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Intracranial Potentials Correlated With an Event-Related Potential, P300, in the Cat

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Intracranial recordings of long-latency, event-related potentials were obtained from paralyzed, artificially respirated cats. A modified oddball paradigm was employed in which cats were presented with a randomized series of two tones, a 'frequent' 4 kHz stimulus and a 'rare' 1 kHz stimulus. A tail shock was administered 700 ms after onset of the rare tone. Under these circumstances the stimulus elicited a positive component at the vertex similar to the human P300. Intracranial potentials associated with the rare tone usually manifested components of greater amplitude than did potentials associated with the frequent tone. A positive component occurring in latency between 200 and 350 ms only accompanied the presentation of the rare stimulus. The P300 component, which was positive at the dura, appeared as a negative component within a few millimeters of the surface over a wide area of the marginal and suprasylvian gyri. Changing the probability of the rare stimulus resulted in a reduction in the amplitudes of both the intracranial negative component and the P300 recorded at the skull. Components of large amplitude associated with the rare stimulus were obtained from the region of the hippocampus. These components reversed polarity, sometimes more than once, as the electrode was advanced. Substantial latency differences were often observed between the P300 recorded at the skull and P300-like intracranial components associated with the rare stimulus. These results suggest that the cortices of the marginal and suprasylvian gyri and the hippocampal region contribute to the generation of the cat P300.

INTRODUCTION

The P300 is a late positive component of the averaged event-related potential recorded from the scalp of humans that reflects the expectancies of the subject rather than the physical parameters of the stimulus[6,28]. These contingencies have led to the designation of the P300 as an 'endogenous component' of event-related potentials[29]. The amplitude of the P300 is inversely proportional to the global probability of the stimulus[5,14,22–24,26], whereas its latency is related, in part, to task difficulty[9,11,25,26] and subject age[12]. Typically, subjects are asked to count rare stimuli, which are randomly intermixed with a series of frequent stimuli. The rare stimulus evokes the P300. Since this component is best elicited when the subject is engaged in the task, the P300 is believed to reflect cognitive events of information processing. Very little, however, is known of the neural structures involved in generating the P300 since intracranial recordings and neuroanatomical identification of the recording sites have only infrequently been employed in humans[13,15,27,32,34].

Models of the P300 with animals conditoned to respond to the rare stimulus have been developed[2,3,7,10,16,18,21], but there are few reports of P300-like potentials recorded from within the brain[10,19]. In this study a cat model, developed earlier in our laboratory[7,8], was employed. Cats exposed to a classical adversive conditioning paradigm showed a late positive component which had an average latency at the midline of the skull of 260 ms. This component's amplitude was large when the signal was task relevant, its amplitude varied inversely with stimulus probability, and it could be evoked by stimuli of different modalities[30], characteristics similar to the P300 in humans. The present study in the cat measures the potentials at several intracranial locations at the time of the skull P300 event to help locate and characterize neural generators of this animal analogue of the human P300.

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MATERIALS AND METHODS

Surgical preparation

Four female cats weighing from 3.3 to 4.3 kg were used in this study. Otoscopic examination verified that each animal was free from ear lice and that the tympanum was clearly visible. Surgical preparation occurred in two stages. Initially under general anesthesia a headholder was implanted, and a stainless steel screw recording electrode placed either 1 or 11 mm behind the bregma to serve as a fixed control recording site. After the development of a P300 component during training a second surgery was performed. The animals were re-anesthetized and from one to five cannulas were implanted in the right side of the skull. The cannulas were constructed from 14 gauge stainless steel tubing. The dura was not penetrated in this procedure, and sterile bone wax was packed into the cannulas to reduce infection. Fig. 1 shows the placement of cannulas relative to the cortical surface. All surgery was performed under sodium pentobarbital anesthesia (40 mg/kg). Cats were allowed at least one week to recover before training or electrophysiological data recording commenced.

General procedures

The cats were paralyzed with gallemine triethiodide (10 mg/kg), intubated, and artificially respirated, and then supported on a piece of foam rubber. Expired carbon dioxide was maintained near 4%. Body temperature was monitored by a rectal thermometer and maintained by a circulating water heating pad. Experiments were carried out in a double-walled sound attenuating chamber with the cat's head held in place by the implanted headholder. The eyelids of the left eye were kept open by a wire loop and a pupillometer positioned in front of the eye to measure pupillary dilation. The pupillometer, described elsewhere[4], measured the amount of light reflected from the iris. To prevent drying of the eye, a thin layer of Terramycin eye ointment was applied.

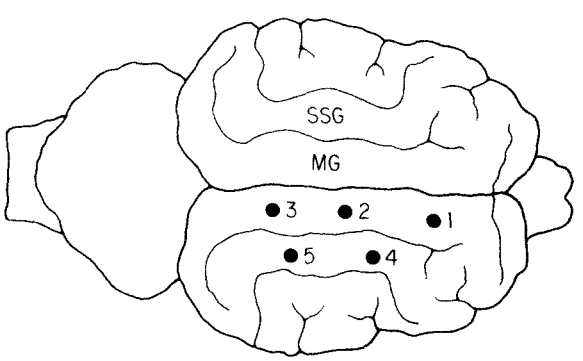

Fig. 1. Location of implanted cannulas relative to the cortical surface. MG, marginal gyrus; SSG, suprasylvian gyrus.

Auditory stimuli consisted of 1 kHz and 4 kHz tones, 50 ms in duration, with a rise time of 5 ms and an intensity of 65 dB SPL. Stimuli were delivered through Beyer earphones coupled to the cat's left ear by a hollow plastic tube. Behavioral reinforcement was produced by a constant current electrical stimulus administered to the tail 700 ms after onset of the 1 kHz tone. The electrical stimulus consisted of a 300 ms train of 15 pulses (2 mA, 2 ms) delivered through a pair of ring electrodes placed 2 cm apart on a shaved portion of the cat's tail.

Behavioral paradigm

A variation of the 'oddball paradigm' employed in human P300 studies was utilized (Fig. 2A). Cats were presented with a random series of two tones, a 'frequent' 4 kHz stimulus and a 'rare' 1 kHz stimulus at two-second intervals. The electrical stimulus to the tail was administered 700 ms after onset of the 4 kHz tone. This procedure led to a conditioning of a pupillary dilation, which began about 300 ms after onset of the 4 kHz tone. When conditioning is established, the rare stimulus elicits a positive potential at the vertex similar to the human P300[7,30]. The pupil response served as a 'behavioral' measure of the animal's conditioning to the rare 4 kHz stimulus.

