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NEW TRENDS IN ADAPTIVE AND BEHAVIORAL DISORDERS

CONTENTS

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TRIGEMINAL EVOKED POTENTIALS IN MAN: A NEW OLFACTORY STIMULATION DEVICE

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The recording of olfactory evoked potentials in healthy humans, using a continuous flow olfactory stimulator, is described. A stimulator pushed inert gas (N2) in a continuous flow through the nose at a rate of 4 l/min. At fixed 30-second intervals, (32 times) the flow was replaced by an equal amount of CO_2, a trigeminal stimulant. Each pulse lasted 200 ms. An electronic timing circuit triggered both the stimulator and the recorder. Signal acquisition was performed using an Evoked Potential Recorder (Nicolet Compact Four by Nicolet Biomedical Instruments), triggered by the stimulator.

Using this stimulator device reliable olfactory evoked potentials can be recorded in a clinical setting. Since this is a non invasive technique which can be used to test olfactory function whether or not the patient cooperates, it is expected to become widely used, particularly in non collaborating patients and in those suspected of malingering.

KEY WORDS: Chemo-somatosensory evoked potentials (CSEPs), man, olfactory evoked potentials, reliability, trigeminal stimuli.

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INTRODUCTION

The functionality of the olfactory system can be evaluated by presenting scales of diluted odorous substances and recording the minimum level required for recognition of the odor by the patient. Currently, most olfactometry evaluations are still based on the so-called "sniff-test" (1-3), which is hampered mainly by patient collaboration and difficulty in keeping volatile substances at fixed dilution in bottles over long time periods. Doty has addressed the latter problem with his microencapsulated sniff test (4).

As early as 1895, a Dutch doctor, H. Zwaardemaker (5), described an apparatus that pushed a known amount of air saturated with an odorous stimulus through the nose of the patient. The volume of air introduced into the patient's nose was a measurable and controlled parameter. This device has been developed further by other researchers. Fortunato and

Niccolini (6) used it for testing awake and collaborating patients of whom verbal responses were required.

The recording of the electric activity of the olfactory pathways in the brain represents a great advance in olfactory testing. Electric potentials have been obtained from isolated receptor cells (7) and in animal models (8), but testing in humans requires a non invasive technique similar to that used to record VEPs and BAEPs from scalp electrodes. When recording event related evoked potentials in man from scalp electrodes, the main technical problem is the small amplitude in relation to the basal electric activity of the brain. The recording of event related potentials of the sensory nerves in man (i.e., VEPs and BAEPs) requires the use of an averaging device in order to amplify the useful responses. Moreover, in order to use the averaging techniques, the stimulus must be presented repeatedly, and synchronized with the activation of the recording and averaging device. The application of the averaging technique in the area of the chemical senses has been limited by the difficulty of stimulus presentation. Gaseous (olfaction) or liquid (taste) substances must be brought into contact with the receptor cells in a specific amount, at a specific moment, and must be removed after a fixed time. This presentation cycle must be repeatable.

Several taste stimulating devices have been described in the literature (9-11). In 1972 and 1973 Heberhold (12,13) described an olfactory stimulator and in 1978 Kobal and Plattig (14) proposed a device presenting odorous stimuli in a continuous flow fashion. More recently Broich et al. (15) described a similar continuous flow stimulator and the results obtained by recording from scalp electrodes.

The aim of this paper is to describe the apparatus for the recording of olfactory evoked potentials and to test preliminarily the reliability of the system in healthy volunteers.

MATERIALS AND METHODS

Stimulation

The olfactory stimulator is schematically represented in Fig. 1. A continuous flow of inert gas (N_2) is completely purified by passing through an adsorption filter FU1 (activated charcoal) in order to remove any odorous contaminant. After the first filter unit, the tube is divided into two branches with two manual valves (V_1;V_2) and flowmeters (F_1;F_2) to regulate N_2 flow. The flow of CO_2 passes through a filter unit (FU_2), a manual valve (V_3) and a flowmeter (F_3). The sum of the flows in branches 2 and 3 must be equal to the flow in branch 1 (set to 4 l/min) but it is possible to regulate the ratio between the branch 2 and branch 3 flows (with V_2 and V_3) in order to dilute the gas stimulus. All the flows are controlled by manual valves and precision electronic flowmeters (Flo-Sensor, McMillan Company, reliability of 0.5% of the maximum scale, that is 0.05 l). LCD displays on the front panel provide information about both the CO_2 and N_2 flow levels.

Both air flows, whether neutral or charged with olfactory stimulants, converge on a two-channel electrovalve (EV) in such a way that one flow is always directed toward the nose and the other discharged. When the stimulus has to be delivered, the flow of CO_2 is directed toward the nose and the flow of N_2 discharged. At the end of the stimulus the plain air flows through the nose and the stimulant is discharged. The switching operation occurs without flow or pressure variation. All the time intervals of the stimulus sequence (duration: 1 to 999 ms; period: 1 to 99s; number of pulses: 1-99) can be set using the panel knobs and numeric display. Once the stimulus sequence is started, it runs automatically until the full test is over.

