. Introduction

Allison (1962) first reported on olfactory evoked potentials. The evoked potentials were obtained by using an averaging technique. Since then , there have been other reports related to this (Kobal, 1981; Plattig and Kobal, 1979; Seta, et al., 1991; Smith, et al., 1971; Tonoike and Kurioka, 1981; Min, et al., 1998). There have been only a few such studies compared with the voluminous studies of evoked potentials referring to visual (e.g., Cobb and Dawson , 1960; Vaughan et al., 1963; Shawkat and Kriss, 1997), auditory (e.g., Celesia et al., 1968; Ninomiya et al., 1997), and somatic (e.g., Allison, 1962; Araki et al., 1997) sensation until quite recently. One reason for this is the confusion surrounding olfactory evoked potential including the many sensations of not only smell but also touch, pressure , and warm senses that have been mentioned. Recently, Tonoike et al..(1998) and measured the cerebral magnetic fields caused by odorant substance stimulus, and they attempted to estimate the site of olfactory response. Although stimuli such as light and sound can be defined in physical quantity, it is difficult for the stimulus of odorant substance to be quantified due to chemical substances. Moreover, the following basic problem should be considered when giving odorant substance to a subjects nasal cavity. Firstly, the timing of triggering for the sake of averaging evoked potentials, the duration of stimulation, and the area of stimulation should be investigated. Secondly, in addition to successive odorant substance stimuli, the fatigue or the adaptation of the nasal cavity, by which the sensation for the odorant substance decreases or completely disappears, occurs and a definite time is required for the recovery from these phenomena. Thirdly, the synchronization of excitation of the individual nerve fiber is difficult, so that the problem of the stimulation method should be considered.

In this paper, we will discuss our recently devised apparatus design to control odorant stimulation and olfactory evoked potentials from the human scalp.

2. Materials and Methods

2.1. Subjects

The subjects were 10 healthy men aged 21 to 26 years with normal olfactory sense. All subjects were non-smokers and were instructed to restrain from drinking or eating for at least one hour prior to the measurement.

2.2. Stimulus conditions

Using the odorant stimulation device, the stimulant was delivered for 0.5sec once every 4 inspirations for a total of 20 stimulations. Methyl cyclopentenolone (B4, Olfactometer T&T), which emits a burnt caramel like smell, was used as the odorant element.

2.3. Experimental apparatus

Problems related to recording the olfactory evoked potentials (OEPs) resulting from odorant stimulation of the human scalp can be summarized as follows: (i) delivering stimulation synchronous with averaging,(ii) sending enough odor to the olfactory receptor cells,(iii) olfactory adaptation or fatigue.

To resolve the above problems, an odorant stimulation controlling device was created in conjunction with this research. Fig 1 shows the external appearance of the device. The device functions by measuring temperature changes at the nostrils during respiration.

Fig.1. Device for controlling odorant stimulation.

The device, a thermistor, records changes in the respiratory temperature as a corresponding electric signal. The inspiration phase is distinguished from the expiration phase in order to restrict administration of the odorant to the inspiration phase only. The function and particular characteristics of this odorant stimulation apparatus are more fully described below.

First, an electromagnetic valve is used to synchronize a specific period of the inspiration phase with the intake of an odor stimulant. Changes in the temperature during respiration are converted into changes in resistance by the thermistor and are output as changes in voltage (Fig.2a). The electric current is supplied by a DC power source. Next, the signal caused by the respiration is passed through the comparator circuit where a positive rectangular voltage output is generated if the respirations voltage is larger than

the standard voltage and a negative rectangular voltage output is generated if the voltage of respiration is smaller than the standard voltage. The size of the standard voltage can be varied depending upon the level of inspiration so that the optimal odor stimulation can be adjusted individually. Only the positive voltage was taken out from the rectangular wave voltage and a pulse signal synchronous with the rising of that voltage was generated by a mono-multi-vibrator. The width of the pulse signal was determined by a time constant circuit and this output width was used as the stimulation time (Fig.2b).