Training began with an habituation phase during which no shock was employed. During habituation approximately 400 stimuli, 10% of which were 'rare', were presented. Usually at the end of such a stimulus series, no conditioned pupil response was apparent to either stimulus as well as no late positive skull potentials. In all cases conditioning was not begun until the averaged pupil response showed stimulus related dilation to be absent. During conditioning stimuli were presented in blocks of 400 and within three or four such blocks the conditioned pupillary response and P300 attained maximum values. Fig. 2 shows the development of the P300 recorded at the skull (B) and the pupillary dilation response (C) in one of the cats. The uppermost records on each side represent the averaged event-related potential and pupillary dila-

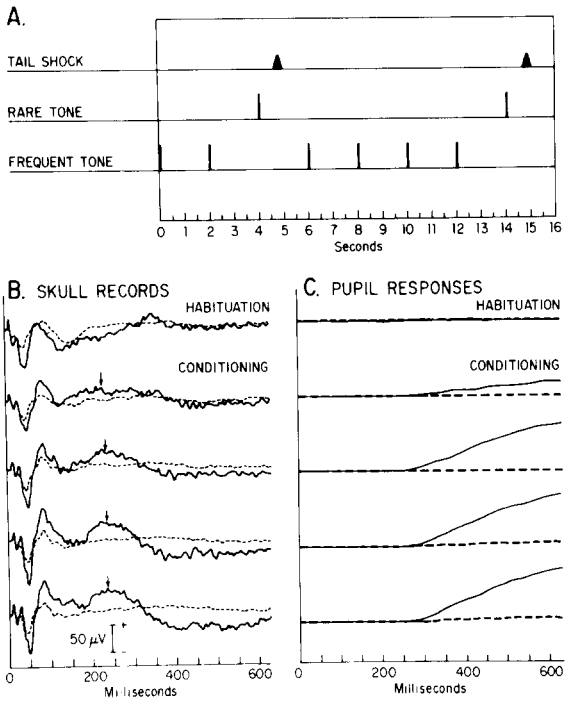

Fig. 2. Diagram of classical conditioning paradigm (A) used in this study. A random series of 'frequent' 4 kHz and 'rare' 1 kHz tones were presented at 2-second intervals. A tail shock occurred 700 ms after onset of the rare tone. Averages of event-related potentials recorded from the skull (B) and pupillary dilation (C) from one of the cats. Stimuli were presented in blocks of 400, 10% of which were rare tones. The interrupted lines represent potentials evoked by the frequent tones. Continuous lines are potentials evoked by the rare tones. The uppermost records represent the initial 400 trials without tail shock. Subsequent records represent sequential blocks of 400 trials with conditioning. In the first set of trials with conditioning a P300 component became discernable (arrow), which increases in amplitude over the next two series along with pupillary dilation.

tion response associated with the rare (continuous line) and frequent (interrupted line) stimuli. Subsequent records show the averaged potentials and pupillary responses to successive blocks of stimuli during conditioning. The arrows in the evoked potential records indicate the latency of the conditioned P300 at approximately 240 ms.

To facilitate training, the duration of the rare stimulus initially persisted until the onset of the electrical stimulus to the tail, that is, 700 ms. Once the P300 appeared, the tone duration was reduced to 50 ms. During data collection the 1 kHz stimulus was presented approximately 10% of the time, except when the effect of increasing the probability to 40% was studied. The paradigm was controlled by a PDP 11/40 computer including randomization of the signal sequence, tone duration, and electric stimulus delivery.

Neural recording

Intracranial electrodes were constructed from 0.005 inch diameter stainless steel wire insulated with epoxylite. The insulation was removed from the terminal 200–300 μm of each electrode. Intracranial electrodes were held in stereotaxic carriers and advanced into the brain through implanted cannulae. The potential recorded from a skull electrode (steel screw) near the vertex was monitored during intracranial electrode advancement. Generally no more than two intracranial electrodes were simultaneously employed. Electrode recordings were obtained relative to an indifferent needle electrode in the back of the neck. A ground electrode was located approximately 1 inch posterior to the indifferent electrode.

Brain potentials were amplified 1000 times with a band pass of 0.1 to 300 Hz (3 dB points, 6 dB/octave slope) and along with the pupillometer output were digitized on-line by the PDP 11/40, sorted with respect to which stimulus had occurred, and averaged. A 625 ms analysis interval beginning at stimulus onset was used with the initial cat. A 650 ms interval beginning 40 ms before stimulus onset was used with the other three animals. During either time base 512 samples of the incoming data were obtained from each channel.

Data was not obtained from all recording sites in all four cats. Data was collected at all five locations in cats T-1 and T-2, at sites 2 and 5 only in cat T-3, and at site 2 only in cat T-4.

During neural recording electrolytic lesions were made at selected intracranial locations for subsequent histological verification of recording sites. When sacrificed, cats were deeply anesthetized and perfused through the heart with saline and 10% buffered formalin and the brains removed. Frozen sections were cut at 50 μm and stained with cresyl violet.

Animal care

All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (DHEW Publication No. 80–23). Due to the use of paralyzed preparations in a classical condi-

tioning paradigm, care was taken to insure that the cats were as comfortable as possible. Typically, the position of the cat was changed at least once during the experimental period, which did not last beyond four hours. Experiments on the same animal were conducted at weekly intervals. The level of electric stimulus employed was not associated with prolonged pupillary dilation. The cats did not resist removal from their normal quarters in the vivarium. All four cats either maintained or slightly increased their body weight during the investigation.

RESULTS

A series of averaged potentials to both the frequent and rare tones are shown in Fig. 3B representing results from several penetrations through the marginal gyrus at site 2 in the same cat on different days. There is a significant amplitude disparity between the potentials associated with the rare and frequent stimuli with the former being of considerably larger amplitude. At the dura there is a negative–positive component occurring at 50–100 ms followed by a late positivity peaking at 200 ms, particularly to the rare tone. An interrupted line has been set at this latter latency. To the rare stimulus there is a reversal of polarity of the late positivity (henceforth termed the P300) recorded at the dura immediately below the cortical surface (4,6, and 8 mm). Additional polarity changes occur at deeper levels. A negative-to-posi-