The stimulator is connected to an external recording device through a specific output trigger.

Recording

Subjects were evaluated with eyes open and fixating a point 2 meters in front of them. Evoked potentials are recorded from the scalp by using two Ag/AgCl electrodes, placed at the positions C_z and A_1 with Fpz as ground. The signals from the electrodes are sent to a recording device (Evoked Potentials Recorder Nicolet Compact Four - Nicolet 4), synchronized with the stimulator. A two-channel low-noise preamplifier unit and analog filters (one with 0.1 - 300 Hz and the other with 30 Hz - 10 kHz frequency range) are available in the Nicolet 4. The preamplified signals are passed through the two analog filters with frequency threshold set in order to obtain a band-pass filtering (0.1 - 100 Hz). A further notch filter is available that can be switched off when it is not needed.

Two other electrodes are placed under and above the eye for monitoring eye movements. If the signal presents artifacts due to blinking it is rejected, otherwise the signal is stored for off-line processing.

Experimental protocol

In order to verify the proposed device, olfactory evoked potentials were recorded in 20 healthy subjects (12 males and 8 females, 20±5 years).

None of the subjects had previous ear, nose and throat (ENT) pathology and all reported normal subjective olfactory function. An ENT examination was performed on all the subjects with endoscopy, rhinomanometry and standard olfactometry by sniff tests to exclude local pathologies, such as inflammation or mechanical obstruction, and hyposmia.

Each test consisted of 16 recordings without stimulant ("blank") and 32 recordings with olfactory stimulation. In order to perform the first part of the test, valve V_3 was closed and the neutral flow passed through branch 2 (Fig. 2). After the first 16 recordings, valve V_3 was partially opened to enable the gas (CO_2) to pass through the flowmeter and then to reach the nostril (Fig. 3).

The gas flow to the nose was fixed at 4 l/min. The electronic timing circuit triggered the stimulation sequence every 30 seconds. The neutral air flow was substituted by an equal amount of CO_2 which is a specific trigeminal stimulant. Each pulse lasted 200 ms and the recording window of the recorder was fixed at 1 s. The overall test lasted about 40 min, including subject preparation time. Training subjects to breathe entirely through the mouth was accomplished easily.

A white masking noise of 80dB through headphones was used to cover the clicking noise of the electrovalve and the sound of the airflow through the tubing. No after-stimulation problems were reported by the subjects. For few subjects avoidance reactions were present only at the beginning of the session and then disappeared as soon as the subjects had become familiar with the experimental setting.

RESULTS

Before recording the evoked potentials, recording without olfactory stimulant was performed on each subject (see Methods). Fig. 2 shows the averaged responses from 15

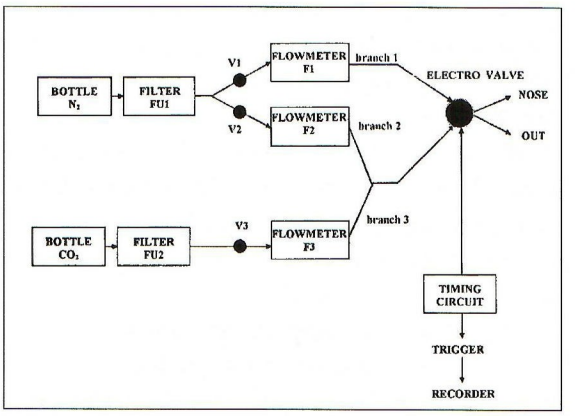

Fig. 1 - Technical scheme of the stimulation apparatus.

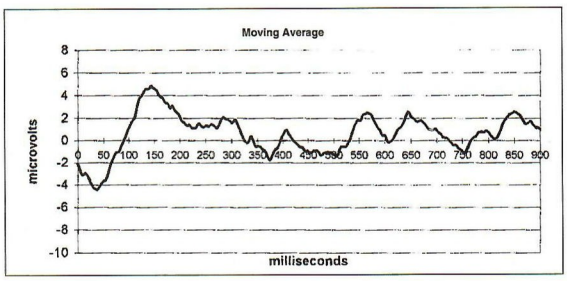

Fig. 2 - Mean electrical responses recorded during the blank test (using N_2) in 20 healthy subjects.

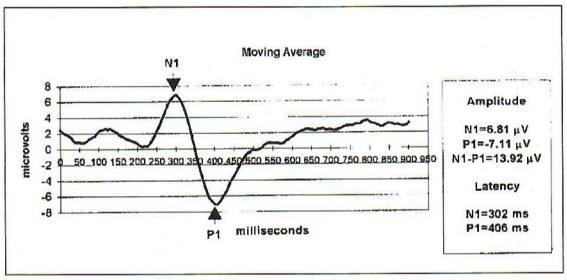

Fig. 3 - Olfactory evoked potentials obtained from the average of 32 successive stimulations for each subject.

subjects in whom no specific pattern and no significant positive or negative peaks can be observed, demonstrating that the air flow (N_2) on its own did not elicit a trigeminal tactile or olfactory stimulation. Besides, only basal activity of the brain and no chemo-somatosensory or event-related potentials could be recorded.

