

# Reliability analysis of event-related brain potentials to olfactory stimuli

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## Abstract

Olfactory event-related potentials (OERP) have been used to investigate olfactory processing in health and disease. However, the reliability of the OERP has yet to be established statistically. The present study examined test–retest reliability of the OERP over a 4-week interval. EEG was recorded from Fz, Cz, and Pz, using a single-stimulus paradigm with amyl acetate. Reliabilities for ERP component latencies and interpeak amplitudes were assessed as intraclass and Pearson product-moment correlation coefficients. Reliabilities were higher for latency than for amplitude. Highest correlation coefficients were observed for P2 latency, specifically at Cz and Pz P3 amplitude and latency exhibited high reliability at Cz and Pz. Fz demonstrated weakest correlation coefficients. The data suggest that OERP reliability is comparable to that of auditory and visual ERPs, supporting the use of OERPs in both basic research and clinical assessment.

**Descriptors:** Olfactory event-related potential (OERP), Reliability, Smell, Olfaction, Aging

In recent years, event-related potentials to olfactory stimuli (OERP) have enjoyed increasing popularity in the study of olfactory processing in healthy and clinical populations (for a review, see Lorig, 2000). OERPs have been found to be sensitive to changes occurring in Down's Syndrome (Wetter & Murphy, 1999), Parkinson's Disease (Barz et al., 1997), and individuals at risk for Alzheimer's Disease (Wetter & Murphy, 2001), and can be used as a means of response-free olfactory testing after traumatic brain injury (Geisler, Schlotfeldt, Middleton, Dulay, & Murphy, 1999). Furthermore, our group has repeatedly reported age- and gender-related differences on this measure (Morgan, Covington, Geisler, Polich, & Murphy, 1997; Murphy et al., 2000; Murphy, Nordin, de Wijk, Cain, & Polich, 1994; Thesen & Murphy, 2001). Using oddball and single-stimulus paradigms (Geisler, Morgan, Covington, & Murphy, 1999; Morgan, Geisler, Covington, Polich, & Murphy, 1999; Pause & Krauel, 2000), the OERP has also been employed in the study of cognitive functions, such as attention (Geisler & Murphy, 2000) and self-identification (Pause, Krauel, Sojka, & Ferstl, 1998).

Despite its extensive use, no study has systematically examined the reliability of this measurement tool. This is somewhat surprising, as the usefulness of any measurement is highly dependent on its reliability, and a lack thereof places a limit on the validity of inferences drawn from measurement results. According to classical

theory of reliability, the score one obtains from a measurement tool, the observed score, consists of true score and error score.

For event-related potential recordings, the error score is largely the summation of variability in (a) stimulus characteristics, (b) measurement procedure, and (c) participant's state. For OERPs, examples of error induced by variability in stimulus characteristics are stimulus concentration and duration, habituation, and breathing technique (Kobal, 1981; Kobal & Hummel, 1991; Lorig, Matia, Peszka, & Bryant, 1996; Tateyama, Hummel, Roscher, Post, & Kobal, 1998; Thesen & Murphy, 2001). Error through measurement procedures can be introduced by variability in electrode placement, impedance values, and measurement point determination (Hall, Rappaport, Hopkins, & Griffin, 1973; Picton et al., 2000; Stecker & Patterson, 1999). Variability in recordings can further be the result of changes in the state of the participant. For example, changes in participants' mood, arousal, and subjective evaluation of the stimulus can all contribute to the error score (Beydoun, Morrow, Shen, & Casey, 1993; Schupp et al., 2000).

For analysis purposes, it is useful to classify changes in scores over time as participant or apparatus dependent and to further divide participant-dependent changes as trait or state related. This results in the following potential sources of error: (a) change in true score as a group effect, (b) idiosyncratic true change, and (c) measurement error (Segalowitz & Barnes, 1993).

