

# Analysis of phase-locking is informative for studying event-related EEG activity

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**Abstract.** A new method is presented for quantitative evaluation of single-sweep phase and amplitude electroencephalogram (EEG) characteristics that is a more informative approach in comparison with conventional signal averaging. In the averaged potential, phase-locking and amplitude effects of the EEG response cannot be separated. To overcome this problem, single-trial EEG sweeps are decomposed into separate presentations of their phase relationships and amplitude characteristics. The stability of the phase-coupling to stimulus is then evaluated independently by analyzing the single-sweep phase presentations. The method has the following advantages: information about stability of the phase-locking can be used to assess event-related oscillatory activity; the method permits evaluation of the timing of event-related phase-locking; and a global assessment and comparison of the phase-locking of ensembles of single sweeps elicited in different processing conditions is possible. The method was employed to study auditory alpha and theta responses in young and middle-aged adults. The results showed that whereas amplitudes of frequency responses tended to decrease, the phase-locking increased significantly with age. The synchronization with stimulus (phase-locking) was the only parameter reliably to differentiate the brain responses of the two age groups, as well as to reveal specific age-related changes in frontal evoked alpha activity. Thus, the present approach can be used to evaluate dynamic brain processes more precisely.

## 1 Introduction

The main goal of this study is to present a new approach for analysis of single-trial electroencephalogram (EEG) epochs in order to evaluate quantitatively event-related phase-locking. Phase-locked EEG activity in the post-stimulus epoch reflects important information about

signal processing in the brain and can be extracted in the time domain by averaging. However, the phase-locking and additive amplitude effects of the EEG response are confounded in the averaged evoked potential. To quantify the phase-locking independently of the amplitude, single-trial EEG sweeps are decomposed into separate presentations of phase relationships and amplitude characteristics. Thus, a combination of two single-sweep parameters can be used to describe specific aspects of single-sweep behavior that are otherwise not separable in the averaged waveform: (1) phase-locking with the moment of stimulus occurrence, and (2) single-sweep amplitude after stimulus presentation.

### 1.1 Theoretical background

The interdependence between the ongoing EEG and evoked brain responses has been emphasized in a variety of studies. According to the concept of induced or evoked brain rhythmicities (Başar 1980; Başar and Bullock 1992), the ongoing EEG is an active signal that determines or controls stimulus- or event-related brain potentials (ERPs). ERPs are regarded as a result of the reorganization of the rhythmic EEG activity and are also thought to originate from the superposition of evoked brain rhythmicities with various frequencies (e.g., Başar et al. 1991). Depending on the internal oscillatory properties of the responding structures, only specific frequencies can produce resonance upon stimulation. Hence, due to variations in the internal properties of the brain and current states, single frequency responses may manifest different degrees of amplitude enhancement and phase-coupling to stimulus. If both strong amplitude enhancement and tight phase-locking are present, strong resonance is defined (Başar 1980), which can be identified through the peaks in the amplitude-frequency characteristics of the evoked responses (Başar 1980; Rösche et al. 1995). Weak resonance is accompanied by either stable or unstable phase-locking to stimulus. Thus, oscillatory EEG responses with different degrees of amplitude enhancement and phase-locking may be produced during stimulus processing.

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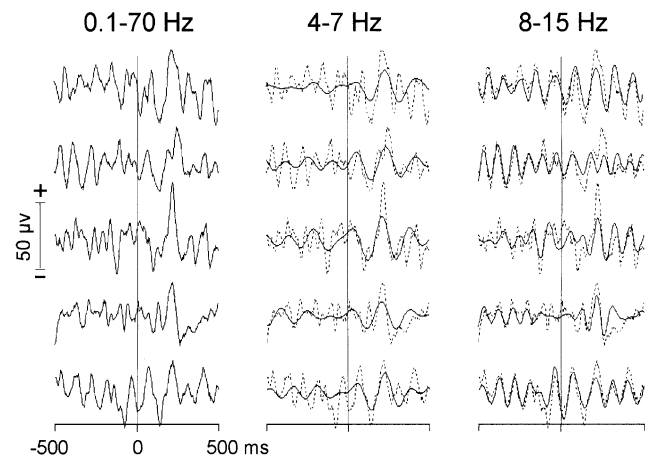
### 1.2 Phase-locked and non-phase-locked activity