Thirty-two responses to olfactory stimulus were recorded for each subject. When the recordings are not affected by eye movements the minimum number of responses needed to get a significant average is 24. For each subject the waveform of the olfactory evoked potentials presents two peaks, classified as N_1 and P_1, rather similar in amplitude and latency to those recorded by Plattig et al. (16). In relation to the N_1 and P_1 components we measured three parameters: the peak-to peak amplitude, the peak-to-baseline amplitude and the latency (Fig. 3). The mean values of these parameters calculated from the population of subjects are reported in Table I. Furthermore these results were filtered by a moving average technique (Fig. 3) and the values we obtained are reported in Table II.

From the comparative analysis of the first four and the last four responses, there did not appear to be any considerable adaptation to the stimuli, i.e. the amplitude and the latency of the responses were constant over the test session (or, at least, did not decrease).

To further support the reliability of this method, 6 subjects were tested twice with a minimum one-week interval between test sessions (Table III). The responses showed good reproducibility both in the waveform and in quantitative parameters, as shown in Fig. 4. In particular, the percentage variation of the latency of N_1 in the intra-individual experiments, with only one exception, was lower than 10% (Table III).

For each subject we examined both the tests with and those without the olfactory stimulant. No blank test gave a positive result. Three of the 20 subjects (15%) failed to show a trigeminal potential following an olfactory stimulus with a peak-to-baseline amplitude lower than 4 µV.

Table I - Mean value and standard deviation of latency and amplitude (peak-to-peak and peak-to-baseline) calculated from the population of subjects (no. = 20)

Latency (ms)	Peak-to-baseline (µV)	Peak-to-peak (µV)
N_1 = 302.67±24.62	N_1 = 8.59±1.72	$N_1 - P_1$ = 18.18±4.19
P_1 = 406.67±29.93	P_1 = -9.59±3.59	

Table II - Average of latency and amplitude (peak-to-baseline and peak-to-peak) in 20 healthy subjects

Latency (ms)	Peak-to-baseline (µV)	Peak-to-peak (µV)
N_1 = 302	N_1 = 6.81	$N_1 - P_1$ = 13.92
P_1 = 406	P_1 = -7.11	

Table III - Test - retest difference in olfactory evoked potentials in healthy subjects (no. = 6)

	TEST 1			TEST 2			% DIFFERENCE		
N°	L (N_1)	P-B (N_1)	P-P (N_1-P_1)	L (N_1)	P-B (N_1)	P-P (N_1-P_1)	L (N_1)	P-B (N_1)	P-P (N_1-P_1)
1	326	11.10	21.43	302	11.60	17.44	7.40	0.60	18.60
2	288	7.01	17.11	316	8.56	21.25	9.72	22.11	24.19
3	296	7.94	16.98	258	4.10	13.80	12.83	48.36	23.42
4	272	3.76	7.23	278	6.06	9.45	2.20	61.10	30.70
5	280	2.33	8.83	278	1.91	9.23	0.71	18.20	4.53
6	330	3.80	6.44	308	3.43	6.56	6.66	10.78	1.86
mean + sd	298.66 ±24.12	5.84 ±3.20	13.00 ±6.28	290.00 ±22.16	5.85 ±3.43	12.96 ±5.60			

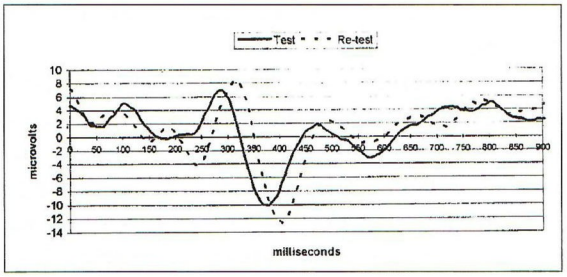

Fig. 4 - Olfactory evoked potentials (each averaged over 32 stimulations) from one subject obtained in two different sessions one week apart.

CONCLUDING REMARKS

The main feature of the device described in this paper is that it provides a non invasive technique for stimulating the olfactory receptors in a controlled manner. The reproducibility of the stimuli allows the use of the averaging technique normally used in the analysis of evoked potentials.

The device was tested on a population of healthy subjects. The analysis of the olfactory evoked potentials and the repeatability of the responses demonstrates the good functionality of the stimulating device that satisfies the fundamental requirements of the protocol, that is constant stimulus intensity and duration values.

This device could be used in the future to quantify olfactory evoked potential parameters in several pathological conditions (17-19).

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