Preservation of P300 event-related potential topographic asymmetries in schizophrenia with use of either linked-ear or nose reference sites

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Summary  Previous studies of the auditory P300 event-related potential (ERP) from our laboratory have reported a left- greater than right-sided attenuation in medicated chronic schizophrenics compared with normal controls. A possible confound in these studies has been the use of the linked-ear reference (LER), which has been criticized on the grounds that it might either induce or suppress topographic asymmetries. To test the effects of LER on P300 asymmetries in schizophrenia, we recorded ERPs with both LER and a nose reference (NR) in a group of 20 chronic medicated schizophrenics and in group of 20 age-matched normal controls. We here report: (1) confirmation of our previous P300 findings of left temporal scalp region deficit using both LER and NR with a 28-electrode montage; this feature was prominent in the wave form associated with the target stimulus, without the use of the wave form subtractions of our previous studies; (2) no statistically significant topographic differences between the LER and NR for either the schizophrenic or normal subjects; and (3) better performance of the LER in differentiating schizophrenics versus normal controls, due to lower wave form variability. We conclude that the LER is preferable for studies using subject groups and methodology similar to the present study.

Key words: Auditory event-related potentials; P300; Scalp topography; Reference electrode sites; Schizophrenia

Two previous studies of the topography of the auditory P300 event-related potential (ERP) from this laboratory have reported a left- greater than right-sided attenuation in schizophrenics compared with normal controls, with a P300 amplitude minimum in the left temporal scalp region (Morstyn et al. 1983; Faux et al. 1988a,b). Both of these studies used a linked-ears reference (LER).

The present study addresses whether the LER used in our previous studies might have distorted ERP scalp distribution and, hence, might have suppressed or induced hemispheric asymmetries. The use of LER in topographic studies has been strongly criticized in that: (1) unequal activity at the scalp could be induced by unequal electrode impedances at the ears (Beamont 1983; Mowery and Bennett 1957) and, conversely, (2) unequal activity at left and right temporal scalp regions could be reduced because ‘forcing the same potential on the two ears ... tends to make the amplitude contours rather more symmetric about the midline’ (from Nunez 1981; see also Rugg 1983; Davidson 1988; Kahn et al. 1988).

Unfortunately there have been few empirical data bearing on these important questions derived from theoretical concerns. Empirical studies of actual EEG or ERP asymmetries rarely compare multiple reference sites (for review see Davidson 1988). Moreover, we know of no study assessing the effect of reference site on ERP asymmetries in psychopathological populations. The question appears especially pertinent since, despite continuing criticisms of LER usage, the LER continues to be
one of the most popular choices among topographic investigators (cf., Kahn et al. 1988).

The purpose of this study was to compare systematically P300 topography using the LER with that of a reference site not likely to induce topographic distortion. The nose (NR) and balanced sternovertebral (SVR; Stephenson and Gibbs 1951) references have the advantage that they do not induce or reduce asymmetrical EP fields and are among the most widely used alternatives to the LER. Each reference has had its advocates among topographic investigators (e.g., NR: Vaughan and Ritter 1970; Vaughan et al. 1980; and SVR: Wolpaw and Wood 1982; Wood and Wolpaw 1982). Clearly, however, the best choice is dependent on the experimental situation and is a compromise between flawed alternatives. For example, Wolpaw and Wood (1982) have argued that the nose lies within the brain electrical fields of the auditory EP and produces voltage gradients which vary across subjects. Scherg and Von Cramon (1985), however, reanalyzed Wood and Wolpaw’s data and showed that no reference site, including SVR, is ‘inactive’ with respect to the auditory EP field if more than two dipole sources are simultaneously active within the epoch of interest, a situation that likely holds for the P300, which may have multiple source generators (reviewed in Vaughan and Arezzo 1988).