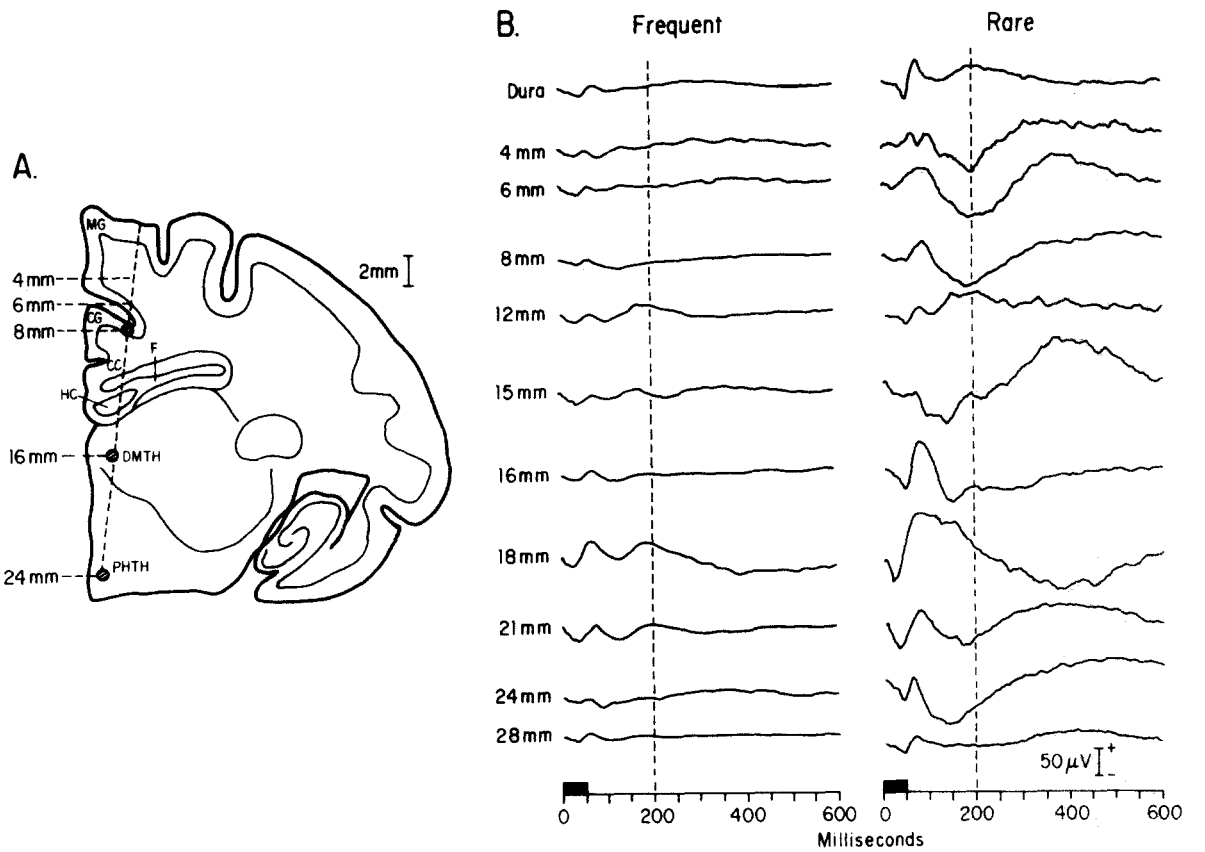

Fig. 3. Drawing of coronal section (A) showing site 2 penetration in cat T-1. The interrupted line indicates the electrode path. Circles indicate sites at which current was passed to mark a particular recording position. MG, marginal gyrus; CG, cingulate gyrus; CC, corpus callosum; F, fornix; HC, hippocampus; DMTH, dorsomedial thalamic nucleus; PHTH, posterior hypothalamic nucleus. Event-related potentials (B) associated with frequent and rare stimuli both at the dura and various depths beneath the cortical surface for the penetration shown in A. The most superficial records (2, 4, 6 mm) show a polarity reversal of the dura P300. In this and subsequent figures the black bar above the time base indicates the occurrence of the stimulus. Similarly, the interrupted vertical lines indicate the latency of the P300 recorded at the dura.

tive shift occurs between 8 mm and 12 mm, and a positive-to-negative change takes place between 18 mm and 24 mm.

The reversal of polarity of the P300 within the marginal gyrus at site 2 was a consistent observation. Of ten such penetrations in four cats, in which records were obtained within 6 mm of the dura, all manifested the polarity reversal. Fig. 4 shows one example from each cat of event-related potentials associated with the rare stimulus at the dura and within the marginal gyrus. With the exception of the 4 mm record from cat T-3, depth records in Fig. 4 represent the most superficial data obtained below the dura in those penetrations. The interrupted lines are at the latency of the P300 recorded at the dura. The solid line represents the latency of the P300 recorded at the same time at the skull. In all four cases an obvious reversal of polarity of the P300 recorded at the dura is apparent in the depth records. The two skull records for each cat replicate well, indicating the stability of the P300 evoked potential latency during movement of the depth electrode.

It is clear from Fig. 4 that latencies of the P300 recorded at the dura and the corresponding negativity in the depth records agree closely with the latency of the P300 recorded at the skull in cats T-3 and T-4. However, the latencies of P300s recorded at the skull for cats T-1 and T-2 occur almost 75 and 100 ms later, respectively, than their dural or depth counterparts. A quantitative comparison of the degree of polarity

