Comparative effects of age on limbic and scalp P3

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Summary We studied the effects of age on the limbic and scalp P3 in 45 patients with intracranial electrodes implanted for pre-surgical investigation of focal seizures. Scalp P3 data from a reference group of 24 healthy control subjects were also analyzed for comparison. An auditory oddball paradigm with infrequent stimuli being presented with a probability of 0.20 was used.

In normals P3 latency increased by 1.34 msec/year ($r = 0.60, P < 0.01$). In the patients limbic and scalp P3 latency increased linearly as a function of increasing age at a rates of 3.85 msec/year ($r = 0.58, P < 0.001$) and 2.71 msec/year ($r = 0.56, P < 0.01$), respectively. The rate of increase of P3 latency with age was significantly lower in the normal controls, as compared to both the patient scalp ($t = 1.79, P < 0.05$) and depth ($t = 2.25, P < 0.005$) ERP data. There was no significant difference between the slopes of the patient P3 latency versus age scalp and depth data ($t = 1.09, P > 0.1$). Unlike for normal controls, there was no relationship between age and limbic P3 amplitude ($r = 0.02, P > 0.1$) or age and scalp P3 amplitude ($r = 0.17, P > 0.1$). The differences between controls and patients could be due to: (i) effects of chronic seizures; (ii) long-term effects of anticonvulsant use; (iii) the use of a relatively long inter-stimulus interval which may have selectively affected the patients. In our patient latency data, the increased slope of the regression line suggests increased slowing of information processing with age in this population of medicated individuals with intractable seizures. The absence of a relationship between P3 amplitude and age may be due to the above-mentioned factors or also the variation of electrode position in the hippocampus.

Key words: Limbic P3; Endogenous ERP; Latency; Age

It is now well established that the latency of scalp P3 increases as a function of chronological age in normal adult subjects (Goodin et al. 1978; Syndulko et al. 1982; Brown et al. 1983; Pfefferbaum et al. 1984; Picton et al. 1984). A progressive decrease in the amplitude of this event-related potential (ERP) with increasing age has also been noted (Goodin et al. 1978; Picton et al. 1984). A P3 ERP may also be recorded from intracranial electrodes sited in the temporal lobes, and studies strongly suggest that the limbic system may be one of the generators of this potential (Halgren et al. 1980, 1986; Squires et al. 1981; Wood et al. 1984). The extent of the contribution of this limbic P3 to the scalp P3, however, is uncertain (Wood et al. 1984; Halgren et al. 1986; Johnson and Fedio 1986; Stapleton and Halgren 1987). There are no studies, to our knowledge, where the effects of aging on limbic P3 have been investigated.

We report on scalp and limbic P3s as a function of age in a series of patients with depth recording electrodes sited for the pre-surgical investigation of intractable focal seizures. Scalp P3s from a series of healthy control subjects were obtained for comparison and analyzed as a function of age.

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Methods

Subjects

Controls. Twenty-four healthy control subjects with no previous history of neurological or psychiatric impairment consented to participate in this study. There were 12 males and 12 females. Average age of subjects was 31.7 years ± 10.7 years, with a range from 16 to 52 years. Subjects over 20 years of age were recruited from hospital staff, while subjects in the 15–20 year age range were children of hospital staff.

Patients. Forty-five patients undergoing presurgical investigation of intractable partial seizures under the Austin Hospital Comprehensive Epilepsy Program (Melbourne, Australia) (Engel 1987) participated in this study. Patients were included in this study using the following criteria: (i) age was greater than or equal to 15 years; (ii) there was no previous history of intracranial infection; (iii) depth electrodes sited for long-term EEG/video monitoring included bilateral mesial temporal placement; (iv) seizure onset was unilateral.

Average age of patient was 29.1 years ± 11.0 years, with a range of 15–64 years. There were 25 males and 20 females. Twenty-four patients had left temporal lobe epilepsy (TLE), 19 had right TLE, and 2 had seizures involving the fronto-temporal areas unilaterally.

