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Olfactory Event-related Potentials in Young and Elderly Adults: Evaluation of Tracking Task versus Eyes Open/Closed Recording

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Abstract

The purpose of the present study was to evaluate olfactory event-related potentials (OERPs) elicited by amyl acetate from subjects performing a visuomotor tracking task compared with the no-task conditions of eyes open and eyes closed. Task condition did not produce any reliable effects for any amplitude measure. Task type weakly influenced only P2 latency. Elder adults evinced smaller P2 and N1/P2 amplitudes and longer N1 and P2 latencies than young adults. The results suggest that tracking task performance is not necessary to obtain robust OERPs from normal subjects of a wide age range.

Introduction

Several sources of subject-induced influence on event-related potentials (ERPs) have been reported (Hall, 1992; Polich and Kok, 1995), but a possibly important factor that is not considered is how variation in background electroencephalogram (EEG) activity can influence the ERP components. In particular, alpha activity (8–13 Hz) has been associated with ERP variability, since this rhythm can reach amplitudes of >100 μV (Markand, 1990; Intriligator and Polich, 1995; Spencer and Polich, 1999). These results may be important for recording olfactory event-related potentials (OERPs), because the rapid sensory adaptation and habituation of the olfactory system necessitates that relatively long interstimulus interval (ISIs) are needed for obtaining accurate OERPs (Morgan *et al.*, 1997). However, the long ISI gives rise to relatively long recording times, which will typically induce increased alpha rhythm activity, but have been found to increase ERP amplitudes (Morgan *et al.*, 1997; Polich, 1997b). Thus, possible interference of background EEG alpha activity with the OERP recordings has been a methodological concern.

To counteract the possible influence of alpha activity on OERPs, a visuomotor tracking task has sometimes been employed to engage attentional processing mechanisms and thereby inhibit or block alpha rhythm production (Kobal *et al.*, 1992; Morgan *et al.*, 1997). A commonly used task requires the subject to move a handheld joystick, slowly and gently, so that a small square remains inside a larger, randomly moving square on a computer terminal screen (Kobal *et al.*, 1992). Because the task engages attentional focus and mild motor movements, it is easy to ensure that the subject is attending to the tracking task by monitoring performance directly during electrophysiological recordings (Wickens *et al.*, 1983). In contrast, 'mental' tasks that theoretically engage attentional operations (e.g. adding or subtracting numbers successively) have been found to block alpha inconsistently—most likely because of variegated cognitive performance across subjects and tasks (Volavka *et al.*, 1967; Marciani *et al.*, 1992). Finally, alpha activity can also be easily blocked or instigated by comparing EEG recording when eyes are open with an eyes closed condition—a method that is simple, noninvasive and easily controlled (Hardle *et al.*, 1984; Penaloza-Rojas, 1990; Marciani *et al.*, 1992; Könönen and Partanen, 1993).

A related issue in this context is how normal aging may alter EEG and OERPs. Previous reports have demonstrated that as normal subjects increase in age EEG power decreases and background (alpha) frequency slows (Celesia, 1986; Dustman *et al.*, 1990), although the association between increasing adult age and EEG frequency slowing has not been observed consistently (Duffy *et al.*, 1984; Giaquinto and Nolfe, 1986; Polich, 1997a; Pollock and Schneider, 1990). More importantly, age-related changes in alpha activity between eyes open and closed recording conditions are also inconsistent (Markand, 1990; Könönen and Partanen, 1993; Polich, 1997b). Given the increased interest in the application of OERPs to assess normative aging and dementia, especially with respect to the olfactory P3 component (Pause *et al.*, 1996; Polich, 1996; Geisler *et al.*, 1999a; Morgan *et al.*, 1999) (C. Murphy *et al.*, submitted for publication), it is important to determine if the addition of a visuomotor task affects OERP components for normal young and elderly adults (Evans *et al.*, 1995; Morgan *et al.*, 1997).

The present study was designed to comprehensively evaluate this issue by recording OERPs in young and elderly adults in different conditions in which they either performed a visuomotor tracking task or did not. Although the P3 OERP component reflects cognitive factors and is of interest, the olfactory N1 and P2 components are likely to be more closely related to olfactory sensitivity/concentration variables and will be assessed here (Lorig *et al.*, 1991, 1996; Kobal *et al.*, 1992; Prah and Benignus, 1992; Schiffman, 1993; Murphy *et al.*, 1994; Lorig and Verspoor, 1996; Pause *et al.*, 1996; Morgan *et al.*, 1997).