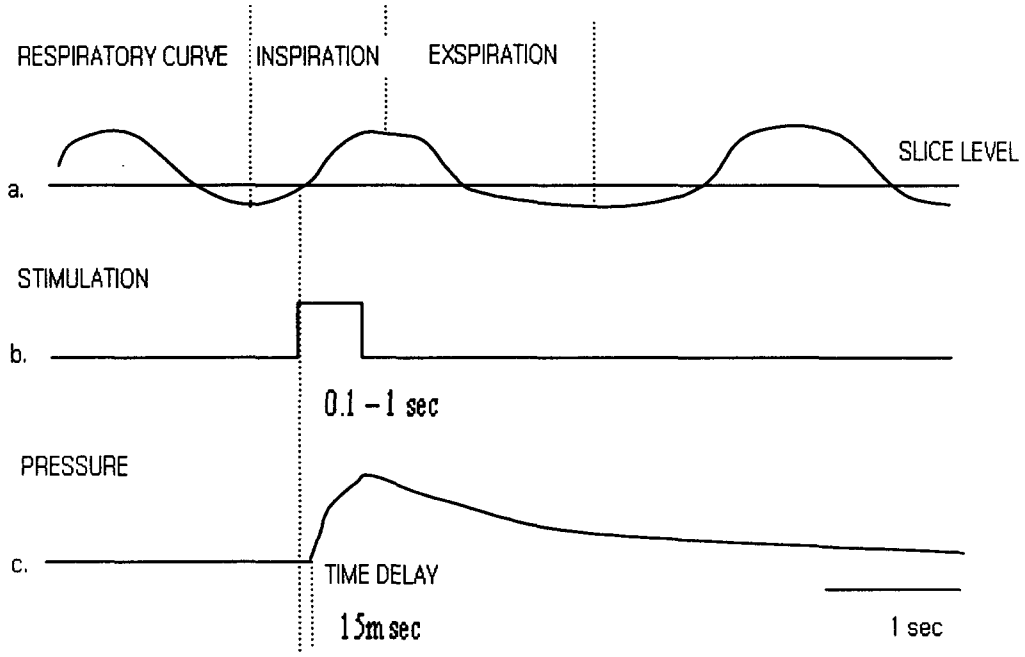

Fig.2. Method of synchronization between odorant stimulation and respirations. A: Translation of temperature changes in respiratory gas into electric signals. The sliced level is the standard voltage to generate a trigger pulse. B: Electronic pulses for opening the solenoid valve. C: The O2 pressure at the end of the tube measured by a pressure transducer.

The fatigue or adaptation of the olfactory sense to the stimulus during the test is taken into consideration: the pulse is divided into an optional number of respiration between 1 and 9 by the N-divided counter. The pulse is then amplified to a voltage high enough to drive the electromagnetic valve and is then outputted.

A block diagram for the entire experimental device is illustrated in Fig.3.