The reference site controversy in normals is thus clearly not resolved. However, in working with psychopathological populations additional considerations must be weighed, namely which site will produce records most free of artifact. Pilot data have led us to use the NR to compare with the LER, since the SVR reference produced more EMG contamination from neck and body muscle activity in patients unable to relax fully.

With respect to reference site, the basic plan of this study was quite simple: to determine whether left- versus right-sided schizophrenic and normal group differences found under the LER and NR were the same or different, and to determine if either reference was preferable for P300 studies. A secondary purpose of this study was to evaluate whether our previous findings based on BEAM technology (e.g., Duffy et al. 1981; Duffy 1982) and a 20-electrode montage were replicable using additional electrodes, a different topographic mapping technology, and in a subject group approximately a decade older.

We here report (1) general confirmation of our previous P300 findings by both LER and NR, and (2) an absence of topographic differences for the two reference sites for either schizophrenics or normals.

Methods

Subjects

The schizophrenic group was composed of 20 male chronic schizophrenics from the Brockton VAMC who met the criteria for schizophrenia of the 3 major diagnostic schemata: Washington University (WU; Feighner et al. 1972), DSM III-R (American Psychiatric Association 1987), and Research Diagnostic Criteria (RDC; Spitzer et al. 1978). All patients were administered the Schedule for Affective Disorders and Schizophrenia (Spitzer and Endicott 1978). This information, in conjunction with chart reviews and videotaped interviews, was used to make WU, DSM III-R, and RDC diagnoses. The normal control group comprised 20 volunteers, primarily recruited from hospital staff. Controls had no personal or family history of psychiatric illness. Further criteria for subject selection were: ages between 20 and 55 years, male, right-handed, no neurological illness, no drug abuse, no history of alcohol abuse, and no medications which would grossly affect the EEG (e.g., reserpine or barbiturates). Subjects with hearing impairments or verbal IQs below 70 were not selected. Subjects were given detailed information concerning the study protocol and gave informed consent.

All 20 schizophrenics were neuroleptic medicated with a mean dose equivalent to 1151 mg/day of chlorpromazine (equivalence calculated by

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1 Twenty-nine patients, meeting the above exclusionary criteria were selected primarily from two inpatient psychiatric wards. Additional exclusions occurred because of inability to perform task accurately (4), refusal to complete the experiment (2), and excessive EMG/EOG artifacts (3).
average of Shader 1975; and Bassuk et al. 1983). The mean age (S.D.) of the schizophrenic group was 40.0 (± 8.7) years and the control group was 37.7 (± 11.6) years (t = 0.73, P > 0.47). Mean scores on the Information sub-scale of the WAIS-R (INFO) were 18.1 (± 5.64) for schizophrenics and 22.3 (± 3.91) for normal controls, yielding projected verbal IQs (VIQ) of 99.5 and 111.0 (t (38) = 2.15, P < 0.04), respectively. Mean score on the Mini-Mental-State exam (Folstein et al. 1975) was 26.9 (± 2.5) out of a possible 30 for schizophrenics, and was 28.5 (± 1.1) for normal controls (t (38) = 2.55, P < 0.02). Mean years of schooling was 11.8 (± 0.9) for schizophrenics and 15.2 (± 3.2) for normal controls (t (38) = 4.53, P < 0.001).

Recording procedures

Evoked potentials were recorded using either linked-ears (LER) or nose (NR) reference sites in an auditory ‘odd-ball paradigm.’ ERPs were produced by tone-pips of 40 msec duration (10 msec rise time) presented at 1 sec inter-stimulus intervals using Etymotic insert headphones. The infrequent (15%) high-pitched tones (1500 Hz, 97 dB SPL) were presented pseudo-randomly, interspersed between frequent low-pitched tones (1000 Hz, 97 dB SPL). There was a continuous background of 70 dB white noise. To allow subjects to adapt to the auditory environment, and for consistency with previous paradigms, subjects read a novel for 20 min while listening to the tones. No instructions for counting the tones were given, but subjects were told they would be asked questions about their reading.