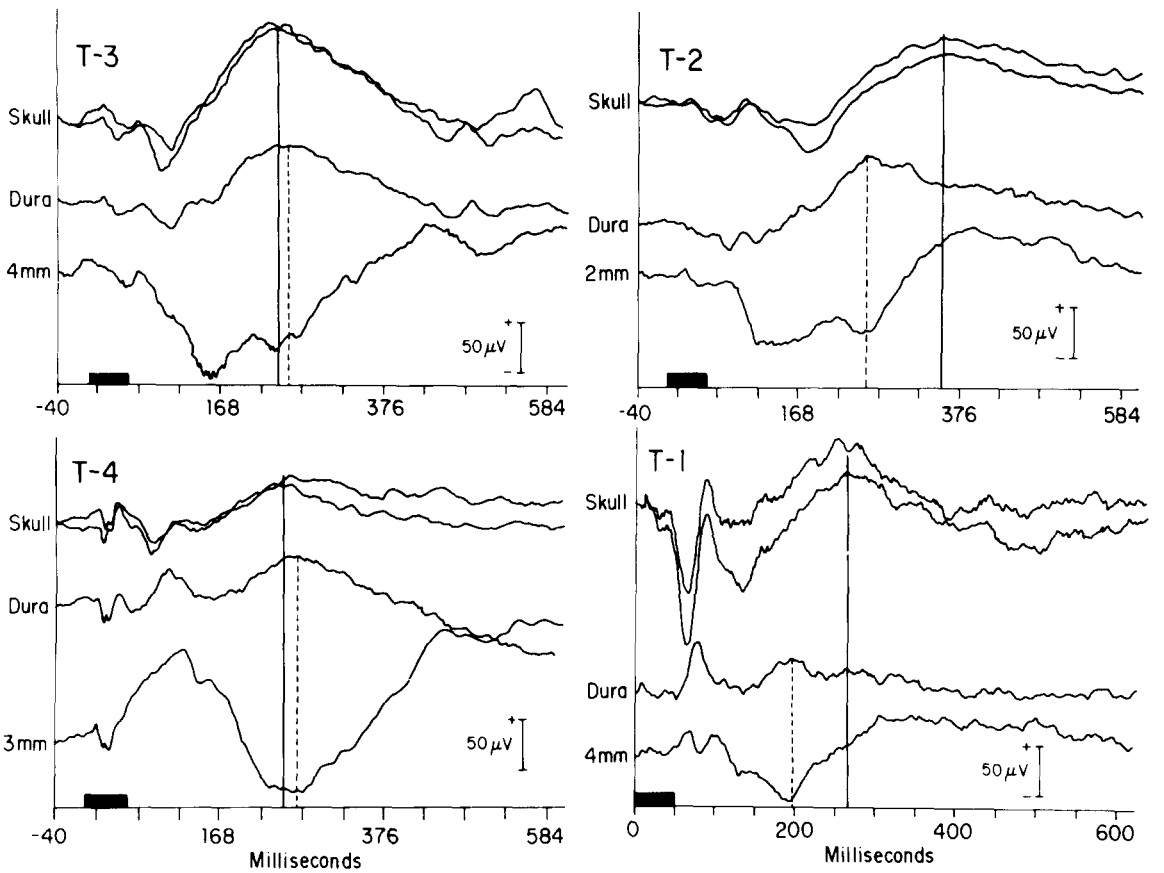

Fig. 4. An example from each cat showing the reversal of polarity of the P300 recorded from the dura as the electrode was advanced into the marginal gyrus at site 2. Also shown are the potentials recorded from a skull electrode which were obtained simultaneously with each pair of dura and depth records. The event-related potentials were elicited by the rare 1 kHz stimulus. In all four examples there is a negative peak in the depth record which occurs near the latency (vertical interrupted line) of the positive component, P300, recorded at the dura. Note that the latency of the P300 recorded at the skull (solid line) occurs close to the latency of the positive component, P300, at the dura in two of the cats (T-3, T-4). In this and subsequent figures data recording began 40 ms before stimulus onset for cats T-2, T-3, and T-4.

TABLE I

Amplitudes of intracranial potentials measured at latency of skull P300

Cat	*Intracranial depth*	*Latency of skull P300 (ms)*	*Amplitude of intracranial event at latency of skull P300 (μV)*	*Depth/dura amplitude ratio at latency of skull P300*
T-1	Dura	257	+22	
	4 mm	273	+32	+1.45
	6 mm	239	−88	−4.00
	8 mm	238	−78	−3.55
T-2	Dura	351	+40	
	2 mm	353	+38	+0.95
	3 mm	353	+11	+0.28
	4 mm	340	+15	+0.38
	5 mm	370	−14	−0.35
	6 mm	337	+ 3	+0.08
T-3	Dura	236	+59	
	2 mm	236	+90	+1.52
	3 mm	233	+25	+0.42
	4 mm	231	−82	−1.39
	5 mm	241	− 8	−0.13
	8 mm	228	−34	−0.58
T-4	Dura	253	+36	
	3 mm	256	−57	−1.58
	6 mm	280	−120	−3.33
	9 cm	280	+ 1	+0.03

reversal observed in the depth records at the latencies of both the dural and skull P300s is contained in Tables I and II.

The second column in Table I lists the depths at which the averages were obtained. The third column lists the latencies of the corresponding P300s recorded at the skull. Column four shows both the polarity and amplitude of the intracranial potentials at the latency of the P300 recorded at the skull. Finally, the ratio of the amplitude of the components recorded within the brain to that recorded from the dura appears in the last column. Table II differs from Table I only in that the latency of the P300 recorded at the dura is the point at which the amplitude ratios were determined. In the last column of both tables a negative value for the amplitude ratio means that the potential within the brain is negative relative to the potential at the dura at the latency of measurement, be it skull (Table I) or dura (Table II).

For cats T-3 and T-4 the amplitude ratios from Tables I and II agree well. In contrast there is less agreement with the ratio measures for cats T-1 and T-2, particularly the latter, in which the latencies of the P300s from the skull and dura differed. Amplitude ratios determined at the latency of the P300 recorded at the skull are mostly positive, while those obtained at the latency of the P300 recorded at the dura are negative.

An examination of the intracranial records from which the amplitude ratios for T-2 were obtained may clarify the reason for this discrepancy. The interrupted line in Fig. 5 marks the latency of the P300 recorded at the dura. The arrow by each depth record indicates the latency of the corresponding P300 recorded at the skull. It is clear that there is a polarity reversal within the marginal gyrus and that the timing of this reversal relates more closely to the peak of the P300 recorded at the dura than it does to the P300 recorded at the skull. These data indicate that a negative component can be obtained within the marginal gyrus which is P300-like in that the maximum ampli-

TABLE II

Amplitudes of intracranial potentials measured at latency of dura P300

Cat	*Intracranial depth*	*Latency of dura P300 (ms)*	*Amplitude of intracranial event at latency of dura P300 (μV)*	*Depth/dura amplitude ratio at latency of dura P300*
T-1	Dura	195	+ 45	
	4 mm	195	− 92	−2.04
	6 mm	195	−100	−2.22
	8 mm	195	−102	−2.26
T2	Dura	255	+ 65	
	2 mm	255	− 54	−0.83
	3 mm	255	−113	−1.74
	4 mm	255	− 31	−0.48
	5 mm	255	−117	−1.80
	6 mm	255	− 25	−0.38
T-3	Dura	250	+ 57	
	2 mm	250	+ 85	+1.49
	3 mm	250	+ 33	+0.58
	4 mm	250	− 74	−1.30
	5 mm	250	− 15	−0.26
	8 mm	250	− 31	−0.54
T-4	Dura	266	+ 39	
	3 mm	266	− 57	−1.46
	6 mm	266	−127	−3.26
	9 mm	266	+ 6	+0.15

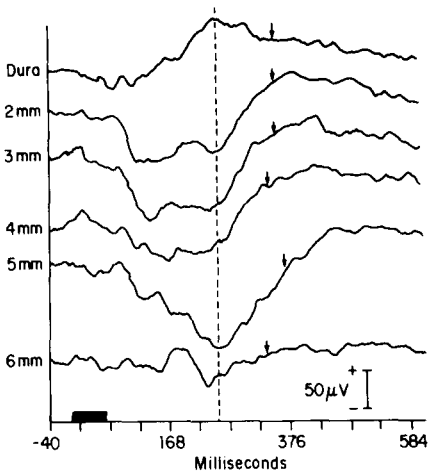

Fig. 5. A series of closely spaced (depthwise) intracranial records from a site 2 penetration in cat T-2. The timing of the polarity reversal in the depth records corresponds to the latency of the P300 recorded at the dura (vertical interrupted line) rather than the latency of the P300 recorded at the skull (arrows indicate latencies of corresponding P300s at the skull).

tude is associated with the rare stimulus, but which does not necessarily occur at the same latency as the P300 recorded at the skull.