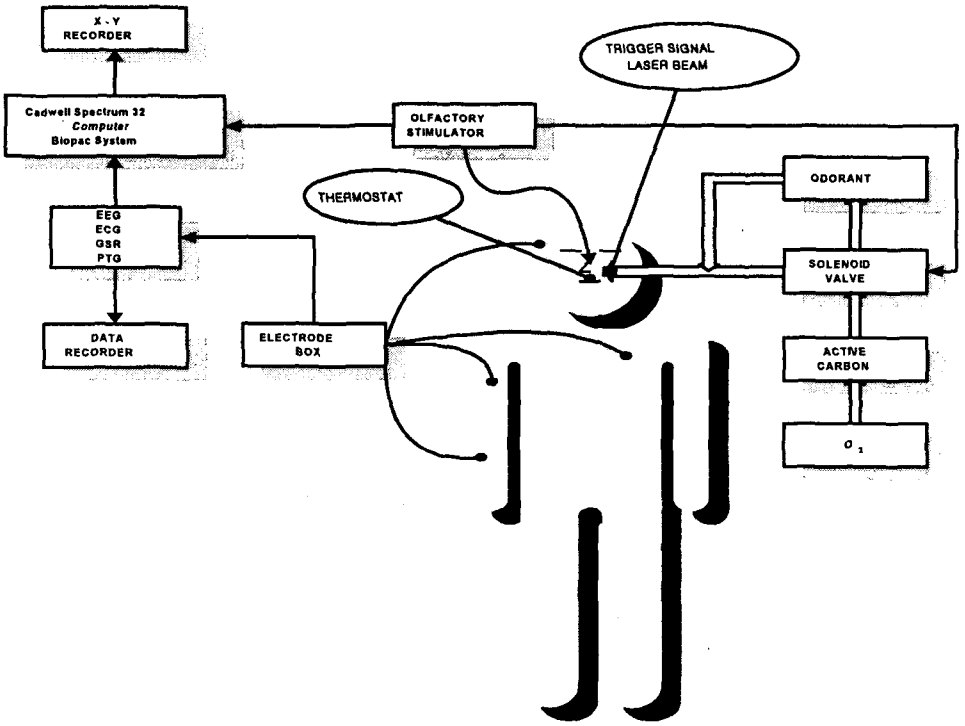

Fig.3. Block diagram showing the method of presentation of odorant pulse to a subject.

The method for odorant stimulation is described as follows: Oxygen is sent out at the flow rate of 3 liters per minute from an oxygen cylinder via a tube with an inside diameter of 5mm. The oxygen is then deodorized by activated charcoal, and the tube is connected with a 1-input 2-output electromagnetic valve. Of the two outputs, one reaches the nostrils via a tube at the end of which is attached a funnel 5cm in diameter. In this way, oxygen can always flow easily into the nasal cavity. To prevent the nasal mucosa from being dry, oxygen is passed through distilled water so that proper humidity in the middle of the tube is maintained. The second output is attached, via a tube, to a beaker containing the odorant element. From this beaker runs an additional tube that is joined to the tube carrying the oxygen near the nostrils. This output routing from the odorant element can be closed by the electromagnetic valve. At a certain fixed point in time during the inspiration phase, the electromagnetic valve is used to switch the flow of air being sent into the nasal cavity from the route of the odorless oxygen to the route containing

the odorant substance. A 15msec delay was noted between the time the electromagnetic valve is activated to the time the tube contents change from pure oxygen to the odorant. This delay is calculated by measuring changes in the tubes pressure at the terminal of the tube containing the odor. A pressure transducer was attached to this tube to measure these changes (Fig.2C.).

2.4. Recording of evoked potentials and statistical analysis of latencies at the peak evoked potentials

EEG was measured in a quiet room with an area of 12㎡. The subject was instructed to sit on a chair in a relaxed state. The subject was requested to sleep well the night prior to the test. A one minute rest time between the tests was taken, so that the results were not affected by fatigue of olfactory sense or adaptation. The temperature of the measurement room was kept at 20-23℃ and the time for the measurement was from 2:00 to 5:00 in the afternoon. The condition of the measurement was the same as used by Hekin and Christiansen (1967). Following the study of Henkin and Christiansen, similar room temperature and experiment time were employed. For example, Yoshii et.al. (1981) experimented at the room temperature of 21±1 degree C. As for the experiment time, Yamamoto et al. (1985) experimented from 2:00 to 4:30 in the afternoon.

The time constant of the amplifier was taken to be 0.3s and the frequency of the high-cut filter was 120Hz. Although the cortical site where the olfactory area exists was the temporal region, the vertex (Cz; 10/20 international system) was used in the study because it is a good site for recording olfactory evoked potential according to Tonoike (1981). In the measurement of evoked potential, the monopolar leading between Cz and A1 (the left lobe) was taken, and the fround was set on diameter (Nihon Kohden, NE-121B) were used.

The trigger pulse that opens the electromagnetic valve for starting the odorant stimulations was also recorded simultaneously with the EEG data by an electroencephalograph (Neuro-Pack Eight Computer, Nihon Kohden Co., Japan). Electrode impedances were adjusted to be less than 5kΩ. The period for analysis of evoked potential was measured to be 1s. The number of odorant stimuli was 20 times and each day evoked potential was recorded and the procedure for average of the evoked potentials was performed after the measurement.