Depth recording electrodes

Depth recording electrodes were constructed from 4 strands of 36-gauge teflon-coated stainless steel wire. The diameter of each depth electrode was 0.8–1.0 mm. Each electrode had 4 exposed recording sites 10 mm apart, with the most mesial recording site being at the tip of the electrode. Depth electrodes were implanted bilaterally in the temporal lobes via an orthogonal approach (Fig. 1), with the electrode tip being targeted at the anterior hippocampus. Correct electrode placement was verified by skull X-ray or CT scan and by postoperative unilateral tissue histology.

Auditory oddball paradigm

An auditory ‘oddball’ paradigm (Duncan-Johnson and Donchin 1977) was generated on a Medelec ST10 stimulator controlled by an Apple IIe microcomputer. The supplied Medelec software had been modified by one of the authors (AP), to include a 500 msec pre-stimulus baseline in ERP recordings. Two pure tones (20 msec duration, rise/fall time 4 msec) of different pitch were presented monaurally through shielded headphones at an average rate of 0.3/sec. The rare stimulus was a 3 kHz ('high') sinusoidal tone burst of 20 msec duration which occurred with a probability of 0.20. The frequent stimulus was a 1 kHz ('low') pure sinusoidal tone burst. Both tones were presented at 50 dB above hearing threshold for that tone in each patient. On a given trial, high tones were presented consistently to one ear, while low tones were presented contralaterally. The rare
tone ERP was recorded from the scalp or depth electrode contralateral to the ear in which the rare (high) tone was presented. The patient silently counted the number of rare tones presented. Tone presentation was halted at two random points during each trial and count reached was tested. Total count reached was also checked at the end of the trial. This ensured that patients maintained sustained attention. Any error in count reached was pointed out to the patient, who then continued to count tones from the correct number. Total number of rare tones presented in each trial was a random number between 20 and 30, so as not to make counted outcome predictable.

**ERPs to both rare and frequent stimuli** were recorded from C3 and C4, with respect to linked earlobes and grounded forehead. Silver/silver chloride electrodes served for extracranial placements in all subjects, and scalp electrode impedance was always less than or equal to 6 kΩ.

A 4-channel Medelec Sensor averager amplified and filtered the signal with a bandpass of 0.1–30 Hz (−3 dB down). ERP data were collected in a 2 sec epoch in 250 points slices (62.5 Hz) sampling rate. A pre-stimulus baseline of 500 msec was included in the recording epoch. ERPs (and EOG) from 2 sets of trials were recorded to both rare and frequent stimuli.

EOG was recorded between the outer canthus and lower eyelid of the right eye and EEG epochs contaminated by eyeblinks and eye movements were rejected by the averager.

**Patients.** Scalp and limbic ERPs were collected in separate recording sessions.

(i) Scalp recordings. Scalp P3s were recorded prior to insertion of depth electrodes from 24/45 patients and recording electrodes and filtering were as described for normal subjects except that: (1) a 500 msec recording epoch without pre-stimulus baseline in 7 patients (250 Hz sampling rate); (2) a 1 sec epoch including a 500 msec pre-stimulus baseline (125 Hz sampling rate) in 5 patients.

(ii) Depth recordings. ERPs were recorded from the most mesial site of each temporal depth electrode, with respect to an extracranial linked earlobe reference in all 45 patients. The forehead served as a ground site. In 11 patients ERPs to both rare and frequent stimuli were averaged from the most mesial recording site of each depth electrode. In 34 patients, ERPs to the rare stimuli from all 4 recording sites on each depth electrode were averaged.

Depth EEG data were filtered using identical filtering to scalp data. The limbic ERP recordings in the 45 patients used the following data epochs: (i) in 11 patients a 500 msec data epoch (without pre-stimulus baseline) was used (250 Hz sampling rate); (ii) a 500 msec pre-stimulus baseline was included in a 1 sec epoch (125 Hz sampling rate) in 6 patients; and (iii) a 2 sec epoch including 500 msec pre-stimulus baseline (62.5 Hz sampling rate) was used in 28 patients. ERPs from 2 sets of trials were recorded from each depth electrode.