Materials and methods

Participants

A total of 40 volunteers participated, half of whom were young (mean = 25.0, SD = 3.6 years) and half of whom were elderly (mean = 72.1, SD = 6.0 years), with equal numbers of each gender in each age group. The young adults were university students and the elderly were recruited from a pool of participants in a longitudinal study on chemosensory function. All participants were screened for nasal/sinus disease by means of endoscopic, rhinomanometric and cytological examinations (Jalowayski *et al.*, 1983; Davidson *et al.*, 1987; Jalowayski and Zeiger, 1988). The elderly participants were also screened for dementia with the Mini-Mental State Exam (Folstein *et al.*, 1975) (cutoff of 26), the Fuld (1978) adaptation of the Information–Memory–Concentration test (Blessed *et al.*, 1968) (cutoff of 3 errors) and the Dementia Rating Scale (Mattis, 1976) (cutoff of 124). All were right handed with the exception of one elderly male.

Stimuli

Amyl acetate was chosen for both threshold determination and OERP recordings because it has been used successfully to elicit OERPs in the target populations (Kobal 1985; Evans and Starr, 1993; Murphy *et al.*, 1994; Morgan *et al.*, 1997). The purpose of threshold assessment in this study was to screen out anosmics. For threshold determination, the amyl acetate was prepared in mineral oil in 18 dilution steps, each step one-third the concentration of the preceding stimulus, starting with a 1% v/v solution. A two-alternative, forced-choice, ascending method of limits was applied to determine the detection threshold (Murphy *et al.*, 1994). The mean threshold in dilution steps was for the young was 13.4 (SD = 3.3) and for the elderly adults was 12.4 (SD = 3.8), with no reliable differences found between the subject groups [$F(1,17) < 1$, $P > 0.1$]. These thresholds are very similar to those previously reported for normal young and elderly adults (Morgan *et al.*, 1997). Thus, the participants in these studies were not anosmic.

Stimulus presentation

Olfactory stimulation for OERP recording was accomplished with a dynamic olfactometer (Murphy *et al.*, 1994; Morgan *et al.*, 1997) which incorporates well-established techniques for stimulus presentation (Kobal and Hummel, 1988; Evans *et al.*, 1993). A digital timer initiates the onset of the OERP recording and simultaneously generates the stimulus from the olfactometer. A tank of breathing air is used to establish a flow rate of 7.4 l/min, with a 80% relative humidity achieved by passing the airstream through deionized water of a constant temperature. A plastic duct delivered the air to the nostril and was heated to raise the air to nasal temperature (36.5°C) before it passes into the nostril through a Teflon tube (1.6 mm inner diameter) placed just inside (2–4 mm) the nostril.

Each stimulus presentation is effected by means of an electromagnetic valve which opens for 200 ms, during which time a portion of the main air flow is replaced by an equal portion of odor flow (2.1 l/min). The switching valves were acoustically isolated and a constant flow rate into the nostril was maintained at all times during OERP data collection. The concentration of amyl acetate delivered by the olfactometer (909 p.p.m.) was safely below the threshold for nasal pungency of 1648 p.p.m. (Cometto-Muñiz and Cain, 1991). Concentration was assessed with an infrared gas spectrometer (Miran) with a ±1.8% error variability, from samples taken in vapor state at the nose-piece. Stimulus rise time was below 20 ms (Murphy *et al.*, 1994). The stimuli were presented with an ISI of 60 s to avoid adaptation/habituation (Morgan *et al.*, 1997).

OERP recordings

EEG was recorded with gold-plated electrodes that were affixed with electrode cream and tape from the Fz, Cz and Pz sites, referenced monopolarly to linked earlobes and grounded at the forehead. Contamination of ERPs from eye movements was monitored with electro-ocular activity (EOG) from electrodes placed at the outer canthus and supraocularly at the left eye. Impedance did not exceed 10 kΩ and was typically below 5 kΩ. EEG epochs were 2000 ms in duration and consisted of a 500 ms pre-stimulus and a 1500 ms post-stimulus period sampled at 1000 Hz, with a bandpass of 0.1–30 Hz (6 dB/octave), amplified 20 000 times and stored on magnetic medium. Trials with eye blinks or artifactual activity exceeding ±50 μV were rejected on-line and were repeated to obtain 20 artifact-free trials. There was little difference among conditions in the number of repeated trials, typically on the order of 5–10 per condition, per subject. Velopharyngeal closure was employed to restrict breathing to the mouth, thereby keeping nasal flow rate constant (Kobal and Hummel, 1989). The side of the nose reported by the participant to be most patent was used for OERP recordings (equal number for both left and right for both young and elderly).