3. Results

The response waveform was not clear with duration of 200ms odorant stimulation, but the waveform during longer than 300 ms was almost the same as that during 500ms. This was the reason that the duration of the odorant stimulation was set at 500ms in the subsequent experiments. The odorant stimulation at a rate of once every 4 respiration was

repeated 20 times. The resulting EEGs recorded from the scalp were put in an electroencephalograph (Neuro-Pack Eight Computer) and averaging was done 8,15, and 20 times off-line. In this way, it became possible to study the waveforms of the olfactory evoked response on the scalp. The analysis entailed studying the waveforms when averaged at 8 and 15 times.

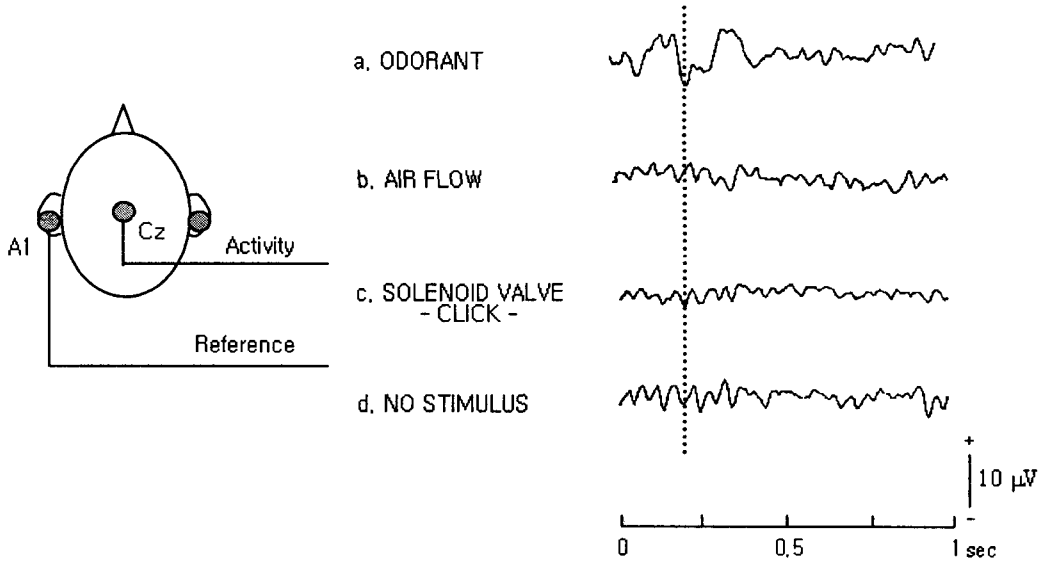

Fig.4. Olfactory evoked potentials and recordings from controls presentations at the Cz electrode site from a young subject. A: Averaged response to odorant stimuli in a normal subject. B: Averaged response to deodorized O2 without odorant stimulation. C: Averaged response to a valve opening without odorant stimulation. D: Averaged response without any stimuli.

An example of an averaged OEP to Methyl cyclopentenolone (B4, Olfactometer T&T) recorded at a Cz from a young normosmic subject is presented in Figure 4. To ensure that the response recorded by EEG averaging induced from the scalp were synchronous with the odorant stimulation, oxygen was made to flow into the odorant circuit element an averaging was done without odorant stimulation (Fig. 4). The positive peak seen in the recordings with the odorant stimulation was not found. Additionally, averaging was done 20 times with neither oxygen nor odorant element flowing to the subject and with only the electromagnetic valve opening and closing. This was done to rule out the possibility of auditory evoked response by the click of the electromagnetic valve. In this case, no response was found. In this experiments, we were able to record a positive peak that had a fixed latency against the odorant stimulation in youngs with

normal olfactory sense and also to prove conclusively that this positive peak is the evoked response to the odorant stimulation.