The two correlation coefficients used in this study, Pearson's  $r$  ( $r$ ) and the intraclass correlation coefficient ( $r_i$ ) have a selective sensitivity to these sources of error. The most commonly used measure to assess test–retest reliability, the Pearson's product-moment correlation coefficient ( $r$ ), represents the stability of the ordering among subjects within the immediate study. Pearson's  $r$  is

This research was supported by NIH grant DC02064 from the National Institute on Deafness and Other Communicative Disorders to CM.

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able to detect idiosyncratic true change and measurement error, but not a change in true score between test sessions common to all group members, and is thus not sensitive to a change common to all subjects. Therefore, Pearson's  $r$  is appropriate for determining the utility of a measurement for use in most experimental investigations where the ordering of subjects within the immediate study is of concern. Intraclass correlation coefficients have been included in many reliability studies. The intraclass correlation coefficient (ICC) takes both intersubject and between-subject variability into account and is sensitive to all three sources of change (Shrout & Fleiss, 1979). This measure of reliability is a reflection of the absolute agreement of scores between tests. Thus, the ICC is appropriate for making inferences about the utility of a measurement as a trait indicator and a clinical diagnostic tool.

Event-related potentials to stimuli of other modalities have been shown to be reliable between sessions (for a listing of previous studies, see Segalowitz & Barnes, 1993). Using auditory stimuli, Carillo-De-La-Pena (2001) tested participants 1 year apart and obtained intraclass correlation coefficients for N1-P2 amplitude ranging from  $r_i = .20$  to  $r_i = .82$ . Similar values for score agreement were found by Segalowitz and Barnes for P300 latency ( $r_i = .71$ ) and amplitude ( $r_i = .61$ ) in an auditory oddball paradigm. Fabiani, Gratton, Karis, and Donchin (1987) reported Pearson's correlation coefficients as high as .81 for P300 amplitude. In the olfactory modality, one study reported correlation coefficients for olfactory ERP comparing ortho- and retronasal stimulation (Heilmann & Hummel, 2001). Administering stimuli at different flow rates, this study obtained Pearson's correlation coefficients for N1-P3 amplitude at Pz of  $r = .92$  to stimulation at 2 ppm and  $r = .88$  to stimulation at 8 ppm. A significant difference between the two measurements was only found for N1 latency.

Reliable presentation of odor stimuli is of special concern in olfactory research and often harder to accomplish than in the visual, auditory, and somatosensory modalities. However, these challenges can be overcome and high reliability estimates obtained, as a comprehensive reliability study of 10 olfactory tests shows (Doty, McKeown, Lee, & Shaman, 1995). Pearson's correlation coefficients for between-session measurements of olfactory tests reported in this study ranged from  $r = .43$  (odor discrimination) to  $r = .90$  (University of Pennsylvania Smell Identification Test). High reliability estimates were also obtained for Phenyl ethyl alcohol staircase odor detection threshold ( $r = .88$ ) and suprathreshold pleasantness ( $r = .78$ ) and intensity ( $r = .76$ ) ratings. These findings show that olfactory tests are not inherently unreliable and that modality specific challenges can be overcome with appropriate methodology.

Olfactometers constructed on the principles developed by Kobal (1981) are the most widely used devices to control olfactory stimuli in ERP/MEG studies. These stimulus delivery devices incorporate vacuum techniques that allow a rapid rise of stimulus concentration below 20 ms. This technique also ensures that odor presentation is independent of tactile or thermal stimulation inside the nose and remains constant across trials, rendering this technique appropriate for stimulus presentation in event-related paradigms. Numerous studies with OERPs have been successfully conducted using the Kobal olfactometer technique and contributed much to the understanding of human olfactory processing in health and disease (for reviews, see Kobal & Hummel, 1991; Lorig, 2000).

The purpose of the present study is to assess the reliability of the event-related potentials to olfactory stimuli and to determine their utility for experimental and clinical studies.