In the aforementioned framework, event-related EEG activity in the post-stimulus period includes frequency responses that are either tightly or loosely phase-coupled, and are called evoked and induced rhythms, respectively (Başar 1980, 1992; Galambos 1992; Bullock and Achimowicz 1994; Pantev 1995). Non-phase-locked responses comprise event-related oscillations that are not strongly synchronized with the moment of stimulus delivery, although they may relate to specific aspects of information processing. These are, for example, induced alpha, beta, gamma, etc., oscillations (Eckhorn et al. 1988; Makeig 1993; Pfurtscheller and Neuper 1994; Pfurtscheller et al. 1994). In the framework of the additive model of evoked potentials (Gevins 1987; McGillem and Aunon 1987), non-phase-locked activity includes the background EEG. For the analysis of only non-phase-locked or both phase-locked and non-locked EEG responses, specific approaches have been used (Pfurtscheller and Aranibar 1977; Jervis et al. 1983; Kaufman et al. 1989; Kalcher and Pfurtscheller 1995; Sinkkonen et al. 1995). These methods are based primarily on power (or amplitude) measurements of the EEG in the post-stimulus period. For quantification of phase-locked activity in ERPs, the averaging procedure is usually applied whereby the phase-locked responses are enhanced and the non-phase-locked ones are attenuated (e.g., Ruchkin 1988).

### 1.3 Phase-locked activity in the averaged ERPs

Although the phase-locked EEG activity is emphasized by the averaging, the shapes of complex waves and responses in the averaged ERPs depend strongly not only on the time relationships or phases of their single-sweep components but also on their amplitudes (Glaser and Ruchkin 1976; Sayers et al. 1979; Beagley et al. 1979). Therefore, the averaged ERP is regarded only as a rough estimation and a first approximation of the brain response (Başar 1980; Steeger et al. 1983; Yu and McGillem 1983). The amplitudes of single ERPs manifest a huge inter- and intra-individual variability and may range from microvolts to tenths of a millivolt. They may also exhibit habituation as well as responsiveness to specific processing requirements, but response amplitude dynamics remain obscured by the averaged waveform (McGillem and Aunon 1987). Similarly, the phase relationships between consecutive single sweeps may be more or less stable, which is also confounded by the averaging. Thus, the phase-locking and power (amplitude) effects cannot be separated in the averaged waveform.

There are only a few investigations focusing on the measurement of phase characteristics at the moment of or shortly after stimulus delivery (Sayers et al. 1974; Beagley et al. 1979; Jervis et al. 1983; Achimowicz 1989; Brandt et al. 1991), although a number of observations demonstrate that amplitude and phase-locking of responses may reflect specific aspects of information processing: Jervis et al. (1983) have shown that the slow ERP components (theta, delta) that usually manifest an addi-