**Analysis**

ERPs to rare stimuli from controls and patients (scalp and depth recordings) were visually scanned for a reproducible P3 occurring in the range of 180–600 msec post stimulus. In patients limbic and scalp P3s from the non-epileptogenic side were included in subsequent analyses. In all subjects P3 latencies were determined by visually selecting the point at which the maximum positive peak occurred in the ERP wave forms. P3 amplitude was measured with respect to 500 msec pre-stimulus baseline. Average pre-stimulus baseline level was calculated by sampling single points of the pre-stimulus baseline amplitude at 50 msec intervals and calculating their mean.

P3 amplitude was measured with respect to pre-stimulus baseline in patients and 24 normal subjects. Scalp P3 amplitude data were available in 17/24 patients in whom a pre-stimulus baseline was included in the averaged ERP wave form. Similarly, limbic P3 amplitude was measured with respect to pre-stimulus baseline in 34/45 patients.

Linear regression was performed on 3 sets of P3 latency and amplitude data ((i) controls (scalp); (ii) patients (scalp); and (iii) patients (depth)) as a function of age. A post-hoc analysis for comparing slopes of the regression lines between the latency plots of the 3 groups (control scalp, patient scalp and patient depth) was performed.
**Results**

Representative scalp and depth ERP waveforms are illustrated in Fig. 2. The limbic P3s in 34/45 patients were absent in the epileptogenic temporal lobe (Fig. 2b), and hence ERP data from the non-epileptogenic side were included in subsequent analyses.

**P3 latency versus age**

*Control subjects.* In control subjects scalp P3 latency was positively correlated with age (Fig. 3) and increased by 1.34 msec/year ($r = 0.60$, $df = 22$, $P < 0.01$).

*Patients.* Limbic P3 latency (Fig. 4a) showed a significant positive correlation with age ($r = 0.58$, $df = 43$, $P < 0.001$) increasing by 3.85 msec/year.

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**Fig. 2.** Examples of representative scalp and limbic ERP recorded from 2 sets of trials in a patient with a left mesial temporal lobe seizure focus. Time of stimulus delivery is indicated by the solid vertical line. a: scalp data recorded from C4 with respect to linked earlobes show a prominent P3 in the rare stimulus ERP (top traces, 21 and 23 averages) as compared to a negligible P3 in the frequent (FREQ) stimulus ERP (middle traces, 92 and 76 averages). EOG (bottom traces, 21 and 23 averages) was recorded from outer canthus with respect to lower lid. The horizontal calibration marker is 200 msec. Note the vertical calibration of 10 ptV for scalp ERPs and 20 ptV for the EOG. b: limbic ERPs recorded from the deepest contacts of both right and left depth electrodes. A right limbic P3 is seen in response to rare stimuli (26 and 20 averages) and is negligible in response to frequent stimuli (72 and 85 averages) as shown in the top 2 traces. Note, however, the absence of the left limbic P3 in response to rare tones (24 and 22 averages) as shown in the third trace. The bottom trace shows the response recorded from the left temporal electrode to the frequent tones (85 and 101 averages). The vertical calibration marker is 30 ptV, and horizontal calibration is 200 msec for all traces.
The limbic P3 data showed similar increases with age for both male and female subjects. In males, limbic P3 latency increased by 3.91 msec/year ($r = 0.56$, $df = 23$, $P < 0.01$) and in females an increase of 3.76 msec/year ($r = 0.66$, $df = 18$, $P < 0.01$) was noted.

Scalp P3 latency in the patient group (Fig. 4b) was also positively correlated with age ($r = 0.57$, $df = 22$, $P < 0.01$) and increased by 2.71 msec/year.

There was no significant difference between the slopes of the regression plots of patient scalp and depth ERP data ($t = 1.09$, $df = 65$, $P > 0.1$). The slope of the regression plot of the controls differed significantly from both the patient scalp ($t = 1.79$, $df = 44$, $P < 0.05$) and depth ($t = 2.25$, $df = 65$, $P < 0.005$) data.