## Methods and Materials

Considering that reliability estimates are contingent upon experimental design, the paradigm for the present study was chosen based on its resemblance to standard OERP experiments, allowing maximum generalization of results to studies from this and other groups. Data in this study were collected for reliability analysis purposes only and special care was taken to ensure constant conditions across measurement sessions.

### Participants

A total of 20 participants (10 young [mean age: 26 years] and 10 elderly adults [mean age: 76 years] with an equal number of men and women) took part. The elderly participants were recruited from a longitudinal study on chemosensory function and have been previously screened for general, nasal, and mental health. All participants reported normal nasal health and the absence of nasal obstructions, head trauma, upper respiratory infections, or current allergies. Participants were paid for participation or received course credit.

### OERP Apparatus and Stimulus

Olfactory stimulation was accomplished by means of an olfactometer described previously (Murphy et al., 1994) that incorporated features used by Kobal and colleagues (Kobal, 1981). Clean air established a flow rate of 7.4 L/min, with an 80% relative humidity achieved by passing the air stream through deionized water of a constant temperature. In a second circuit, liquid amyl-acetate in its pure form was substituted for water. Plastic tubing delivered the air, which was heated to body temperature (36.5°C) before it passed through a Teflon tube (1.6 mm inner diameter) placed just inside the nostril. At each stimulus presentation, a solenoid valve opened for 200 ms, during which time a portion of the main air flow was replaced by an equal portion of odor flow (2.1 L/min). Excess air/odor was exhausted via a vacuum pump that led to an exhaust vent located in another room. 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 (1,493 ppm) was safely below the threshold for nasal pungency of 1,648 ppm (Cometto-Muniz & Cain, 1991). Stimuli rise time was below 20 ms (Murphy et al., 1994). The stimuli were presented with a long interstimulus interval (ISI) of 60 s to avoid adaptation and habituation (Morgan et al., 1997).

### OERP Recordings

Electroencephalographic (EEG) activity was recorded using gold-plated electrodes, affixed with Grass electrode cream and tape, from the Fz, Cz, and Pz electrode sites, referenced monopolarly to linked earlobes and grounded to the forehead, according to the international 10/20 system. Impedance was kept below 5 k $\Omega$ . Neuroelectric activity and nasal respiration were recorded for 2,000 ms (500 ms prestimulus and 1,500 ms poststimulus), amplified 20,000 times (Astro-Med Grass Instrument, Model 12 Neuro-Data Acquisition System) through a 0.1–30 bandpass filter (6 db per octave), digitized at 1000 Hz (Biopac Systems, MP100), and stored on disk. Artifactual activity was assessed between trials at all electrode sites and electro-ocular activity was monitored with electrodes placed at the outer canthus and supraocularly to the right eye. Trials with eye blinks or EEG activity exceeding  $\pm 50$  mV were excluded from further analysis.

### Procedure

Participants were tested twice with a between-session interval of 4 weeks. Test-retest occurred during the same time of the day to avoid circadian effects. Room temperature and humidity, as well as medication status of participants, were kept constant across sessions. All participants were tested by the same experimenter. Twenty-five individual trials were recorded for each testing session. Participants were seated comfortably in a reclining chair adjacent to the olfactometer arm to reduce muscle movement. Before each trial, participants placed their right nostril on the nasal piece. Stimulus onset occurred randomly within a 10-s time window. Random presentation was chosen to reduce expectancy effects (Loveless & Sanford, 1974). Before each session, participants were trained to perform velopharyngeal closure, a special breathing technique to avoid nasal respiration (Thesen & Murphy, 2001). A thermistor ( $t_c = 6$  s; Model F-TCT, Grass Instruments, USA) was placed inside one nostril, which monitored nasal air flow at all times. All participants were able to perform velopharyngeal closure consistently and no trials had to be excluded due to nasal respiration.