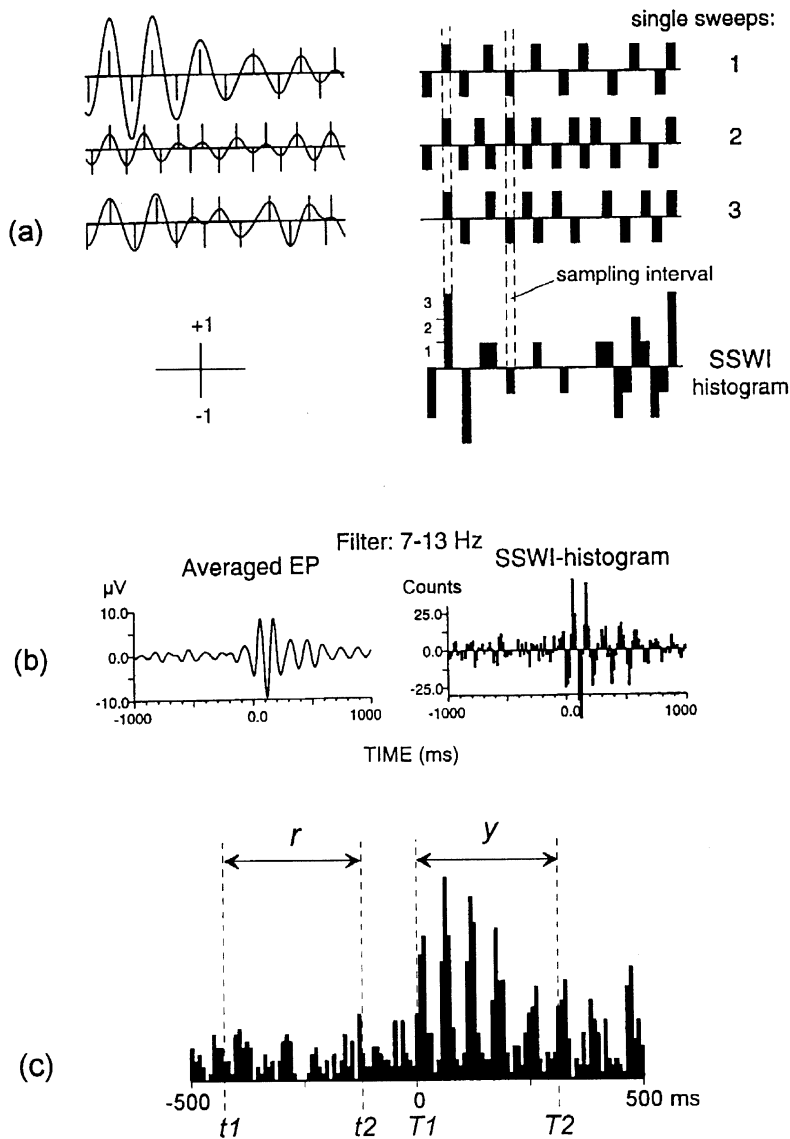


**Fig. 1.** Effect of bandpass filtering on single electroencephalogram (EEG) sweeps: several single sweeps are filtered in a wide frequency band (0.1–70 Hz). The same wide-band filtered sweeps presented as *dashed curves* in the two rightmost columns are superimposed with the corresponding bandpass-filtered sweeps in the frequency ranges of 4–7 and 8–15 Hz. Stimulus occurrence is at 0 ms

tive power effect also have a strong phase-locking effect. However, higher-frequency components with only a phase-locking effect have been revealed. Such components cannot be reliably identified in the averaged potentials because they are masked by the high-power components, as is the case with the evoked gamma band (40 Hz) response (e.g., Başar et al. 1987; Pantev et al. 1991). Hence, relevant phase-locked components may be confounded by power factors. In addition, estimation of component stability at the level of single-sweep analysis has demonstrated significant differences between variability (or stability) of exogenous and endogenous components (Michalewski et al. 1986). Specific contributions of factors such as aging (Pfefferbaum and Ford 1988; Fein and Turetsky 1989; Smulders et al. 1994) or pathology (Ford et al. 1994; Unsal and Segalowitz 1995) to either phase-locking or power of single responses have also been suggested. Taken together, these findings suggest that quantification of both aspects of single-sweep behavior in an independent manner would be informative for revealing significant information. In the present study, one possible method is presented.

## 2 Method

To obtain the oscillatory EEG responses in different frequency bands, digital filtering is commonly used (e.g., Barrett 1986; Ruchkin 1988; Cook and Miller 1992; Farwell et al. 1993). To allow the precise evaluation of response phase-locking, only filters with zero phase shift should be applied (Başar and Ungan 1973; Başar 1980). In addition, filter characteristics especially for narrow bandpass filters, as well as for abrupt amplitude changes typical of averaged ERPs, should be chosen so as to avoid the production of filter-related oscillations (Wastell 1979; de Weerd 1981; Farwell et al. 1993). Figure 1 presents examples of single EEG epochs before and after ideal (rectangular) filtering (Başar and Ungan 1973) in the theta and alpha frequency ranges as analyzed in the present study. It can be seen that even when ideal filtering with zero phase shift is applied to single EEG epochs, the filtered waveforms correspond very closely to the original (wide-band filtered) EEG, which



**Fig. 2a–c.** Method for evaluation of single-sweep phase-locking. **a** Three single sweeps (1, 2, 3) filtered in the alpha range (*left*), the identified extrema presented by their locations with bars equal to  $\pm 1$  (*right*), and the sums of bars building the single sweep wave identification (SSWI) histogram. **b** A typical result from an adult subject: averaged evoked potential filtered in the alpha range (*left*) and the corresponding SSWI histogram (*right*). **c** Illustration of the quantification of phase-locking: after the histogram from **b** is rectified, the sums in the reference ( $r$ ) and post-stimulus ( $y$ ) intervals are calculated. The number of single sweeps averaged and used for SSWI histogram building is 96. Stimulus is presented at 0 ms

indicates that real EEG frequency responses but not filter artifacts are present in the single ERP epochs.