After reading, subjects were instructed to stare at a central fixation point and silently count each high-pitched tone. The experimenter stopped the task after every 20–35 (randomly determined) target tone presentations and asked subjects to report their counts. Correct or near correct (± 2) answers were rewarded with $1.00 or the equivalent in VA canteen coupons. An investigator sitting behind the patient monitored the patient for compliance with the counting task, assisted in coaching subjects to minimize muscle movement artifacts, and provided reward payments for accuracy. Schizophrenic subjects were able to perform this task with a median accuracy of 97%; no subject scored lower than 82% accuracy on any one run of 20–35 infrequent tones. Normal controls performed the task with a median accuracy of 99% with the lowest performance at 89%.

In the counting task, 600 tones, yielding between 80 and 100 (randomly determined) infrequent tones, were presented in each of the 2 reference site conditions. Order of reference site was counterbalanced within groups, such that one-half of the subjects from each group received the LER condition during the first block of 600 tone presentations, and the remaining received the NR first. The 2 reference sites were never simultaneously active, and electrical continuity between earlobe leads existed only in the LER condition.

ERPs were recorded from 28 scalp electrodes using an Electrode Caps International, Inc. electrode cap with tin plate electrodes. Scalp electrode placements included all electrodes in the international 10–20 system with 8 additional interpolated electrodes. Cap electrodes Fp1, Fp2, T3, Cz, and T4 were located by precise international 10–20 measurements, and all other 10–20 electrodes were positioned automatically at standard relative distances. For the remaining interpolated electrodes: FTC1 was placed at the intersection between F3-T3 and C3-F7; and TCP1 was similarly placed between C3-T5 and P3-T3. CP1 and PO1 were placed at the midpoints of the diagonals formed by Cz-P3 and Pz-O1, respectively. Homologous electrodes were placed symmetrically on the contralateral side (FTC2, TCP2, CP2, PO2). Vertical EOG was recorded for each eye using supra- and infra-orbital electrodes. Electrodes on the external canthi recorded horizontal EOG. Placements on the left and right masseter muscles of the jaw were used to monitor EMG. Electrode impedance was maintained between 2 and 4 kΩ. The EEG was filtered using a bandpass of 0.3–40 Hz (Neuroscience, Inc., EEG amplifiers, 36 dB/octave roll-off for low-pass and 6 dB/octave for high-pass). Evoked potential averages for each channel were

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Accuracy was defined by the formula: \( c = (1.0 - \frac{|p - a|}{a}) \), where \( c \) equals the proportion correct (overall accuracy), \( p \) equals the subject’s count, and \( a \) equals the actual count.
constructed from 2 msec samples over a 616 msec time interval, yielding 308 sample points. Sampling began 16 msec prior to stimulus presentation (a duration fixed by the hardware manufacturer), and the average of this pre-stimulus interval was used to establish the baseline. Sample segments from any channel which had voltages in excess of \( \pm 50 \mu V \) were excluded from the average. Based upon this rejection criterion, individual and grand-averaged EMG and EOG wave forms from the artifact monitoring channels showed no activity \( > \pm 2 \mu V \) about the baseline. Evoked potentials were averaged separately for the following conditions: reference electrode site (LER and NR) and tone probability (infrequent and frequent) to produce infrequent attentive (Ia) and frequent attentive (Fa) wave forms.

Two P300 peak component latency measures were used for each Ia wave form. The first measure (maximum amplitude latency (MAL)) was defined as the data point at the Cz electrode with the largest positive voltage between 300 and 500 msec. The second measure (Woody latency [WL]) was calculated using a variation of Woody cross-correlation filtering (Woody 1967). Each individual's wave form at Cz was cross-correlated with a template wave form consisting of the grand-averaged wave form of the normal controls. An individual wave form segment between 300 and 500 msec was shifted one point in time along the corresponding segment in the template wave form, and a cross-correlation was calculated with each time shift (lag). Woody latency was then defined as the maximum amplitude latency in the template wave form minus the lag having the largest cross-correlation.