Procedure

On the first day, the threshold and neuropsychological assessment data were obtained. On the second day, OERPs were recorded from each participant in three different conditions: (i) while performing a tracking task in which a small circle and large square appeared on a video screen, located 1 m in front of the participant's eyes, and the participant was instructed to use a joystick to keep the small circle inside the large square that moved across the screen randomly (Kobal *et al.*, 1992); (ii) with eyes open and viewing a 2 cm circular dot at a distance of 1 m; and (iii) with eyes closed. In all conditions, the participant was instructed to raise a finger when an odor was perceived to ensure stimulus perception in the absence of adaptation/habituation. The condition order was counterbalanced across participants and age group. These conditions were designed to empirically assess possible effects of task-related EEG changes and OERP measures in two clinically important populations. Although direct measures of background EEG were not obtained because of equipment limitations, the task conditions were modeled directly after those employed in many previous studies so that a comprehensive evaluation of their putative effects on the OERP could be assayed empirically (Kobal *et al.*, 1992; Morgan *et al.*, 1997; Polich, 1997a, 1997b).

Table 1 *F*-Values from three-factor ANOVAs with Geisser–Greenhouse correction

	Amplitude			Latency	
	N1	P2	N1/P2	N1	P2
Task	2.55	1.91	0.25	3.24	3.77*
Age group	0.92	18.22***	17.98***	39.26***	24.11***
Electrode site	12.39***	1.32	11.79***	2.13	14.56***
T × A	0.59	0.66	2.41	0.25	1.90
T × E	0.58	0.40	1.15	3.20	1.78
A × E	2.32	0.21	2.33	2.11	4.15*
T × A × E	0.75	1.79	1.38	0.54	0.19

$^*P < 0.05$, $^{***}P < 0.001$ for both ANOVAs and post-hoc analyses.

Analysis

The OERPs from each participant, task condition and electrode site were adjusted so that the average voltage from the 500 ms pre-stimulus baseline equaled zero. Ampli-

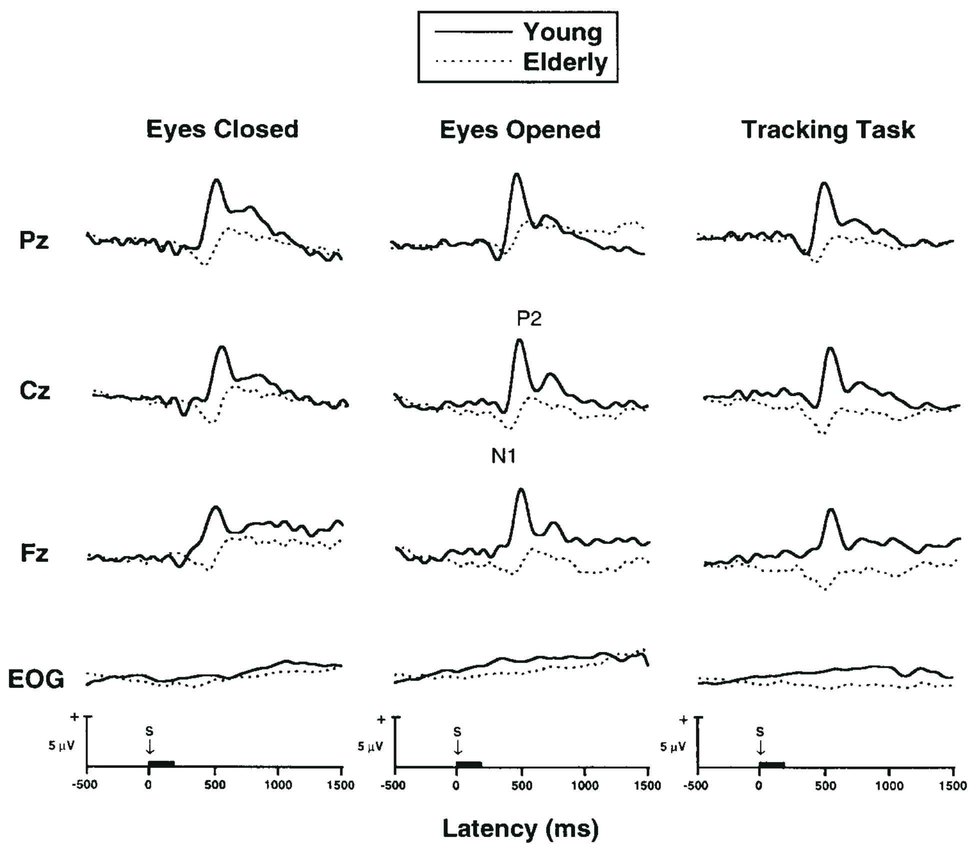

Figure 1 Grand averaged olfactory event-related potentials and EOGs for each task condition and recording site in young and elderly adults. The arrow indicates stimulus onset.