### 2.1 Identification of phase relationships in single sweeps

The first step is to present each filtered single sweep with its phase relationships. To present single sweep phase characteristics in the time domain, a min/max identification is performed (e.g., McGillem and Aunon 1987): For each single sweep all wave extrema are determined with their polarity, absolute amplitudes, and position on the time axis. The positions of the wave extrema (or wave phases) along the time axis are defined such that each modified single sweep is given by  $x_n(i\Delta t)$ , where  $n$  is the consecutive sweep number,  $\Delta t$  is the sampling interval, and  $i$  is an integer representing the consecutive (discrete) measurement points:

$$x_n(i\Delta t) = \begin{cases} +1 & \text{if a maximum at the moment } i\Delta t \text{ is detected,} \\ -1 & \text{if a minimum at } i\Delta t \text{ is detected, or} \\ 0 & \text{if no extremum at } i\Delta t \text{ is detected} \end{cases} \quad (1)$$

This procedure is illustrated in the left-hand panel of Fig. 2a, where three representative filtered sweeps are shown along with the identified extrema (maxima and minima). The right upper panel of Fig. 2a

displays the detected latency points without the signals. In the filtered waveform supposed to reflect enhanced and damped oscillations of the EEG frequency response, each extremum is identified. If non-filtered signals are analyzed, time and amplitude criteria might be manipulated in order to make the identifications insensitive to the impact of very small amplitudes and/or high-frequency components (McGillem and Aunon 1987; Daskalova 1988; Kolev and Daskalova 1990). After the min/max identification, each single sweep is presented by a coded string of (+1), (−1) or (0) of its wave extrema along the time axis. The wave 'phase' codes are stored with their latency values for further processing.

### 2.2 Stability of phase-locking

As a next step, the modified single sweeps  $x_n(i\Delta t)$  from a single experiment including  $n = N$  sweeps can be summed

$$y(i\Delta t) = \sum_{n=1}^N x_n(i\Delta t) \quad (2)$$

or averaged

$$y'(i\Delta t) = (1/N) \sum_{n=1}^N x_n(i\Delta t) \quad (3)$$

so that phases with stable locking are emphasized in the respective time periods, and nonstable ones are attenuated. As a result, a histogram (single sweep wave identification, or SSWI, histogram) is constructed. The sum of the identified coded extrema at each time point  $i\Delta t$  is determined and assigned to the corresponding histogram bar. This procedure is illustrated in the right-hand panel of Fig. 2a.

A typical SSWI histogram obtained from the auditory single sweeps filtered in the alpha (7–13 Hz) range of an adult subject is shown in Fig. 2b. The strong phase-locking of alpha responses in the first 250–300 ms after stimulus presentation is clear. In the prestimulus period, where the phases of the alpha waves are random and non-phase-locked, the SSWI histogram contains markedly smaller values in comparison with the post-stimulus epoch. In this particular case, a prolongation of the phase-locked alpha oscillations until about 500 ms can also be observed.

According to (3), a normalized SSWI histogram can be obtained by dividing the bar values into the number of sweeps  $N$ . For data reduction, during construction of the histogram it is possible to increase  $\Delta t$  appropriately according to the analyzed frequency band, so that  $\Delta t \leq 1/(2F)$ , where  $F$  is the highest frequency in the signal of interest (cf. Regan 1989).

### 2.3 Quantitative assessment of phase-locking

For quantitative assessment of phase-locking, sums  $y(i\Delta t)$  (2) or averages  $y'(i\Delta t)$  (3) can be used. The relevant information about phase-locking is reflected by both the negative and the positive values. To avoid cancelling out possible significant effects when longer time intervals are analyzed, the absolute values are used for phase-locking quantification. For a given time interval  $T_1 T_2$ , the phase-locking  $y(T_1 T_2)$  will be measured as