P300 integrated voltage was defined as the mean voltage between 300 and 400 msec in the Ia condition. This time-window captured the rising phase and peak of the P300 component in most subjects and was similar to that of previous studies (Morstyn et al. 1983; Faux et al. 1988a).

The first step in analysis was to examine the voltage topography using color-coded maps (Duffy et al. 1981; Duffy 1982) of the voltages at the 28 scalp electrodes. We then examined mean differences as a function of the variance at each site using a \( t \)-statistic significance probability map (SPM; Duffy et al. 1981) to determine the area of maximal \( t \)-statistic separation between schizophrenics and normal controls. SPM analysis was used to insure that the planned subsets of electrodes for hypothesis testing were sufficient to describe group differences at entire scalp.

The presence of left temporal P300 deficits in Morstyn et al. (1983) and Faux et al. (1988a) was the basis for selecting, prior to data collection, a subset of electrodes for MANOVA. Specifically, in the Ia condition and for each reference site condition we examined the mean P300 integrated voltages at electrodes T3, C3, Cz, C4, and T4. The set of analyses used paralleled our previous studies: a simple ratio of left versus right amplitudes and a series of tests derived from MANOVA. The first MANOVA consisted of a 'within-groups' analysis to test the effects of reference site on P300 distribution at the scalp, i.e., to test for the presence of a scalp site \( \times \) reference site interaction. (Since MANOVA is conservative with respect to declaring statistical significance, we also performed paired \( t \) tests (NR-LER) of P300 integrated amplitudes at each of the 28 scalp electrode sites, in effect biasing the outcome toward finding some statistically different sites as a result of multiple \( t \) tests and greater statistical power.) The second MANOVA focused on 'between'-group differences, where Hotelling's \( T^2 \) test provided information on 3 kinds of differences: (1) overall group amplitude differences; (2) group amplitude differences at each scalp electrode site ('protected' \( t \) contrasts); and (3) group topographic differences based on a test of a group \( \times \) electrode site interaction (profile analysis of parallelism). We also used McCarthy and Wood's (1985) algorithm for normalizing (scaling) the data to eliminate amplitude effects which might be misinterpreted by MANOVA as an interaction.

In a previous study (Faux et al. 1988a) a P300 voltage criterion of 2 \( \mu V \) at the T3 electrode correctly categorized 7/9 normals and 9/11 schizophrenics. The same 2 \( \mu V \) criterion level at T3 was used to determine the degree of differentiation of schizophrenic and control individuals in the LER condition.

An important methodological point is that, in the present study, our analyses focus on the P300
component derived from the Ia condition, e.g., the wave form associated with the target stimulus; we will refer to this simply as the 'P300.' This is in contrast to the 'P300g,' a subtraction wave form previously used by us, and one that combined wave forms to frequent and infrequent stimuli and in counting and non-counting conditions (see Faux et al. 1988a,b).

**Results**

(1) **P300 topographic distribution and t-SPM analyses**

For the normal control group P300 component distribution was symmetrical throughout the P300 interval and was similar for both linked-ears (LER) and nose reference (NR) conditions (Fig. 1). In both LER and NR conditions maximum amplitude was at about 380 msec and showed a centroparietal midline maximum.

The schizophrenic group P300 topography was generally similar for both LER and NR conditions (Fig. 1). However, both conditions showed more positive activity in the right temporal scalp regions than in the left. We next computed t-SPMs to compare schizophrenic vs. control group difference topography for all scalp electrode sites (see Fig. 1). In the LER condition the largest \( t \) values were in the left frontotemporal region (F7, FTC1, and T3) and in the NR condition they were in the left frontotemporal and centroparietal regions (electrodes F7, FTC1, T3, C3, TCP1, and P3). In both NR and LER reference site conditions, left-sided electrodes (involving T3) showed larger \( t \) values than did midline or right-sided electrodes. Since our exploratory analyses were consistent with left vs. right group differences, subsequent statistical analyses were reduced to the band of electrodes beginning at T3 and progressing contralaterally to include C3, Cz, C4 and T4, as in Faux et al. (1988a) 3.