### Magnitude Estimation and Single-Stimulus Paradigm

Immediately after each trial, participants were asked to report the perceived intensity of the stimulus they had just received on the Labeled Magnitude Scale (Green et al., 1996). In addition to eliciting a subjective measure of the participant's olfactory perception, the estimation of odor magnitude for each stimulus ensured that the participant was attending to the stimulus, eliciting cognitive OERP components in a single-stimulus paradigm (Morgan et al., 1999; Polich & Heine, 1996). Allowing long ISIs, the single-stimulus paradigm is especially useful for cognitive ERP testing in the olfactory modality (Geisler et al., 1999; Geisler & Murphy, 2000; Morgan et al., 1999), where a rapid succession of stimuli would produce strong adaptation and habituation effects.

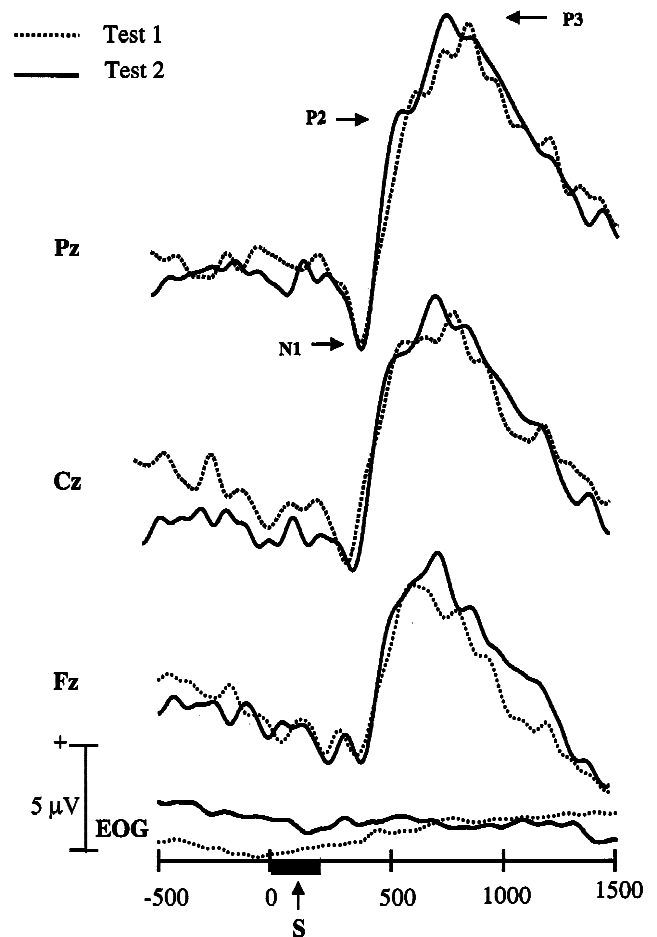
### Results

A total of 20 trials (trials 1–20) from each participant were averaged. Measurements included N1, P2, and P3 latencies and N1-P2 and N1-P3 interpeak amplitudes. The latency window for N1 was 320–500 ms, for P2 450–700 ms, and for P3 750–1,100 ms after solenoid activation. Two raters, naïve to test session and participant's age and gender, independently determined the peaks of the individual averages. All statistics were calculated using values from defined measurement points of individual averages. Figure 1 shows grand-averaged ERP waveforms for recordings separated by 4 weeks at each recording site.

A multivariate analysis of variance (MANOVA) was utilized for each OERP component, with test as within-subject factor and age group as between-subject factor. Greenhouse-Geisser adjustments were made to correct for degrees of freedom.

No significant differences were found between tests for latency: N1,  $F = 1.08$ ,  $p > .05$ ,  $\eta^2 = .06$ ; P2,  $F_{1-19} = 0.77$ ,  $p = .41$ ,  $\eta^2 = .041$ ; P3,  $F_{1-19} = 0.33$ ,  $p = .57$ ,  $\eta^2 = .01$ ; or amplitude: N1-P2,  $F_{1-19} = 2.06$ ,  $p = .57$ ,  $\eta^2 = .10$ , N1-P3,  $F_{1-19} = 0.21$ ,  $p = .98$ ,  $\eta^2 > .01$ .