$$y(T_1 T_2) = \sum_{i\Delta t=T_1}^{T_2} |y(i\Delta t)| \quad (4)$$

or

$$y'(T_1 T_2) = (1/N) \sum_{i\Delta t=T_1}^{T_2} |y(i\Delta t)| \quad (5)$$

If equal sweep numbers are used,  $y(T_1 T_2)$  and  $y'(T_1 T_2)$  would be the appropriate measurable parameters for inter-individual or inter-group comparisons, because of the lack of phase-locking in the non-stimulus-related EEG. When a different number of sweeps is used (depending on the experiment),  $y(T_1 T_2)$  is higher for the higher number of sweeps because of the summing, and  $y'(T_1 T_2)$  is higher for the lower number of sweeps because of the averaging. In this case, a reference measure of the phase-locking,  $r(t_1 t_2)$  for a time interval of the same length,  $t_1 t_2 = T_1 T_2$  taken from the spontaneous or prestimulus EEG is appropriate to be used (Fig. 2c). Then the reference value is

$$r(t_1 t_2) = \sum_{i\Delta t=t_1}^{t_2} |y(i\Delta t)| \quad (6)$$

and the normalized parameter evaluating the phase-locking  $y''(T_1 T_2)$  is calculated as

$$y''(T_1 T_2) = \{y(T_1 T_2)\} / \{r(t_1 t_2)\} \quad (7)$$

## 3 Auditory evoked potentials in young and middle-aged adults

In the following example, it will be demonstrated that (i) among a variety of parameters of averaged and single EEG responses, age-related effects can be detected only by analyzing the phase-locking, (ii) single-sweep amplitude and phase-locking manifest independent responsiveness to age and topology factors, and (iii) alpha and theta responses can be differentiated on the base of the

specific effects of age and topology on single-sweep behavior.

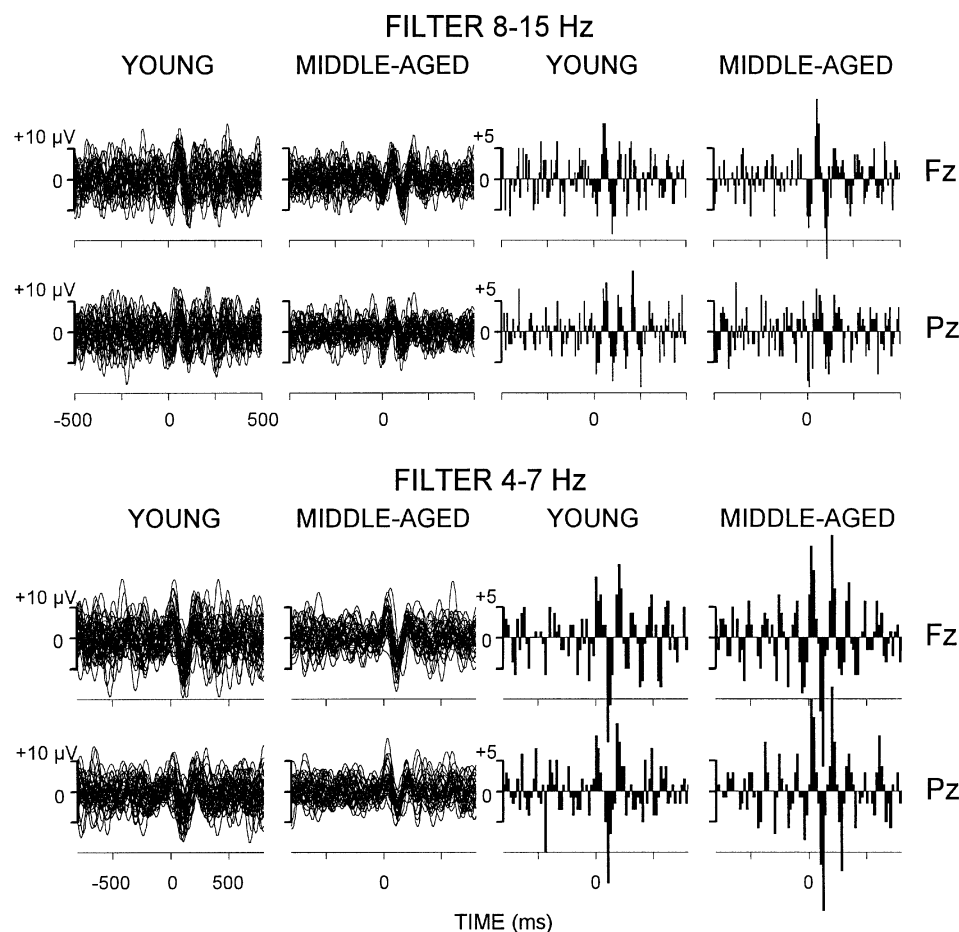
Twelve middle-aged adults from 50 to 55 years of age (mean 53.6, SD 2.2 years) were examined along with 14 young adults from 18 to 30 years of age (mean 24.8, SD 5.6 years). Subjects were colleagues or students. They were all healthy, drug free, and without any neurologic, psychiatric, or hearing problems in the past. No difference existed between the hearing thresholds of young and middle-aged adults. Auditory evoked potentials were recorded from Fz, Cz, and Pz electrode sites, with linked mastoids as reference. The stimuli were 60 tone bursts (800 Hz frequency, 70 dB SPL intensity, 50 ms duration, 10 ms r/f, 3.5–5.5 s inter-stimulus intervals) presented via loudspeakers in a free sound field. During the stimulation session subjects were instructed to relax and remain silent with their eyes open. EEG epochs from 1024 ms before to 1024 ms after stimulus were amplified with cutoff frequencies of 0.1 and 70 Hz, digitized with a sampling frequency of 500 Hz (12 bit ADC), and analyzed off line. Trials contaminated with ocular, muscular or any other type of artifacts, as well as those exceeding  $\pm 45 \mu\text{V}$  in any EEG or electrooculogram (EOG) lead, were rejected. After the artifact rejection procedure, 45–47 artifact-free sweeps were accepted for further analysis.