(2) **P300 grand-averaged and individual time-domain wave forms and latency measurements**

Grand-averaged wave forms are displayed for frontal, lateral, and midline electrode sites in Fig. 2 for the LER condition and in Fig. 3 for the NR condition. In both reference conditions normal controls showed P300 component maxima (300–400 msec) at the centroparietal midline (Cz and Pz) with lateral placements showing reduced but approximately symmetrical amplitudes. In contrast, schizophrenics showed P300 component amplitude reductions at all sites compared to normal controls, with greater right- than left-sided P300 component amplitudes. These findings were consistent with the LER and NR topographic maps of Fig. 1.

Fig. 4 shows the evoked potential average from each individual which was used to compute the group grand averages at T3 and T4. The uniformly low amplitudes over the 300–400 msec interval in

3 In the NR condition, TCP1 had a slightly larger \( t \) value \( (t_{(38)} = 4.13) \) than did T3 \( (t_{(38)} = 3.93) \); these sites accounted for nearly equal portions of the variance (29% and 25%, respectively; each \( P < 0.0005 \)). For consistency with previous work and with the LER analysis we have used T3 for the ratio and multivariate analyses in the NR condition.
Fig. 2. Grand-averaged wave forms (La condition) for schizophrenics and normal controls referred to LER. Note that schizophrenic versus normal control group differences over 300–400 msec are generally larger at left-sided electrode sites than at homologous sites on the right. Voltage origins at time zero were determined by a 16 msec prestimulus interval (not shown). Compare with Fig. 3, NR values.

Scoring of P300 peak component latency for each subject's P300 wave form showed no statistical differences between groups in peak component latency at any electrode site for either the maximum amplitude latency (MAL) or the Woody latency (WL). For example, at Cz, mean WL and mean MAL (values given in parentheses) were: (1) in LER condition, 386.2 (383.8) msec for controls and 388.6 (371.6) msec for schizophrenics (WL: $t = 0.20$, $P < 0.84$; MAL: $t = 0.99$, $P < 0.33$), and (2) in NR condition, 377.0 (385.8) msec for controls and 380.0 (385.0) msec for schizophrenics (WL: $t = 0.28$, $P < 0.78$; MAL: $t = 0.07$, $P < 0.95$). Moreover, there were no statistical differences between WL and MAL measurements within groups.

(3) Analysis of left versus right amplitude ratios

The first statistical measure testing for a left-right voltage asymmetry was a simple log ratio of left- versus right-sided electrodes for P300 integrated amplitudes. In Table I negative values of
Fig. 3. Grand-averaged wave forms (Ia condition) for schizophrenics and normal controls referred to NR. Note that the general trends in scalp distribution and group differences are the same as those for LER displayed in Fig. 2.

TABLE I

P300 left/right voltage ratios in schizophrenics and normal controls. Figures in parentheses indicate the geometric mean. The probability levels are from a one-sided Mann-Whitney U test, since previous results predicted that schizophrenics would show smaller left versus right voltage ratios than controls.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Log_e geometric mean (geometric mean in parentheses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Linked-ears reference</td>
</tr>
<tr>
<td></td>
<td>Sz Controls Mann-Whitney</td>
</tr>
<tr>
<td>T3/T4</td>
<td>-0.13 0.02  ( P &lt; 0.03 )</td>
</tr>
<tr>
<td></td>
<td>(0.74) (1.04)</td>
</tr>
<tr>
<td>C3/C4</td>
<td>-0.07 0.00  ( P &lt; 0.06, \text{ ns} )</td>
</tr>
<tr>
<td></td>
<td>(0.83) (1.00)</td>
</tr>
<tr>
<td></td>
<td>Nose reference</td>
</tr>
<tr>
<td></td>
<td>Sz Controls Mann-Whitney</td>
</tr>
<tr>
<td></td>
<td>-0.19 0.01  ( P &lt; 0.01 )</td>
</tr>
<tr>
<td></td>
<td>(0.64) (1.03)</td>
</tr>
<tr>
<td></td>
<td>-0.15 0.02  ( P &lt; 0.005 )</td>
</tr>
<tr>
<td></td>
<td>(0.78) (1.04)</td>
</tr>
</tbody>
</table>