In this study an emphasis was placed on collecting data from the middle of the marginal gyrus, site 2. However, an attempt was made to determine the distribution of the P300 recorded over a portion of the cortex by sampling electrical activity at several other locations (see Fig. 1). In T-1 the amplitudes of P300s recorded at the dura averaged between 28 and 33 μV for sites 2 through 5 while the amplitude rostrally in the marginal gyrus at site 1 was 20 μV. In cat T-2 amplitudes of P300s recorded at the dura were also, for the most part, closely grouped, although the magnitudes were substantially larger than in T-1. For sites 1 through 4, P300 amplitudes averaged 60 to 70 μV. In contrast, the P300 amplitude at site 5 averaged only 35 μV. In the third cat the amplitude at site 5 was again smaller, averaging 37 μV compared to 63 μV from site 2. It is obvious that P300 components can be recorded from the dura over a wide area of cortex without any consistent amplitude difference. Moreover, a reversal of polarity of the dura positivity was seen at all recording sites as the electrode penetrated through the cortex.

With respect to latency, a wide range of values was observed across dura recording sites, especially in cat T-1. In this animal latencies ranged from as low as 135 ms at site 4 to 268 ms at site 3, whereas the values at the skull were between 238 and 265 ms. In cat T-2 the latencies at the dura varied from 235 to 315 ms, whereas at the skull the latencies were longer, ranging from 310 to 370 ms. In contrast in both cats T-3 and T-4 the latencies of P300s recorded at the dura at sites 2 and 5 corresponded to those recorded from the skull.

In three of four cats the amplitudes of P300s recorded at the skull were about the same as amplitudes recorded at the dura. In cats T-1, T-2 and T-4 the amplitudes at the skull/dura were 34/28, 60/61, and 28/38 μV, respectively. Only in cat T-3 was the difference between skull, 100 μV, and dura, 63 μV, substantial.

Latency as the sole criterion for defining a P300-like potential recorded from the brain is insufficient. It is necessary to demonstrate that potentials represented in the depth records, as in the marginal gyrus in these experiments, reflect neural events underlying the P300 phenomenon. Factors such as stimulus probability should have similar effects on skull and depth records if the correspondence is to be made. This is an important criterion for examples like the data from cat T-2, in which the latency of the polarity reversal differs substantially from the latency of the P300 recorded at the skull. That such an amplitude change can be obtained by manipulating the probability of the rare stimulus is shown in Fig. 6 for two depth records from cat T-2, each representing a different experiment. Also shown are the associated P300s recorded from the skull and the pupil responses.

Increasing the probability of the rare tone from 0.10 to 0.40 was associated with an attenuation in the amplitudes of both the P300 recorded at the skull and the negative potential in the depth record. When measured at the latency of the P300 recorded at the dura (vertical interrupted line), the reduction in amplitude at a depth of 3 mm (Fig. 6A) was 83%. The reduction in amplitude of the P300 recorded at the skull (arrows) was 63%. On another day (Fig. 6B) the amplitude reductions at a depth of 5 mm and at the skull were 57% and 48%, respectively.

Table III summarizes the results of changing stimulus probability. The amplitudes of the negative P300-like events recorded intracranially were meas-

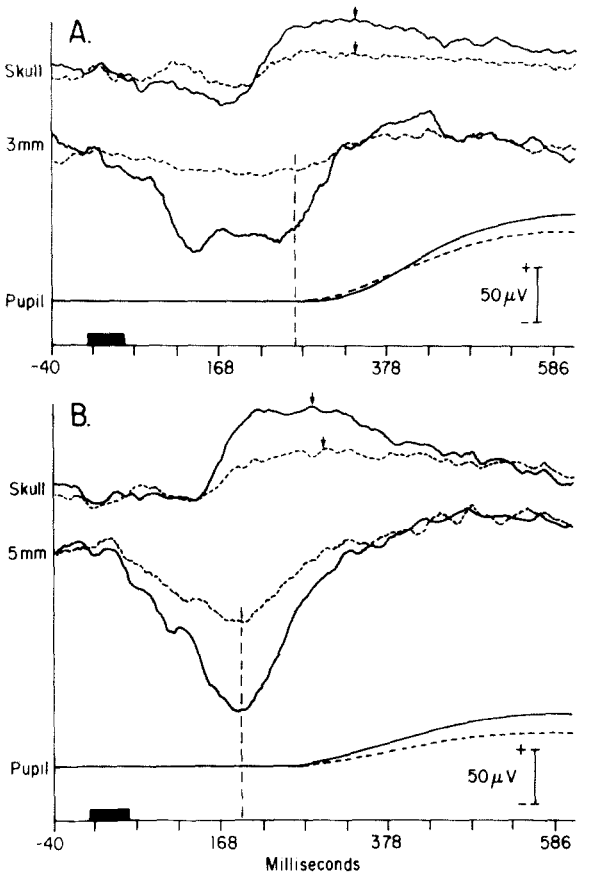

Fig. 6. The effects of varying stimulus probability on event-related potentials and pupillary dilation during two penetrations at site 2 in cat T-2. The continuous lines represent data obtained when the probability of the rare 1 kHz stimulus was 0.1. The interrupted lines represent data obtained when the probability was increased to 0.4. Note the substantial reductions in both the amplitudes of the P300 recorded from the skull and the corresponding records obtained from within the marginal gyrus (3, 5 mm) as probability of the rare tone increased from 0.1 to 0.4. The arrows refer to the latencies of the P300s recorded at the skull while the vertical interrupted line refers to the latency at the dura. In these examples there is also a decrease in the amplitude of the pupillary dilation response associated with increased probability of the rare tone.

ured at the latency of the P300 recorded at the dura. The results in Table III show the anticipated reduction in amplitude at both the skull and in the depth records as probability of the rare stimulus is increased. There was no consistent change in the pupil response, that is, both increases and decreases in amplitude were observed.