A significant main effect of age was found for latency on N1,  $F = 10.56$ ,  $p < .01$ ,  $\eta^2 = .37$ ; P2,  $F = 31.61$ ,  $p < .001$ ,  $\eta^2 = .637$ ; and P3,  $F = 29.08$ ,  $p < .001$ ,  $\eta^2 = .62$ , and the interpeak amplitudes of N1-P2,  $F = 5.38$ ,  $p < .05$ ,  $\eta^2 = .23$ ; and N1-P3,  $F = 11.8$ ,  $p < .01$ ,  $\eta^2 = .39$ , with elderly subjects showing smaller amplitudes and longer latencies.



**Figure 1.** Grand averaged olfactory event-related potential waveforms at each electrode site for both test sessions. S: stimulus.

No significant interactions were observed between age group and test session for latency: N1,  $F = 0.11$ ,  $p = .76$ ,  $\eta^2 = .01$ ; P2,  $F = 0.27$ ,  $p = .78$ ,  $\eta^2 = .02$ ; P3,  $F = 0.02$ ,  $p = .89$ ,  $\eta^2 = .0$ ; and interpeak amplitude: N1-P2,  $F = 2.7$ ,  $p = .12$ ,  $\eta^2 = .13$ ; N1-P3,  $F = 2.64$ ,  $p = .12$ ,  $\eta^2 = .13$ .

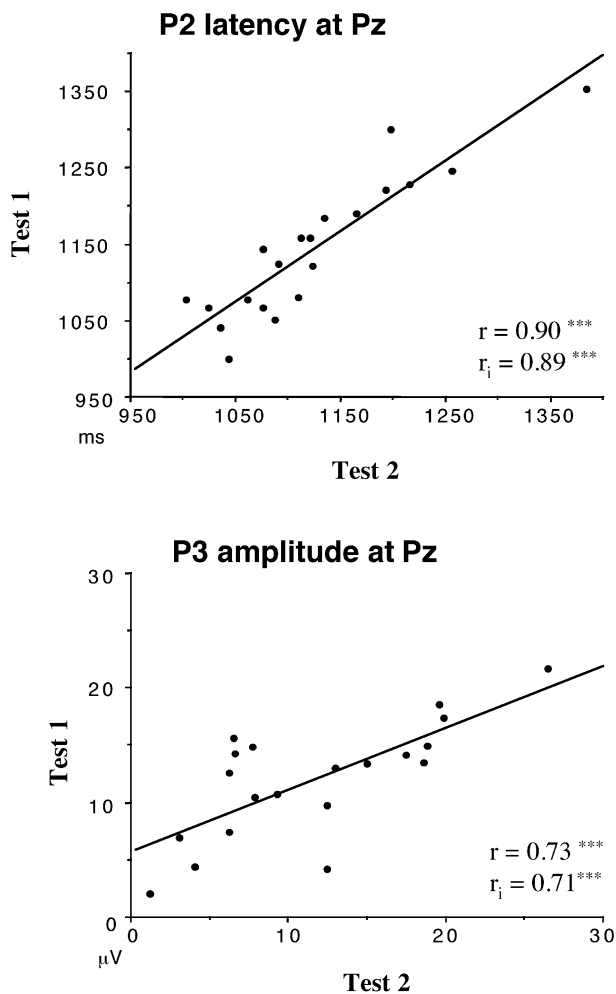
Thus, the MANOVA showed no significant interactions of age with test session for either amplitude or latency measures; hence data were combined for computation of reliability indices. Table 1 presents Pearson's product-moment and intraclass correlation coefficients computed between both test sessions for component latencies, interpeak, and baseline-to-peak amplitudes.