Note: The appropriate summary statistic for a ratio measurement is the geometric mean, computed by taking the exponential of the mean of the logarithms of the ratios. To prevent division by zero in computation of ratios, a constant value of +0.1 \( \mu V \) was added to the voltage at each electrode site.
mean ratios in logarithmic form indicate a left < right amplitude and positive values indicate a left > right amplitude. Schizophrenics showed a clear trend toward left < right P300 component amplitudes, whereas normal controls tended to show more symmetrical amplitudes, with left slightly greater than right for T3 versus T4. The NR appeared to have slightly more L-R asymmetry for schizophrenics than did the LER; the appropriate statistical analyses of LER/NR topographic differences within groups are reported in section 4 below.

(4) P300 multivariate analyses using Hotelling’s $T^2$ test
Two types of MANOVA were performed. The first MANOVA consisted of a ‘within-groups’ analysis to test the effects of reference site on P300 distribution at the scalp, and the second set of analyses focused on ‘between-group’ differences.

Within-groups analysis. Control and schizophrenic groups were tested separately for the effects of reference site on P300 distribution. Normal controls showed no evidence of a scalp site × reference site interaction (Hotelling’s $T^2$ equivalent $F(4, 16) = 0.58, \ P > 0.68$). Schizophrenics also showed no scalp site × reference site interaction ($F(4, 16) = 1.33, \ P > 0.30$). Paired $t$ tests (NR – LER) of P300 integrated amplitudes for
TABLE II
P300 protected t test comparisons at 5 electrode sites for schizophrenic vs. normal control group: control—schizophrenic group difference (C – S), pooled standard deviation and Hotelling’s T contrast values.

<table>
<thead>
<tr>
<th>Electrode sites</th>
<th>T3</th>
<th>C3</th>
<th>Cz</th>
<th>C4</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linked-ears reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference C – S</td>
<td>3.03</td>
<td>3.54</td>
<td>3.03</td>
<td>3.11</td>
<td>2.44</td>
</tr>
<tr>
<td>Pooled S.D.</td>
<td>1.48</td>
<td>2.15</td>
<td>2.92</td>
<td>2.49</td>
<td>1.76</td>
</tr>
<tr>
<td>Hotelling’s T</td>
<td>6.45 *</td>
<td>5.20 *</td>
<td>3.38</td>
<td>3.95 *</td>
<td>4.38 *</td>
</tr>
<tr>
<td>Nose reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference C – S</td>
<td>2.97</td>
<td>3.69</td>
<td>2.85</td>
<td>2.62</td>
<td>2.03</td>
</tr>
<tr>
<td>Pooled S.D.</td>
<td>2.39</td>
<td>3.03</td>
<td>3.50</td>
<td>3.01</td>
<td>2.34</td>
</tr>
<tr>
<td>Hotelling’s T</td>
<td>3.93 *</td>
<td>3.85 *</td>
<td>2.58</td>
<td>2.74</td>
<td>2.75</td>
</tr>
</tbody>
</table>

Note: Asterisked Hotelling’s T contrasts indicate C – S differences which are statistically greater than zero; T contrast criterion for $P < 0.05$ is 3.74.

Each group at each of the 28 scalp electrode sites were not statistically significantly different at any electrode site in either the schizophrenic or control group (t (19) values varied from 0.1 to 0.6 in schizophrenics, and from 0.1 to 0.5 in normal controls). Thus there was no statistical evidence to suggest that LER and NR were different in P300 voltage topography for either group.