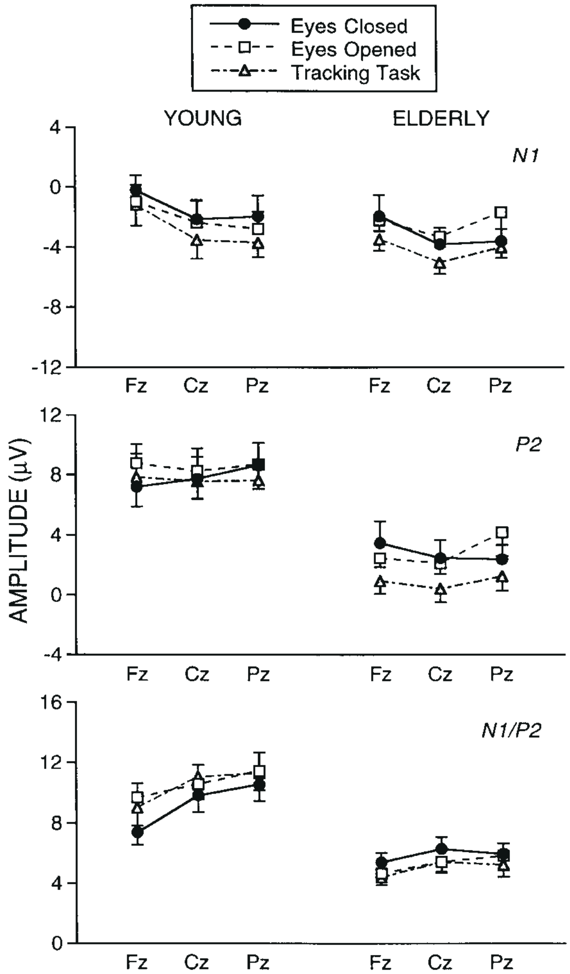

Figure 2 Mean (±1 SE) N1, P2 and N1/P2 amplitudes (Cz) for each task condition in young and elderly adults.

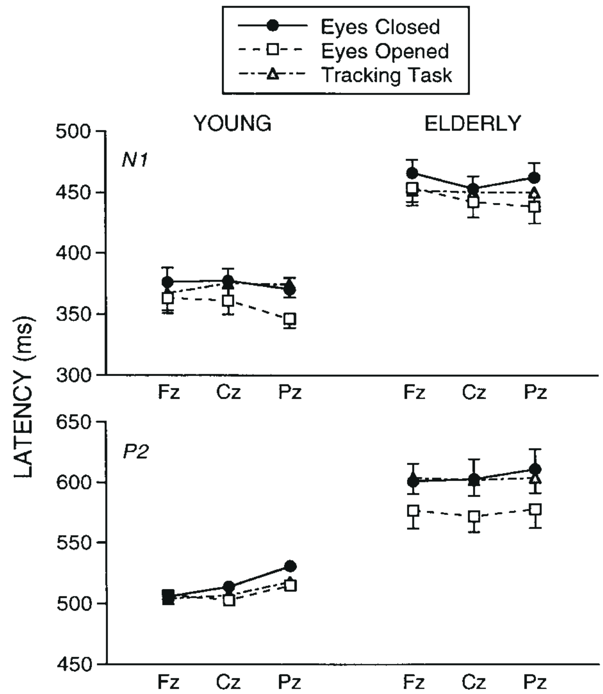

Figure 3 Mean (±1 SE) N1 and P2 latencies (Cz) for each task condition in young and elderly adults.

tudes of N1, P2 and N1/P2 (peak-to-peak), as well as peak latencies relative to stimulus onset, were assessed visually. A three-factor ANOVA (3 task conditions × 2 age groups × 3 electrode sites) was conducted on each dependent variable, with Geisser–Greenhouse corrections applied to the degrees of freedom for repeated measure factors. Post-hoc analyses were conducted with the Newman–Keuls means comparison procedure. Table 1 summarizes the ANOVA results. Figure 1 presents the grand averaged OERPs from each task condition, age group, and electrode site. Figure 2 illustrates the mean (+ 1 SE) N1, P2 and N1/P2 amplitudes. Figure 3 illustrates the mean (+ 1 SE) N1 and P2 peak latencies.