Results of human studies have been interpreted as indicating that the hippocampus might be a generator

TABLE III

Changes in amplitude of P300 recorded at the skull, negative P300-like intracranial potential, and pupil dilation as stimulus probability changes from 0.1 to 0.4

Cat	*Recording site*	*Recording depth*	*Amplitude change of skull P300 (%)*	*Amplitude change of intracranial potential (%)*	*Amplitude change of pupil dilation (%)*
T-1	2	16 mm	-27	-64	+17
T-1	5	16 mm	-20	-64	+17
T-2	2	3 mm	-63	-83	-19
T-2	2	5 mm	-48	-57	-38
T-2	3	8 mm	-43	-20	+11
T-2	5	4 mm	-58	-23	+11
T-4	2	9 mm	-17	-41	-72

of the P300 recorded from the scalp[13,15,27]. Accordingly, in three cats penetrations were made through the posterior suprasylvian gyrus (site 5) and into the region of the hippocampus. These penetrations resulted in polarity reversals and steep voltage gradients at levels corresponding to the hippocampus and associated structures. Since the recording sites were not the same across animals, the pattern of polarity shifts differed from cat to cat. The evoked potentials for both frequent and rare stimuli for the experiment in cat T-3 are shown in Fig. 7. The interrupted lines indicate the latency of the P300 recorded at the dura. Arrows indicate the latencies of the corresponding P300s recorded at the skull. The first several records (2–8 mm) associated with the rare stimuli show a polarity reversal similar to that seen in marginal gyrus tracks at the vertex. Deeper, at 16–20 mm, a negative-positive-negative sequence of polarity changes was observed. The steepness of the voltage gradients is underscored by the fact that the 18 mm record would be four times as large as shown here if drawn to scale.

It is obvious from Fig. 7 that the peak amplitude in the depth records, whether negative or positive, occurs, for the most part, earlier than the latency of the P300 recorded at the dura. The latencies of P300s recorded at the skull rather closely approximate peaks in the depth records at several levels (8, 12, 16 and 18 mm), but not all. In particular, the peaks in the more superficial records occur much earlier than the corresponding P300 recorded at the skull. Table IV con-

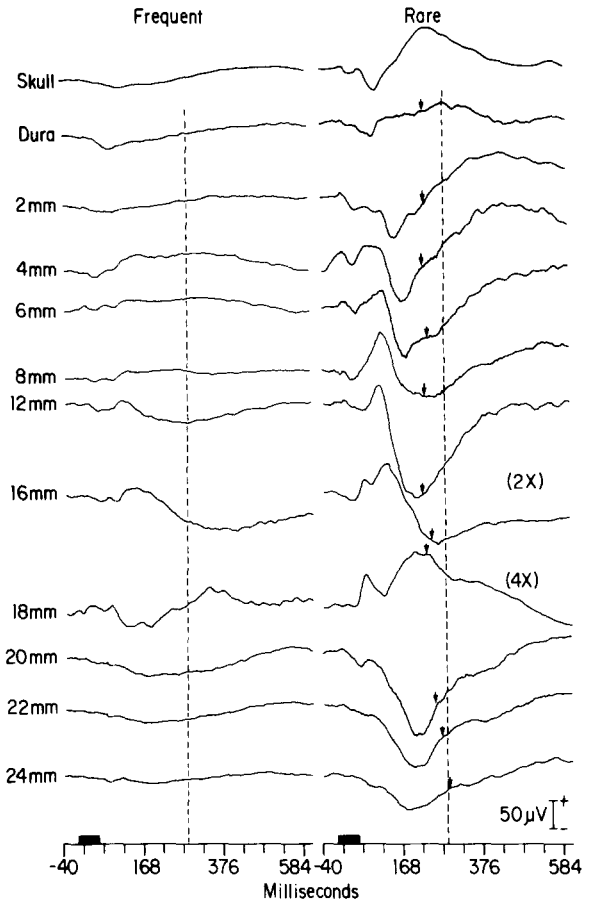

Fig. 7. A series of intracranial potentials evoked by both frequent and rare stimuli as the electrode passed through the suprasylvian gyrus (site 5) to the hippocampus. The skull records represent an average of all the skull potentials obtained during this penetration. The latency of the individual P300 recorded at the skull corresponding to each intracranial record is shown by an arrow. The interrupted lines indicate the latency of the P300 recorded at the dura. The most superficial records (2, 4, 6 mm) show a polarity reversal of the dura P300. At deeper levels in the brain, corresponding to the region of the hippocampus (≥ 12 mm), a negative-to-positive-to-negative sequence of polarity changes is seen. Potentials of especially large amplitude were obtained in this region (16, 18 mm). Note the change in calibration at the 16 mm and 18 mm depths.

tains the depth/dura amplitude ratios calculated at the latencies of the P300s recorded at both the dura and the skull. Unlike the marginal gyrus penetrations summarized in Tables I and II, here it is the P300 recorded at the skull which seems to be more closely related temporally to the peaks in the depth records at the level of the hippocampus. Although the ratios agree in sign except for the first two measures, the ratio determined at the latency of the P300 recorded at

TABLE IV

Amplitudes of intracranial potentials measured at latencies of both skull P300 and dura P300

Intracranial depth	*Latency of skull P300 (ms)*	*Amplitude of intracranial event at latency of skull P300 (μV)*	*Depth/dura amplitude ratio at latency of skull P300*
Dura	237	+ 16	
2 mm	237	- 19	- 1.18
4 mm	232	- 18	- 1.13
6 mm	242	- 72	- 4.5
8 mm	234	- 53	- 3.3
12 mm	229	-186	-11.6
16 mm	250	-186	-11.6
18 mm	242	+393	+24.6
20 mm	248	-133	- 8.3
22 mm	268	- 65	- 4.1
24 mm	295	- 26	- 1.6
	Latency of dura P300 (ms)	*Amplitude of intracranial event at latency of dura P300 (μV)*	*Depth/dura amplitude ratio at latency of dura P300*
Dura	284	+ 34	
2 mm	284	+ 23	+0.67
4 mm	284	+ 22	+0.64
6 mm	284	- 48	-1.41
8 mm	284	- 43	-1.26
12 mm	284	-124	-3.65
16 mm	284	-180	-5.29
18 mm	284	+208	+6.12
20 mm	284	- 85	-2.5
22 mm	284	- 56	-1.65
24 mm	284	- 32	-0.94

the skull is larger than the ratio determined at the latency of the P300 recorded at the dura at every level. Therefore, it is the former which more clearly represents the degree of polarity and amplitude change which occurred in the depth records. The results from T-2 were similar to T-3 in that P300s recorded from the skull were more closely related temporally to peaks in the depth records than were P300s recorded from the dura. The data from cat T-1 was ambiguous. It was not clear which of the P300s corresponded more closely to the peaks in the depth records. Like the data depicted in Tables I and II, these results underscore the extent to which latencies of P300-like components recorded within the brain may or may not correspond closely to the latencies of P300s recorded at the dura or skull.