Individual measurements of each parameter were subjected to a repeated-measures analysis of variance with one between-subjects variable, age (young vs middle-aged), and one within-subject variable, electrode (Fz, Cz, and Pz). The Greenhouse-Geisser procedure was applied to the analyses with repeated measures, with corrected probability values reported here. Only the age and age  $\times$  electrode effects will be dealt with in the present study.

### 3.1 Analysis of averaged auditory ERPs

According to a methodologic approach developed and applied previously, ERPs were studied in the time and frequency domains, which included: averaging, calculation of amplitude-frequency characteristics, and response adaptive filtering (Başar 1980). Peak amplitude and latency values of N1 and P2 components were measured. Averaged ERPs were filtered in the 1.5–4 Hz, 4–7 Hz, 8–15 Hz, and 15–50 Hz frequency ranges and the maximal peak-to-peak amplitudes in the time window 0–300 ms after stimulus were computed. The time window 0–500 ms was used for the 1.5–4 Hz range.

No differences were revealed between the two age groups for peak latency and amplitude values of N1 and P2 [age: N1 latency,  $F(1/24) = 0.38$ ,  $P = 0.55$ ; N1 amplitude,  $F(1/24) = 3.26$ ,  $P = 0.08$ ; P2 latency,  $F(1/24) = 1.56$ ,  $P = 0.22$ ; P2 amplitude,  $F(1/24) = 0.01$ ,  $P = 0.93$ ]. Similarly, no age-related changes were found for the amplitudes of delta, theta, alpha or faster frequency components in the averaged ERPs [age: delta (1.5–4 Hz),  $F(1/24) = 2.34$ ,  $P = 0.14$ ; theta (4–7 Hz),  $F(1/24) = 0.72$ ,  $P = 0.41$ ; alpha (8–15 Hz),  $F(1/24) = 1.01$ ,  $P = 0.32$ ; faster components (15–50 Hz),  $F(1/24) = 0.57$ ,  $P = 0.46$ ].



**Fig. 3.** Superimposed single sweeps (*left*) with the corresponding SSWI histograms (*right*) in alpha (8–15 Hz) and theta (4–7 Hz) frequency ranges for two representative adults, young and middle-aged. Recordings are from Fz and Pz leads. Stimulus occurs at 0 ms. Superimposed single sweeps illustrate the lack of pronounced age differences in the maximal amplitudes, whereas a stronger phase-locking of alpha responses at the frontal site, and of theta responses at both locations, is clearly demonstrated in the SSWI histograms