Between-groups analysis. (i) Overall group differences. Fig. 5 plots the mean amplitude values (by group, electrode site, and reference) which were used in the ‘between-groups’ MANOVA analyses for both the LER and NR conditions. Overall P300 amplitude differences comparing schizophrenics and normal controls were statistically significant for both LER (Hotelling’s $T^2$ equivalent $F (5, 34) = 9.44, P < 0.05$) and NR ($F (5, 34) = 5.23, P < 0.05$). Fig. 5 also shows a relatively symmetrical L = R voltage distribution for normal controls, while schizophrenics showed an L < R pattern, suggesting the presence of a group × scalp electrode site interaction.

(ii) Group amplitude differences at each scalp electrode site (‘protected’ t contrasts). Table II shows that protected t contrasts comparing individual scalp regions under both reference conditions were maximal at T3, a finding consistent with the t-SPM topography of Fig. 1. With respect to reference site, t contrasts were uniformly smaller for NR than for LER, a finding related to the slightly higher wave form variability of NR compared to LER, as shown by the pooled standard deviations (Table II) and wave form variability (Fig. 4, see also below). However, $F (max/min)$ tests showed that NR and LER variances were only statistically different at T3 ($F (19, 19) = 2.61, P < 0.05$).

![Fig. 6](image-url)

Fig. 6. Normalized P300 integrated amplitudes (300–400 msec) for LER and NR conditions. Data were normalized according to the following formula: $N_{ijk} = (V_{ijk} - \text{Min})/(\text{Max} - \text{Min})$, where N equals a normalized voltage for the kth person, the jth electrode and the ith group; $V_{ijk}$ equals the raw integrated voltages for each level of i, j, and k; Min equals the smallest mean voltage for each i and j; Max equals the largest mean voltage for each i and j. Group × electrode site interaction effects were tested using a MANOVA profile analysis of parallelism. Normal controls (panel A) showed an L = R symmetry in contrast to schizophrenics (panel B) who showed L < R amplitudes.
Distributions of normalized amplitudes for LER and NR were nearly identical within the normal control group (Fig. 6A) and within the schizophrenic group (Fig. 6B), but the schizophrenic groups' L < R topography was in contrast to the L = R topography of normals. Group differences in normalized topographic distribution tested by profile analysis of parallelism were statistically significant for both LER ($F(4, 35) = 3.72, P < 0.05$) and NR conditions ($F(4, 35) = 3.89, P < 0.05$), indicating the presence of a strong group × electrode site interaction. These results provide statistical confirmation that the topographic differences between schizophrenics and normal controls that are apparent in color maps (Fig. 1), grand-averaged wave forms (Figs. 2 and 3), and in ratio data are approximately the same for both reference site conditions.

(5) P300 separation of individual in the schizophrenic and control groups

Fig. 7 (left panel) shows that the LER correctly categorized 18/20 individuals in both groups using the previously established 2 μV criterion level. In the right panel (Fig. 7) we have displayed the NR condition using the TCP1 electrode (adjacent to T3, and slightly posterior and medial), since, as noted in footnote 3, this electrode site had the largest t value. Comparison of LER and NR shows that the visual separation between groups is worse in the LER condition. This visual impression is borne out by the magnitude of respective t values (LER: $t(38) = 6.45$; NR: $t(38) = 4.13$). This difference in individual categorization is consonant with the slightly greater variability of the NR condition shown in Table II.

Discussion

These results confirm previous findings of P300 asymmetry in schizophrenia (Morstyn et al. 1983; Faux et al. 1988a) and suggest that the LER does not greatly influence the detection and measurement of P300 asymmetry relative to the NR. These results do not suggest that past criticism of the LER is unfounded, but they do suggest that in some studies the possibility of an experimental confound caused by the LER may be a greater theoretical than practical concern.