DISCUSSION

The major finding to emerge from this study is the reversal of polarity of a P300-like potential recorded at the dura when the recording electrode was positioned within 6 mm of the surface. This occurred over a broad strip of cat cortex, including the marginal and suprasylvian gyri. The fact that P300s recorded from the skull replicated well while the electrode was advanced into the brain indicates that the polarity reversal reflected a change in the position of the electrode with respect to the location of a generator rather than to a major change in the activity of the generator itself. The reversal of polarity suggests that the electrode passed through a dipole generator oriented roughly parallel to the electrode track. Considering the relatively superficial depths at which polarity reversals were obtained, the logical site for such a P300 generator would be the cortex itself.

In an earlier study Farley and Starr[7,8] recorded the cat P300 from an array of fixed skull electrodes. The largest P300 amplitude was obtained from the center of the array at the vertex. Although slightly off the midline site 2 in the present study corresponded closely to Farley and Starr's central recording site. Our attention was focused on this site initially because it seemed to offer the highest probability of obtaining an intracranial polarity reversal. However, Farley and Starr's results also showed that the decrease in amplitude associated with recording from electrodes immediately surrounding the central site was not great. Their electrodes were spaced 11 mm apart. Recording sites 1, 3, 4 and 5 in the present study were no more than 11 mm from site 2. It is not surprising, therefore, that the amplitude of P300s recorded from the dura at these locations did not consistently differ much from the amplitude of the P300 recorded from the central site 2 position. The occurrence of a P300 at the dura over a wide area of cat cortex and the relative similarity of amplitude across dura recording sites are both consistent with Farley's results.

Human studies have shown that increasing the probability of the rare stimulus causes a decrease in the amplitude of the P300 (refs. 5,14,22–24,26). Earlier work[30] in our laboratory produced the same result for the cat P300 recorded from the skull and is substantiated by the present study. In addition we have presented evidence that the amplitude of the negative potential obtained superficially within the brain at sites 2 and 3 in the marginal gyrus and site 5 in the suprasylvian gyrus is also reduced by increasing stimulus probability. No probability tests were conducted at sites 1 and 4. In our view it is important that a reduction in amplitude of an intracranial component occur when probability of the rare stimulus is increased if the component does in fact reflect activity of a P300 generator.

Depth records from the region of the hippocampus contained components which occurred at the latency of the P300 recorded at the skull and manifested a large amplitude in response to the rare stimulus. The multiple polarity reversals of these components observed as the electrode traversed the limbic structures are consistent with the location of a P300 generator in the region of the hippocampus. Moreover, the complex arrangement of dendate gyrus, hippocampus, and parahippocampal gyrus provide an anatomical substrate consistent with our observations. Depending upon the angle of penetration an electrode could easily traverse successive layers of cortex. If a dipole generator were distributed across these cortical layers, the result would be multiple polarity reversals, as we have observed. No attempt was made to vary stimulus probability while records were being obtained from this area.

Depth recording in human subjects suggest that the hippocampus, parahippocampal gyrus, and amygdala could be involved in generating the P300. Large P300-like potentials manifesting steep voltage gradients and polarity reversals across recording sites have been reported[13,15,27]. In one study single unit activity from these structures also correlated with the behavior of the potentials[13]. The hippocampus has also been implicated using the analysis of magnetic fields associated with ionic current flow in the brain[20]. In contrast substantial excisions involving these structures failed to reduce the P300 recorded from the scalp[33]. Also, on the basis of intracranial records other investigators[34] argue for a thalamic origin of the human P300. In an animal study[10] P300-like potentials have been recorded from the cingulate cortex and anterior thalamus of the rabbit. Clearly more effort is needed in animal subjects to identify possible subcortical generators of the P300.

If the cat P300 originated from a single source, it

would be expected that the latencies of P300-like potentials recorded from the skull and various intracranial locations would be very nearly identical because of the instantaneous transmission of potentials by volume conduction[17]. However, in the present study P300-like potentials from the skull, dura, and depth records often differed substantially in latency. We suggest that these latency disparities reflect the existence of multiple generators of the cat P300. The same point has been made with respect to human data[32]. Evidence has been presented in this study for at least two possible generators, a large area of cortex including the marginal and suprasylvian gyri and the region of the hippocampus.

If multiple sources contribute to the cat P300, summation and cancellation of activity from different generators would be expected at each recording site and complex changes in the evoked potentials could occur with relatively small movements of the electrode[31]. This could explain the substantial latency and polarity differences among records from different recording sites often observed in this study and underscores the importance of using functional criteria, as opposed to simply latency and polarity criteria, to determine whether an intracranial component is related to the P300. What is essential is that the intracranial component be differentially sensitive to rare, task relevant stimuli, as is the P300 recorded at the skull. In this regard it is useful to increase the probability of the rare stimulus and look for a diminished amplitude in the intracranial P300-like component.

To the best of our knowledge a reversal of polarity of a P300-like potential at the cortical level in either human or animal studies has not been reported elsewhere. In part this may reflect the limited extent of P300 studies in animals. Species differences may also be involved. In addition the P300 from a classically conditioned, paralyzed preparation may not be directly comparable to P300s obtained under other circumstances[7].

While the results of this study suggest that a large area of cat cortex and the hippocampal region contribute to the generation of the cat P300, certain considerations limit this interpretation. Conceivably, an electrode could pass outside of the region of an active generator containing a dipole layer, yet penetrate at an angle more or less parallel to the orientation of the dipole and still record a reversal of polarity of a P300-like component. Presumably the amplitude of the component would be less than if the electrode had penetrated the active region. This suggests the importance of closer spacing of recording sites to specify more exactly the locations of the generators of P300-like components. Also, it should be noted that even if a subcortical component closely resembles a component recorded at the surface (dura or skull), it may actually contribute little or nothing to the surface potential[1,31]. Finally, the recording of P300-like single unit activity from suspected generator sites as well as ablation studies involving those structures are needed to corroborate the results of the present study.

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REFERENCES

1 Arezzo, J., Legatt, A. D. and Vaughan, Jr. H. G., Topography and intracranial sources of somatosensory evoked potentials in the monkey. I. Early components, *Electroenceph. clin. Neurophysiol.*, 46 (1979) 155–172.

2 Arthur, D. L. and Starr, A., Task-relevant late positive component of the auditory event-related potential in monkeys resembles P300 in humans, *Science*, 223 (1984) 186–188.

3 Buchwald, J. and Squires, N., Endogenous auditory potentials in the cat: a P300 model, In C. Woody (Ed.), *Conditioning*, Plenum Press, New York, 1982, pp. 503–515.