### 3.2 Analysis of single sweeps

Since the specific functional roles of theta and alpha frequencies have recently been emphasized (Klimesch et al. 1994; Başar and Schürmann 1994), ERPs filtered in the 4–7 Hz and 8–15 Hz ranges were additionally analyzed at the level of single-sweeps by using two parameters:

- (i) Single-sweep amplitudes. The maximal peak-to-peak amplitude within the time window 0–300 ms after stimulus was measured for each single alpha and theta response. Then the mean amplitude value was calculated for each subject at each electrode site.
- (ii) Single-sweep phase-locking. For quantitative evaluation of the phase-locking of single responses, the above-described procedure was applied. For statistical evaluation, the integral values of the normalized histograms (5) within the 0–300 ms post-stimulus epoch were calculated for each subject and lead.

Figure 3 presents single alpha and theta responses and the corresponding SSWI histograms of two representative subjects (young and middle-aged) to illustrate typical group differences.

- (i) Single-sweep amplitudes. Single alpha responses tended to be smaller in middle-aged adults [age,  $F(1/24) = 3.42$ ,  $P = 0.08$ ]. No age difference was found for the amplitudes of single theta responses.
- (ii) Phase-locking. A significant age  $\times$  electrode interaction was found for alpha responses [ $F(2/48) = 4.73$ ,  $P = 0.02$ ], because only at the frontal site were the differences between the age groups significant [single age effect at Fz,  $F(1/24) = 6.75$ ,  $P = 0.01$ ]. The stronger alpha phase-locking at the frontal site is illustrated in Fig. 3. The phase-locking of theta responses at each lead was significantly stronger in middle-aged than in young subjects [age,  $F(1/24) = 4.91$ ,  $P = 0.04$ ].

To summarize: (a) In contrast to the analysis of averaged potentials, the analysis of single-sweep parameters (amplitude, phase-locking) reveals significant differences between young and middle-aged adults during auditory stimulus processing. (b) Alpha and theta response systems of the brain undergo specific changes with age in adults, as revealed by the stronger phase-locking of the evoked frequency patterns of middle-aged subjects. (c) These changes show topographic localized selectivity, being less localized for the theta responses but focused over the frontal brain area for the alpha responses.

#### 4 Discussion and conclusions

The most common way to study phase-locked EEG responses to external stimulation is to analyze averaged potentials. In this study, the separate evaluation of single-sweep phase and amplitude characteristics is proposed as a more informative approach compared with ERPs obtained by signal averaging. To allow separate assessment, phase relationships of single responses are identified along the time axis. Then a subsequent coding of their amplitudes is performed. This step enables the independent analysis of the phase-locking stability, with the amplitude measurements assessed separately. The Fourier transform also permits phase relationships in the frequency domain to be analyzed (Beagley et al. 1979; Jervis et al. 1983; Achimowicz 1989). However, although the phase spectrum obtained yields information about the phase of different frequencies in the signal, the time dimension is lost and time dynamics of between-sweep synchronization cannot be evaluated. If single-sweep phase history obtained after the method of complex demodulation is used, further analysis has been shown to be critical (Nogawa et al. 1976).

The method proposed here is simple and reliable for assessing both the time and phase dimensions in specific frequency bands at the level of single sweeps. The major advantages are: (a) Information about the stability of the phase-locking can be used for demonstrating the presence of event-locked oscillatory activity. (b) The method permits the localization of phase-locking along the time axis in relation to an event. (c) A global assessment and comparison of the phase-locking of ensembles of single sweeps elicited in different processing conditions is possible. (d) By analyzing subgroups of single sweeps, evaluation of the dynamics of the phase-locking during an experimental session or condition is possible.

Furthermore, the functional changes in dynamics of single alpha and theta responses in the two age groups under study demonstrate that single-sweep parameters can provide evaluation of the effect of age on stimulus information processing, which is more precise than if only averaged ERPs are analyzed. As shown by the present results, the power of responses tended to decrease, whilst the phase-locking increased significantly with age. These two effects could not be separated in the averaged waveforms. The stability of phase-locking was the only parameter to differentiate reliably the brain responses of the two age groups, as well as to reveal the specific age-related changes in frontal alpha responses. Given the possible simultaneous and independent variations in power and phase-locking of brain responses during sensory and cognitive information processing, or during development, aging, pathology, etc., the present approach may reveal new aspects of single-sweep behavior that will allow dynamic brain processes to be evaluated more precisely.

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