Within groups, direct comparison of LER and NR P300 integrated amplitudes using repeated-measures MANOVA (conserving type I errors) and paired t statistic maps (conserving type II errors) showed that there was no statistical difference between LER and NR scalp topography. Between-group differences also were similar for LER and NR. Both reference sites obtained the following results: (1) t statistic mapping showed that left temporal scalp regions maximized the statistical separation between groups. (2) Analysis of left-right log ratios showed a P300 asymmetry in schizophrenics' amplitudes, and MANOVA profile analysis of P300 'normalized' topography validated the presence of a group × scalp region
interaction. (3) At the region of maximal statistical separation in LER, schizophrenics were discriminated from normals with 90% accuracy using a 2 $\mu$V criterion. Since the NR resulted in less group discriminative power than the LER, it may be less preferable in these studies than the LER. (4) No statistical differences between groups were found in P300 peak component latency.

Additionally, these results extended the generalizability of our previous studies in that they were acquired from: (1) patients who were a decade older than patients previously studied; (2) patients in a VA hospital, as opposed to a state hospital setting; and (3) completely different EEG instrumentation with an extended electrode montage. These data thus strengthen the argument that P300 asymmetries in schizophrenia are robust to a variety of experimental parameters.

There is suggestive evidence that the P300 left temporal feature may be related to lateralized brain limbic system abnormalities in schizophrenia. McCarley et al. (1989) report a high correlation of P300 amplitude with left sylvian fissure enlargement on CT scans, a presumptive indicator of left temporal lobe tissue loss. Depth recordings have indicated major generator loci for the P300 in temporal lobe limbic structures (reviewed in Halgren et al. 1986).

Other data support the presence of temporal lobe limbic system abnormalities in schizophrenia, including several recent morphometric studies (Bogerts et al. 1985; Brown et al. 1986; Crow 1986), with Brown et al. (1986) reporting lateralization and greater left-sided pathology. Converging lines of evidence suggesting left hemisphere abnormality in schizophrenia have come from measures as diverse as cerebral blood flow (Gur et al. 1985) and reaction time to a visual spatial task (Posner et al. 1988).

Despite the statistical strength of the P300 findings, several cautions must be emphasized regarding their clinical and electrophysiological interpretation.

(1) We have not yet compared data in the same patients on and off medication, nor have we tested patients without previous exposure to neuroleptics. However, current evidence suggests that the P300 abnormalities we have observed in schizophrenia are not an effect of neuroleptic medication. We have now observed similar topographic asymmetries ($P < 0.05$) in 10 unmedicated ($> 2$ weeks) schizophrenics compared with 8 age-matched normal controls (Faux et al. 1989; see also Blackwood et al. 1987; Brecher et al. 1987 for midline electrode data).

(2) We have also not compared the P300 topography of schizophrenics with other psychopathological populations. Thus, the specificity of our P300 findings remains a question.

(3) ERP scalp distributions cannot unambiguously localize underlying source generators. However, the presence of an asymmetric P300 scalp distribution is consistent with the notion that a pathological process has modified, perhaps unilaterally, the characteristics of source generators in schizophrenia. These characteristics might include (i) unilateral changes in dipole orientation, (ii) unilateral tissue reduction at the source, (iii) the activation of different or fewer generators, and/or (iv) changes in the synchronization of normally coactive generators. Thus, we believe that the presence of a P300 asymmetry in schizophrenics implies a pathological process, even if that process cannot be specifically localized by ERP techniques alone.

In sum, these data suggest: (1) LER does not influence the measurement of P300 asymmetries in schizophrenia; (2) P300 asymmetries in schizophrenia are a robust finding, now having been seen in 3 different studies; (3) the LER is probably more suitable than the NR in these studies, since it maximized schizophrenic vs. normal control group differences; and (4) the P300 differences found in previous studies using subjects with a mean age in late twenties also hold for subjects who are a decade older and for a different montage and topographic mapping technology.

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