4 Cassaday, J. M., Farley, G. R., Weinberger, N. M. and Kitzes, L. M., Pupillary activity measured by reflected infra-red light, *Physiol. Behav.*, 28 (1982) 851–854.

5 Courchesne, E., Hillyard, S. A. and Galambos, R., Stimulus novelty, task relevance and the visual evoked potential in man, *Electroenceph. clin. Neurophysiol.*, 39 (1975) 131–143.

6 Donchin, E., Ritter, W. and McCallum, C., Cognitive psychophysiology: the endogeneous components of the ERP. In E. Callaway, P. Tueting and S. H. Koslow (Eds.), *Event-Related Potentials in Man*, Academic Press, New York, 1978, pp. 349–411.

7 Farley, G. R. and Starr, A., Middle and long latency audi-

tory evoked potentials in cat. I. Component definition and dependence on behavioral factors, *Hearing Res.*, 10 (1983) 117–138.

8 Farley, G. R. and Starr, A., Middle and long latency auditory evoked potentials in cat. II. Component distributions and dependence on stimulus factors. *Hearing Res.*, 10 (1983) 139–152.

9 Friedman, D., Simpson, R., Ritter, W. and Papin, I., The late positive component (P300) and information processing in sentences, *Electroenceph. clin. Neurophysiol.*, 38 (1975) 255–262.

10 Gabriel, M., Sparenborg, S. P. and Donchin, E., Macropotentials recorded from the cingulate cortex and anterior thalamus in rabbits during the 'oddball' paradigm used to elicit P300 in humans, *Soc. Neurosci. Abstr.*, 9 (1983) 1200.

11 Gomer, F. E., Spicuzza, R. J. and O'Donnell, R. D., Evoked potential correlates of visual item recognition during memory-scanning tasks, *Physiol. Psychol.*, 4 (1976) 61–65.

12 Goodin, D., Squires, K., Henderson, B. and Starr, A., Age-related variations in evoked potentials to auditory stimuli in normal human subjects, *Electroenceph. clin. Neurophysiol.*, 44 (1978) 447–458.

13 Halgren, E., Squires, N. K., Wilson, C. L., Rohrbaugh, J. W., Babb, T. L. and Crandall, P. H., Endogenous potentials generated in the human hippocampal formation and amygdala by infrequent events, *Science*, 210 (1980) 803–805.

14 Hillyard, S. A. and Galambos, R., Effects of stimulus and response contingencies on a surface slow potential shift in man, *Electroenceph. clin. Neurophysiol.*, 22 (1967) 297–304.

15 McCarthy, G., Wood, C. C., Allison, T., Goff, W. R., Williamson, P. D. and Spencer, D. D., Intracranial recordings of event-related potentials in humans engaged in cognitive tasks, *Soc. Neurosci. Abstr.*, 8 (1982) 976.

16 Neville, H. J. and Foote, S. L., Auditory event-related potentials in the squirrel monkey: parallels to human late wave responses, *Brain Research*, in press.

17 Nunez, P. L., *Electric Fields Of the Brain. The Neurophysics of EEG*, Oxford University Press, Oxford, 1981.

18 O'Brien, J. H., P300 wave elicited by a stimulus-change paradigm in acutely prepared rats, *Physiol. Behav.*, 28 (1982) 711–713.

19 O'Connor, T. and Starr, A., Generator of P300 in the cat, *Soc. Neurosci. Abstr.*, 9 (1983) 1195.

20 Okada, Y. C., Kaufman, L. and Willimson, S. J., The hippocampal formation as a source of the slow endogenous potentials, *Electroenceph. clin. Neurophysiol.*, 55 (1983) 417–426.

21 Paller, K. A., Zola-Morgan, S., Squire, L. R. and Hillyard, S. A., Late positive event-related potentials in cynomolgus monkeys *(Macaca fasicularis)*, *Soc. Neurosci. Abstr.*, 8 (1982) 975.

22 Picton, T. W. and Hillyard, S. A., Human auditory evoked potentials. II. Effects of attention, *Electroenceph. clin. Neurophysiol.*, 36 (1974) 191–200.

23 Ritter, W. and Vaughan, Jr., H. G., Averaged evoked responses in vigilance and discrimination. A reassessment, *Science*, 164 (1969) 326–328.

24 Squires, K. C., Donchin, E., Herning, R. I. and McCarthy, G., On the influence of task relevance and stimulus probability on event-related potential components, *Electroenceph. clin. Neurophysiol.*, 42 (1977) 1–14.

25 Squires, K. C., Hillyard, S. A., and Lindsay, P. H., Vertex potentials evoked during auditory signal detection: Relation to decision criteria, *Percept. Psychophys.*, 10 (1973) 445–452.

26 Squires, N. K., Donchin, E., Squires, K. C. and Grossberg, S., Biosensory stimulation: Inferring decision-related processes from the P300 component, *J. exp. Psychology: Human Perception and Performance*, 3 (1977) 299–315.

27 Stapleton, J. M., Derrick, B. E., Crandall, P. H. and Halgren, E., Endogeneous limbic potentials in humans: component and site specificity, *Soc. Neurosci. Abstr.*, 9 (1983) 1199.

28 Sutton, S., Braren, M., Zubin, J. and John, E. R., Evoked potential correlates of stimulus incertainty, *Science*, 150 (1965) 1187–1188.

29 Sutton, S., Tueting, P., Zubin, J. and John, E. R., Information delivery and the sensory evoked potentials, *Science*, 155 (1967) 1436–1439.

30 Wilder, M. B., Farley, G. R. and Starr, A., Endogeneous late positive component of the evoked potential in cats corresponding to P300 in humans, *Science*, 211 (1981) 605–607.

31 Wood, C. C. and Allison, T., Interpretation of evoked potentials: a neurophysiological perspective. In C. L. Nash and R. H. Brown (Eds.), *Proceedings of the Spinal Cord Monitoring Workshop: Data Acquisition and Analysis*, Case Western Reserve University Press, 1979. pp. 253–301.

32 Wood, C. C., Allison, T., Goff, W. R., Williamson, P. D., and Spencer, D. D., On the neural origin of P300 in man, In H. H. Kornhuber and L. Deecke (Eds.), *Progress in Brain Research, Vol. 54.*, Elsevier, Amsterdam, 1980 pp. 51–56.

33 Wood, C. C., McCarthy, G., Allison, T., Goff, W. R., Williamson, P. D. and Spencer, D. D., Endogeneous event-related potentials following temporal lobe excisions in humans, *Soc. Neurosci. Abstr.*, 8 (1982) 976.

34 Yingling, C. D. and Hosobuchi, Y., A subcortical correlate of P300 in man, *Electroenceph. clin. Neurophysiol.*, 59 (1984) 72–76.