**P3 amplitude versus age**

*Control subjects.* Scalp P3 amplitude was negatively correlated with age in controls (Fig. 5)
and decreased by 0.28 μV/year ($r = 0.58$, $df = 22$, $P < 0.01$).

**Patients.** Patient limbic P3 amplitude versus age data are plotted in Fig. 6. There was no relationship between limbic P3 amplitude (Fig. 6a) and age ($r = 0.02$, $df = 32$, $P > 0.1$). When the results of patients were separated according to sex, no correlation was noted between limbic P3 amplitude and age for either males ($r = 0.03$, $df = 17$, $P > 0.1$) or females ($r = 0.13$, $df = 13$, $P > 0.1$).

Limbic P3 amplitude data (Fig. 6a) appeared to exhibit a bimodal distribution, i.e., amplitudes either greater or less than 30 μV. A 1-tailed $t$ test for uncorrelated samples found that these groups were significantly different ($t = 9.72$, $df = 32$, $P < 0.0005$) and on this basis separate regression analyses were performed on the 2 groups of amplitude data. No significant correlations were found, however, between age and ‘low’ P3 amplitude ($y = 24.17 - 0.30x$, $r = 0.35$, $P > 0.1$) or ‘high’ P3 amplitude ($y = 35.49 + 0.46x$, $r = 0.39$, $P > 0.1$) subgroups.

There was no correlation between scalp P3 amplitude (Fig. 6b) and age ($r = 0.37$, $df = 15$, $P > 0.1$) in the patients studied.

**Discussion**

In our normal control subjects, P3 latency increased with chronological age by 1.34 msec/year and amplitude decreased by 0.28 μV/year. Our data are consistent with other normative scalp P3 data using the auditory oddball paradigm where P3 latency increased at rates of 1–1.8 msec/year and decreased at about 0.2 μV/year in normal subjects with ages ranging from 15 to 80 years (Goodin et al. 1978; Syndulko et al. 1982; Brown et al. 1983; Pfefferbaum et al. 1984; Picton et al. 1984).

P3 data from the non-epileptogenic side in the patient population were analyzed as the limbic P3 is often absent in the epileptogenic temporal lobe in these patients (Squires et al. 1981; Meador et al. 1987b; Puce and Bladin 1987; Puce et al. 1989). A linked earlobe reference was chosen to facilitate comparison with other studies of P3 and aging (e.g., Goodin et al. 1978; Brown et al. 1983; Pfefferbaum et al. 1984; Picton et al. 1984), even though there is some evidence from studies using non-cephalic references that polarity reversals can occur in scalp and nasopharyngeal P300-like ERPs.
EFFECTS OF AGE ON LIMBIC AND SCALP P3s


Limbic and scalp P3 latencies were found to both be positively correlated with age and increased as a function of age at rates of 3.85 and 2.71 msec/year, respectively. The scalp and depth recordings of patients had a significantly higher rate of increase with age as compared to the healthy controls. There was, however, no difference between the slopes of the regression lines of scalp and depth recordings in the patients. The higher rate of increase of limbic and scalp P3 latency with age observed in the patients could be due at least 3 factors: (i) the length of inter-stimulus interval (ISI) used in this study; (ii) the effects of underlying pathology and seizures; (iii) the long-term effects of anticonvulsant medication.

The results of our normals were comparable to the other studies already cited, although a relatively long ISI of 3 sec was used in this study. The ISI used here was chosen because in initial pilot studies the patients tested had difficulty in executing the task when the tones were presented at a faster rate (ISI = 1 sec). The use of longer ISIs has been shown previously to increase scalp P3 latency (Polich 1987) in normal subjects. It is possible, but unlikely, that the longer ISI may have selectively affected the patients and not the controls in this study in an age-dependent manner. The longer ISI would perhaps require a more sustained effort in maintaining attention and this could be disrupted in the patients by anticonvulsant use, particularly in cases of polytherapy (Reynolds 1983; Gillham et al. 1988), by seizures themselves (Bornstein et al. 1988) and by transient inter-ictal EEG abnormalities (Aarts et al. 1984). In normal subjects, however, the (visual) P3 has been shown to be unaffected over long, sustained recording periods requiring sustained levels of attention (Pritchard et al. 1986).