Results show generally higher reliability for latency compared to amplitude. Highest correlation coefficients were observed for P2 latency at Pz,  $r_i = .89$ ,  $p < .001$  (Figure 2) and Cz,  $r_i = .83$ ,  $p < .001$ . For amplitude, the N1-P3 peak to peak measures at Pz,  $r_i = .71$ ,  $p < .001$  (Figure 2), and Cz,  $r_i = .67$ ,  $p < .001$ , exhibited higher correlation coefficients than the N1-P2 peak to peak measures did for amplitude at the same recording sites (for Pz:  $r_i = .56$ ,  $p < .005$ ; for Cz:  $r_i = .59$ ,  $p < .005$ ). Nonsignificant correlations were evident at Fz for N1 latency and P3 amplitude. Consistently higher reliability estimates were obtained from the posterior electrode sites Cz and Pz. Figure 3 shows intraclass correlation coefficients of individual components at different electrode sites.

**Table 1.** Test-Retest Reliabilities Estimated by Pearson's  $r$  ( $r$ ) and Intraclass Correlation Coefficients ( $r_i$ ) for Latencies and Amplitudes of Olfactory Event-Related Potential Components

	Fz		Cz		Pz	
	$r$	$r_i$	$r$	$r_i$	$r$	$r_i$
Latency						
N1	0.36 n.s.	0.34 n.s.	0.70***	0.67***	0.49*	0.48*
P2	0.83***	0.74***	0.87***	0.83***	0.91***	0.89***
P3	0.64**	0.65***	0.75***	0.75***	0.78***	0.78***
Amplitude						
Baseline to peak						
N1	0.60*	0.59**	0.66**	0.65***	0.23 n.s.	0.28 n.s.
P2	0.52 n.s.	0.16 n.s.	0.41 n.s.	0.37*	0.30 n.s.	0.28 n.s.
P3	0.81*	0.60 n.s.	0.37 n.s.	0.36*	0.53*	0.54*
Peak to peak						
N1-P2	0.49*	0.49*	0.64*	0.59*	0.66*	0.56**
N1-P3	0.19 n.s.	0.18 n.s.	0.69**	0.67***	0.79***	0.71***

\* $p < .05$ , \*\* $p < .005$ , \*\*\* $p < .001$ , n.s.:  $p > .05$ .



**Figure 2.** ICC and linear regressions for between-session measurements at Pz electrode site for P2 latency (above) and P3 amplitude (below). \*\*\* $p < .001$ .

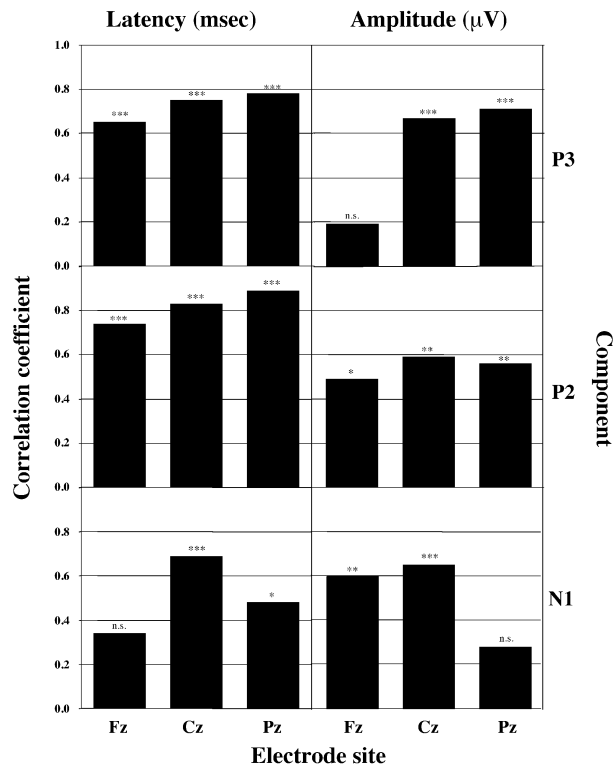
## Discussion

The purpose of the present study was to establish for the first time reliability estimates for event-related brain potentials to olfactory stimuli. A standard paradigm was used for this test–retest study to allow the generalization of results to other OERP studies.