Results and discussion

Task condition did not produce any reliable effects for any amplitude measure, $P > 0.10$ in all cases). P2 latency was shorter for the tracking task condition overall [$F(2,76) = 3.8$, $P < 0.05$], although this outcome appeared to originate primarily from the elderly subjects, since age and electrode produced a significant interaction for P2 latency [$F(2,76) = 4.2$, $P < 0.05$]. Young subjects produced larger P2 [$F(1,38) = 18.2$, $P < 0.001$] and N1/P2 [$F(1,38) = 17.9$, $P < 0.001$] amplitudes than elderly subjects. Young subjects demonstrated shorter N1 [$F(1,38) = 39.3$, $P < 0.001$] and P2 [$F(1,38) = 24.1$, $P < 0.001$] latencies than elderly subjects. As indicated by the scalp topography patterns in Figure 2, N1 amplitude [$F(2,76) = 12.4$, $P < 0.001$], N1/P2 amplitude [$F(2,76) = 11.8$, $P < 0.001$] and P2 latency [$F(2,76) = 14.6$, $P < 0.001$] varied in the usual fashion with electrode position. No other reliable effects or interactions were observed. In sum, task type weakly influenced P2 latency, with the usual effects of subject age and electrode observed.

Much of the work on olfactory event-related potentials has utilized amyl acetate as the stimulus (Kobal 1985; Evans and Starr, 1993; Murphy *et al.*, 1994; Morgan *et al.*, 1997); thus, the current study sought to investigate the effects of the tracking task versus eyes open/closed condition using this prototypical stimulus. Psychophysical work with amyl acetate, presented for 2 s duration in squeeze bottles in anosmic individuals, indicates thresholds for nasal pungency of 1648 p.p.m. (Cometto-Muñiz and Cain, 1991). The shorter duration of stimulation from the olfactometer in the current study (200 ms) would be expected to generate even higher thresholds for pungency. Thus, the potentials evoked from 909 p.p.m. amyl acetate would be expected to be olfactory rather than trigeminal in nature. Furthermore, in persons anosmic following traumatic brain injury we have observed no OERP response to amyl acetate at a concentration slightly higher than was used in the current study, in spite of robust ERP responses to trigeminal stimulation (Geisler *et al.*, 1999b), thus the event-related brain potentials observed in the present study were olfactory.

In order to afford the participant every advantage in performance, the side of the nose that he or she reported as most patent, or open, at the beginning of testing was used for stimulus presentation. Nasal patency was not measured at the beginning, during or at the end of OERP testing, and we are aware of no studies in the literature that have made such measurements during OERP experiments. This might be an interesting variable to pursue. Such measurements were outside the scope of the present study.

The current study employed thresholds for amyl acetate in order to screen for anosmia. Effects of aging on olfactory thresholds have been observed in the general aged population (Murphy, 1986, 1993a, 1993b; Schiffman, 1986, 1993). These studies did not screen for dementia or nasal sinus health. In studies such as the present, where the elderly have been screened to eliminate the confounding of dementia effects from pre-clinical and early Alzheimer's disease, other dementias of old age and the confounding of nasal sinus disease, observed age effects on psychophysical thresholds are substantially smaller, though occasionally not statistically significant, than in studies of the general aging population (Morgan *et al.*, 1997; Covington *et al.*, 1999). It is of interest that age effects on the OERP are robust in studies of pre-screened elderly persons.

The elderly generated smaller P2 and N1/P2 amplitudes and longer N1 and P2 latencies than did the young adults, as has been observed previously (Murphy *et al.*, 1994; Evans *et al.*, 1995; Morgan *et al.*, 1997, 1999; Covington *et al.*, 1999) (C. Murphy *et al.*, submitted for publication). In addition, the present results are also in accord with respect to the influence of recording site and latency (Kobal *et al.*, 1992). More important for the present purposes, it is noteworthy that the tracking task versus eyes open/closed condition did not produce any reliable interactions with subject age. Thus, either of these recording conditions can be used with normal young or elderly adults.

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