The patients studied had confirmed focal seizures, usually with underlying temporal lobe pathology. The presence of microscopic or submicroscopic lesions in the temporal lobe or elsewhere in the brain could affect the neurophysiological mechanisms involved in P3 generation. Seizures themselves could disrupt P3 processes and possibly even cause further pathology. Certainly generalized tonic-clonic seizures may increase neuronal loss, particularly in hippocampal field H1, in chronic epileptics (Dam 1987). The role of chronic partial seizures causing neuronal loss and effects on cerebral information processing, however, remains undetermined.

These patients have also been using anticonvulsants chronically, and it is possible that certain anticonvulsants may affect the P3. There are few studies, to our knowledge, that address this question. Barratt et al. (1986) found visual scalp P3s to be unaffected by a single dose of phenytoin in normal volunteers. Anticonvulsant use, however, is known to increase the latencies of earlier auditory evoked potential components in both chronically and newly treated patients (Wolpaw and Penry 1978). The effects of long-term anticonvulsant use on the P3 ERP, if any, are yet to be determined.

The P3 latency increases with age in our patient; scalp and limbic data are similar to those of scalp P3 in old subjects published by Brown's group (1983). Brown et al. (1983) reported a slope of 3.14 msec/year in subjects over 45 years of age. They found no significant correlation in younger subjects (< 45 years) and their overall P3 latency increase for all subjects was 1.12 msec/year. The majority of our patients were under 45 years of age and the corresponding P3 latency versus age plot was effectively shifted to the right of normals. It may be that the combined effects of seizures, pathology and anticonvulsants accelerate the changes seen in P3 with aging in this epileptic population, perhaps due to alteration of cholinergic activity (Meador et al. 1987a,b). It is not possible to say from the presented data which of the variables could have contributed to the effects seen in the latency data.

We were unable to demonstrate any correlation of limbic or scalp P3 amplitude with age of patient, unlike in our normal controls. Although the limbic P3 data showed an apparent bimodal amplitude distribution, when a linear regression analysis was performed separately on these 2 groups no correlation between amplitude and age was demonstrated. It is possible that any relationship between age and P3 amplitude may be have been
obscured in our data due to reasons already outlined above, in the effects of anticonvulsants, seizures, pathology, or perhaps exogenous stimulus factors. In normal subjects P3 amplitude can be manipulated by altering ISI (Fitzgerald and Picton 1981) and stimulus intensity (Roth et al. 1982). Longer ISIs produce larger P3s (Fitzgerald and Picton 1981), as do increases in stimulus intensity (Roth et al. 1982). ISI remained unaltered during our recordings. Although the patients did not have a full hearing assessment prior to ERP recording, their pure tone thresholds were determined for both tone frequencies (1 kHz and 3 kHz). Hence, during the oddball paradigm, tones were given to the patients at an equivalent level of loudness, and it is unlikely that stimulus intensity had an effect on our recordings. In the case of our limbic P3 data another important factor may have come into play. Limbic P3 amplitude (and polarity) changes markedly when hippocampal recording position is altered even by a few millimeters (Halgren et al. 1986; Stapleton and Halgren 1987). Hence, there may be large variations in limbic P3 amplitude even with stereotaxic placement of electrodes. The limbic P3 amplitudes from the most mesial recording contact were included in the data analyses, although recordings from the other recording contacts were also performed. The amplitude of limbic P3 becomes increasingly smaller with a transition from mesial to more superficial or lateral position in the temporal lobe (Halgren et al. 1986).

We have demonstrated an increase in limbic P3 latency with age in epileptic subjects, which parallels that of scalp recordings. The increase in P3 latency with age in the scalp and limbic P3s of the epileptic patients was higher than that of normals, suggesting an increased slowing of information processing with age in the epileptic subjects. These results have implications for studies of cognitive processing and indicate that the effects of age, chronic epilepsy and long-term anticonvulsant usage may be significant.

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References

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