4. Discussion

Recording the olfactory evoked potential (OEP) from the scalp through stimulation has many problems. The smell sensation is generated when an odorant element contained in the air contacts with the mucosa located in the ceiling of the nasal cavity. Therefore, applying a stimulus is difficult. In humans, this method of experimentation poses many problems such as pain, hemorrhaging, and the placement of the electrodes. Consequently, examinations involving direct stimulation of the olfactory epithelium are difficult to perform for several reasons. First, when recording the evoked response from the human scalp by odorant stimulation, one must determine how to apply the odorant stimulation to the target area. Second, there is a possibility that the odorant stimulation will irritate the trigeminal nerve located in the nasal cavity. Finally, olfactory adaptation and the possibility of an auditory evoked response to the click from the electromagnetic valve must also be taken into consideration. Kobal and Hummel (1988) have mentioned that the evoked response recorded on the human scalp could be due to the trigeminal nerve or olfactory response, or both, in cases of strong stimulants such as phenylethyl alcohol, limonen, anethol, and menthol, all of which have been used in odorant stimulation. Regarding the olfactory adaptation when recording OEP on the human scalp, Gerull et al., (1974) have stated that OEP can be recorded with no resulting adaptation phenomenon if the odorant stimulation is applied once every two respirations. Taking these problems into consideration, the averaging is performed simultaneously with odorant stimulation in the present study. This was achieved by synchronizing respiration with the intake of methyl cyclopentenlone (T&T olfactometer, B4), a sweet burnt-smelling odor considered not to stimulate the trigeminal nerve as much as smell stimulating substance. The temperature change of the inspiration was converted into an electric signal by a thermistor fitted to the nostril of the subject. This electric signal was used as a trigger pulse to the electromagnetic valve which simultaneously opened the route to the odorant element and started the averaging. This odorant stimulation controlling device was designed to freely change the interval from once every respiration of odorant stimulation to once every nine respirations. However, when olfactory adaptation occurs or when the length of time taken for the experiment and the time taken for a series of experiments become tool long, it is difficult to keep the subjects condition constant.

The evoked potential to the odorant stimulation recorded on the human scalp is mostly reported as a positive peak in the literature. The latency of this positive peak varies considerably according to the experimental methods used by the report. Tonoike and Kurioka (1981) has reported a positive peak with latencies of 730 and 830ms. Others have mostly reported one positive peak, with 345-455ms by Kobal and Hummel (1988). These

authors stated above reported a long latency of more than 200ms. The reason why conventional studies have obtained a longer latency compared with latency of 180 ± 23ms obtained in our study is explained as follows: The odorant substance was controlled by means of an electromagnetic valve in the apparatus for the presentation of the odorant substance. The odor passed through a long tube, and it arrived to the mucosa of nasal cavity. Through the time that odorant substance was given was considered to be the time of the opening the electromagnetic valve, the correct stimulus time given should have been the moment that the odorant substance touched the surface of the mucosa. There existed a time difference between the opening time of the electromagnetic valve and the touching time of the odorant substance on the mucosa. Therefore, the conventional studies usually included the time lag for the dose of the odorant substance, and they did not give the correct latency in the response for the odorant substance stimulus. The amplitude did not increase when the concentrations exceeded certain value when the evoked potential gives the maximum amplitude. Namely, the amplitude showed the tendency for the saturation in the concentration to exceed a certain value. A similar result was reported for the visual evoked potentials of P100 (Kurita et al., 1992). Our results show the close values obtained by the new apparatus and technique.

References

[1] Allison, T.; "Recovery function of somatosensory evoked response in man," Electroencephalogr Clin Nrurophysiol, 14: 331-343, 1962

[2] Allison, T., Goff, W. R.; "Human cerebral evoked responses to odorous stimuli," Electroencephalogr Clin Nrurophysiol, 23: 558-560, 1967

[3] Araki, A., Yamada, T., Ito, T., Urushibara, N., Kohira, R., Hsusp., and Yeh, M.; "Dissociation between upper and lower neck N13 potentials following paired median nerve stimuli," Electroencephalogr Clin Nrurophysiol, 104: 68-73, 1997

[4] Celesia, G. G., Broughton, R. J., Rasmussen, T., and Branch, G.; "Auditory evoked response from the exposed human cortex," Electroencephalogr Clin Nrurophysiol, 24: 458-466, 1968