Our data suggest that event-related potentials to olfactory stimuli have good reliability in terms of both stability and score agreement as estimated by Pearson's  $r$  and intraclass correlation coefficients. Most notably, the highest reliabilities were obtained for P2 latency. P2, here defined as the first positive peak occurring between 450 and 700 ms, has been identified as an exogenous component mediated by stimulus characteristics (Tateyama et al., 1998) and showing strong positive correlation with olfactory threshold tests (Murphy et al., 1994). The high reliability estimates for the latency of the P2 component,  $r_i = .89$  and  $r = .90$ , are supportive of its use as a trait index in most clinical and experimental studies.

Similarly, the olfactory P3 exhibited a high degree of stability in respect to its temporal occurrence, yielding test–retest correlation coefficients for latency of  $r_i = .75$  and  $r_i = .78$  at Cz and Pz, respectively. Thus, it seems reasonable to suggest that P3 latency is acceptable as a stable measure of central olfactory processing. The development of a P3 component to olfactory stimuli has been related to endogenous variables, such as attention (Geisler & Murphy, 2000; Krauel, Pause, Sojka, Schott, & Ferstl, 1998) and stimulus probability (Pause, Sojka, Krauel, & Ferstl, 1996), and reflects cognitive aspects of odor processing. Interestingly, because stimulus characteristics have been well controlled in the present investigation compared to variables influencing cognitive processing of the stimulus, higher test–retest reliability estimates can be expected for those components reflecting sensory processing of the olfactory stimulus and more variability should be evident for components reflecting cognitive processing. Indeed, reliability estimates were higher for P2 latency than for P3 latency, supporting the classification of P2 as an exogenous and P3 as an endogenous component. Studies on the stability of the OERP manipulating endogenous variables could be useful to further elucidate the nature of these waveform components.





**Figure 3.** Direct comparison of intraclass correlation coefficients ( $r_i$ ) by olfactory event-related potential component and electrode site for latency and amplitude (baseline-N1 amplitude; N1-P2, and N1-P3 interpeak amplitude). \* $p < .05$ , \*\* $p < .005$ , \*\*\* $p < .001$ , n.s.:  $p > .05$ .

Importantly, results suggest that peak-to-peak amplitude measurements are preferable over measurements taken from a pre-stimulus baseline. Furthermore, the results of the present study also identified electrode sites from which the most stable record-

ings are possible. Specifically, the posterior electrode sites proved to generate more consistent recordings compared to the frontal Fz site. Therefore, when using a standard OERP paradigm similar to the one used in the present study, in young and older adults, results of measurements taken from frontal electrode sites should be interpreted with caution.

The least reliable component measured was the early N1. However, when looking at the grand-average waveforms, reproducibility of N1 is rather high. This discrepancy can be explained by the low signal-to-noise ratio of the small component, which can introduce variability during measurement point determination. As a result, an investigator interested in early processing of olfactory stimuli should increase the number of trials per average, and not necessarily the number of participants, to obtain a better signal-to-noise ratio for this component. However, the investigator has to consider that increasing the recording time might compromise the alertness of the subject.

The finding that reliability estimates varied considerably for different components and recording sites urges investigators to see the OERP as a heterogeneous measurement with differential reliabilities of its components. Thus, when making inferences about any OERP measurement results, the reliability of the individual component on which inferences are based should be considered. Furthermore, the reliability estimates from the present study, together with mathematical equations that estimate the reliability of a measurement as sample size changes, such as the Spearman-Brown prophecy formula, can be used to predict the appropriate increase in number of participants to satisfy specific reliability standards for making sound inferences.

In conclusion, the data suggest that certain components of the olfactory event-related potential can be recorded with high reliability when precise control over the olfactory stimulus is exerted. Reliability estimates comparable to those obtained for event-related potentials of other modalities support the use of the OERP in both basic ERP research and in the clinical assessment of chemosensory disorders.

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(RECEIVED June 26, 2001; ACCEPTED April 19, 2002)