[5] Cobb, W. A., and Dawson, G. D.; "The latency and form in man of the occipital potentials evoked by bright flashes," J Physiol, 152: 108-121, 1960

[6] Gerull, G., Gisen, M., and Mrowinski, D.: "E.R.O.(Evoked Response Olfactometry)-Untersuchung der Adaptation fur olfaktorische Impulsrezung," HNO, 22: 233-235, 1974

[7] Henkin, R. I., and Christiansen, R. L.; "Taste localization on the tongue palate and pharynx of normal man," J Appl Physiol, 22: 316-320, 1967

[8] Min, B. C., Park, S.J., Kim, C. J., Wada, M.; "Chemosensory event related potentials to olfactory stimulations," Korean J of the Sci. of Emotion and Sensibility, 1: 113-119, 1998

[9] Ninomiya, H., Onitsuka, T., Chen, C. H., and Kinukawa, N.; "Possible overlapping potentials of the auditory P50 in humans:factor analysis of middle latency auditory evoked potentials," Electroencephalogr Clin Nrurophysiol, 104: 23-30, 1997

[10] Plattig, K. H., and Kobal, G.; "Spatial and temporal distribution of olfactory evoked potentials and technique involved in their measurement," In Lehmann, D., and Callaway, E. (eds), Human Evoked Potentials: Application and Problems. New York, 149-177, 1979

[11] Seta, N., Tonoike, M., and Kizuka, I.; "A test of olfactory evoked potentials using odor stimulation," Proc. 13th IEEE-EMBS, 13: 541-542, 1992

[12] Smith, D. B., Allison, T., Goff, W. R., and Principato, J. J.; "Human odorant evoked response: effects of trigeminal or olfactory deficit," Electroencephalogr Clin Nrurophysiol, 30: 313-317, 1971

[13] Shawkat, F. S., and Kriss, A.; "Interocular interaction assessed by VEPs to pattern onset, reversal, and offset in normally sighted and amblyopic subjects," Electroencephalogr Clin Nrurophysiol, 104: 74-81, 1997

[14] Tonoike, M., and Kurioka, Y.; "Precise measurement of human olfactroy evoked potentials for odorant stimuli synchronized with respirations," Jpn J EMG, 9: 214-223, 1981

[15] Tonoike, M., Yamaguchi, M., Matsumoto, Y., Kaetsu, I., Seo, R., Koizuka, I., and Kida, H.; "Odorant perception and recognition indicated by olfactory evoked potentials and event - related magnetic fields in humans," In Recent Advances in Human Neurophysiology, Hashimoto, I., and Kakigi, R. (Eds), Elsevier Sciences Publishers B.V., The Netherlands, 835-843, 1998

[16] Kobal, G.; "Elektrophysiologische untersuchungen des Menshlichen Geruchssinns," Thieme. Stuttgart, 1981

[17] Kobal, G., and Hummel, C.; "Cerebral chemosensory evoked potentials elicited by chemicalstiulation of the human olfactory and respiratory nasal mucosa," Electroencephalogr Clin Nrurophysiol, 71: 240-250, 1988

[18] Kurita, S., Tobimatsu, S., Nakayama, M., and Kato, M.; "The neurophysiologic significance of frontal negativity in pattern-reversal visual evoked potentials," Invest Ophthalomol Vis Sci, 33: 2423-2428, 1992

[19] Vaughan, H. G., Katzman, R., and Taylor, J.; "Alterations of visual evoked response in the presence of homonymous visual defects," Electroencephalogr Clin Nrurophysiol, 15: 737-746, 1963

[20] Yamamoto, T., Kato, T., Matsuo, R., Kawamura, Y., and Yoshida, M.; "Gustatory reaction time to various sweeteners in human adults," Physiol Behav, 35: 411-415, 1985

[21] Yoshii, K., Kobatake, Y., and Kurihara, K.; "Selective enhancement and suppression of frog gustantory responses to amino acids," J Gen Physiol, 77: 373-385, 1981