PERIODICITY OF HIGH-ORDER FUNCTIONS IN THE CNS

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INTRODUCTION

This report describes the results of recent studies on perceptual and integrative processes in the central nervous system, studies which represent part of the continuing research in this laboratory supported by the National Aeronautics and Space Administration. The purpose of these studies has been to elucidate basic neural mechanisms that underlie human attentive processes in order to develop techniques for monitoring the functional state of these processes. Such techniques would have widespread applications, particularly in the space program, where relatively high levels of sensory-motor performance are required.

The electroencephalogram (EEG), a recording of the electrical activity of the brain, provides one of the few nondestructive techniques for assessing brain function. Widely used in diagnosing relatively gross brain dysfunctions, the EEG has proven to be of little benefit in assessing subtle alterations of higher nervous function, such as the cognitive processes.

The application of new recording and data-analysis techniques has resulted in a major breakthrough in this area. Using methods which permit the recording of relatively slow brain electrical potentials while a subject performs a reaction-time task, a surface-negative wave, maximal in amplitude over the frontal regions of the brain, called the contingent negative variation (CNV), is revealed. The CNV has been shown (by us and others) to be a consistent phenomenon, easily recorded in children as well as adults, and to bear a definite relationship to the processes of attention and alertness in man. The elucidation of the physiological significance of this slow potential and the neural mechanisms underlying its production has been the subject of intensive research in this laboratory for several years, much of which has been summarized in previous reports to NASA.

A basic understanding of the physiological significance of the CNV is necessary before proceeding to the development of an alertness-monitoring test utilizing these recording techniques. Previous work in this laboratory established the existence of a wave analogous to the CNV in rhesus monkeys. The presence of the CNV in monkeys has permitted a more rigid control of variables than is possible in human subjects and has allowed us to investigate the cerebral origins of this potential.

Recent work (Borda, 1970, reprint enclosed) suggests that the potential typically recorded at the scalp is not, as previously supposed, unitary, but is rather a composite of several waves. The research presented below further

differentiates these components and establishes the cortical origin of the potential.

METHODS

Six monkeys (Macaca mulatta) served as subjects. They were trained to press a lever during a 2500 c/sec tone (S_2) to receive a 45 mg sucrose pellet. An auditory "click" (S_1) preceded the tone by 1 sec. A lever press (R) between the click and tone caused the trial to end immediately, with no tone or reward. The training paradigm was essentially identical to that described by Borda (1970) (reprint enclosed). The animals were trained in a heavy restraining chair to which was attached a head holder and a track designed for use with the Kopf electrode carrier. Four connectors attached to the head holder matched four bolts implanted in the skull of the animal. The chair, head holder, and animal preparation are described in an enclosed reprint (Rettig, 1971). When the subjects reached 85% correct responses, they were prepared by epidural implantation of a 1.25 cm ID stainless-steel cannula and four 8-32 stainless-steel bolts. Transcortical electrodes, like those described by Borda (1970) were implanted contralateral to the cannula. After recovering from surgery, the animals were placed in the restraining chair, and their heads were fixed in the holding device described by Rettig (1971). An electrode consisting of four wires with bared spots at 0-1 (#1), 2-3 (#2), 4-5 (#3), and 6-7 (#4) mm from the tip was fixed in a Kopf electrode carrier and lowered in 1 mm increments through the cortex. A paraffin plug was used in the cannula to reduce brain pulsations. At each level, 16 or more trials were recorded for averaging. Brain electrical activity was amplified by a Brush, 8-channel, RC-coupled (time constant 1.6 sec) amplifier and stored on magnetic tape with a PI-400, 12-channel, FM recorder. Recordings were made as anterior as the frontal pole and as posterior as the precentral rolandic gyrus.

After three penetrations through the cortex, the animal was anesthetized and perfused with saline and then formalin. The brain was removed, inspected grossly, and prepared for histological confirmation of the type of tissue penetrated and the depth reached by the electrode.

RESULTS

Histology

Of the five monkeys that served as subjects, four had cannulas implanted, were explored with three penetrations of the cortex, and were prepared for histology. In the last monkey, the cannula was implanted twice over different areas of cortex. There were three frontal cannula positions and three precentral positions. Of the 18 penetrations, the probe penetrated only 3 mm into cortex on two occasions, and therefore the data were of only marginal value. Eleven penetrations went straight through gray matter into white matter, three passed through gray matter along the wall of sulci and into the white at the bottom, and three passed from gray matter to white matter and into gray matter again. One penetration extended to the cingulate gyrus. The reference pairs were generally contralateral to the penetrations; however, the "subcortical" members of two of the reference-electrode pairs in two of the premotor monkeys were in the gray of either principal or cingulate sulci. Because of space limitations, the third premotor reference pair was located in the parietal lobe. The reference pairs for the precentral experiments had the subcortical members in the white matter. Fig. 1 shows the positions of the penetrations. The reference electrodes are represented by a loop with a central point.

Table I presents the apparent histological depths and the depths estimated from the electrophysiological data. The latter were calculated from the first position at which the pairs of electrodes on the shaft, those referred to the tip of the probe, showed significant activity. Estimating this as 1 mm into the cortex and calculating each subsequent position from the new electrode-carrier position, the total length of the intracortical run could be computed. The histological preparations were subject to some distortion due to post-mortem changes and reconstruction from multiple slides. Fig. 2 depicts the reconstructed electrode paths.

The error of the electrophysiological estimate is approximately 1 mm. With the exception of Monkey E, penetration 2, all anatomical approximations are within 2 mm of the physiological estimates. The exception, Monkey E, penetration 2, involves a 7 mm run through the gray matter of the superior wall of sulcus arcuatus and 3 mm more through white matter. The electrophysiological data suggest that the probe penetrated 4 mm into the gray matter on the first entrance, implying that there was a greater than usual distortion of the cortex before the penetration of the electrode.

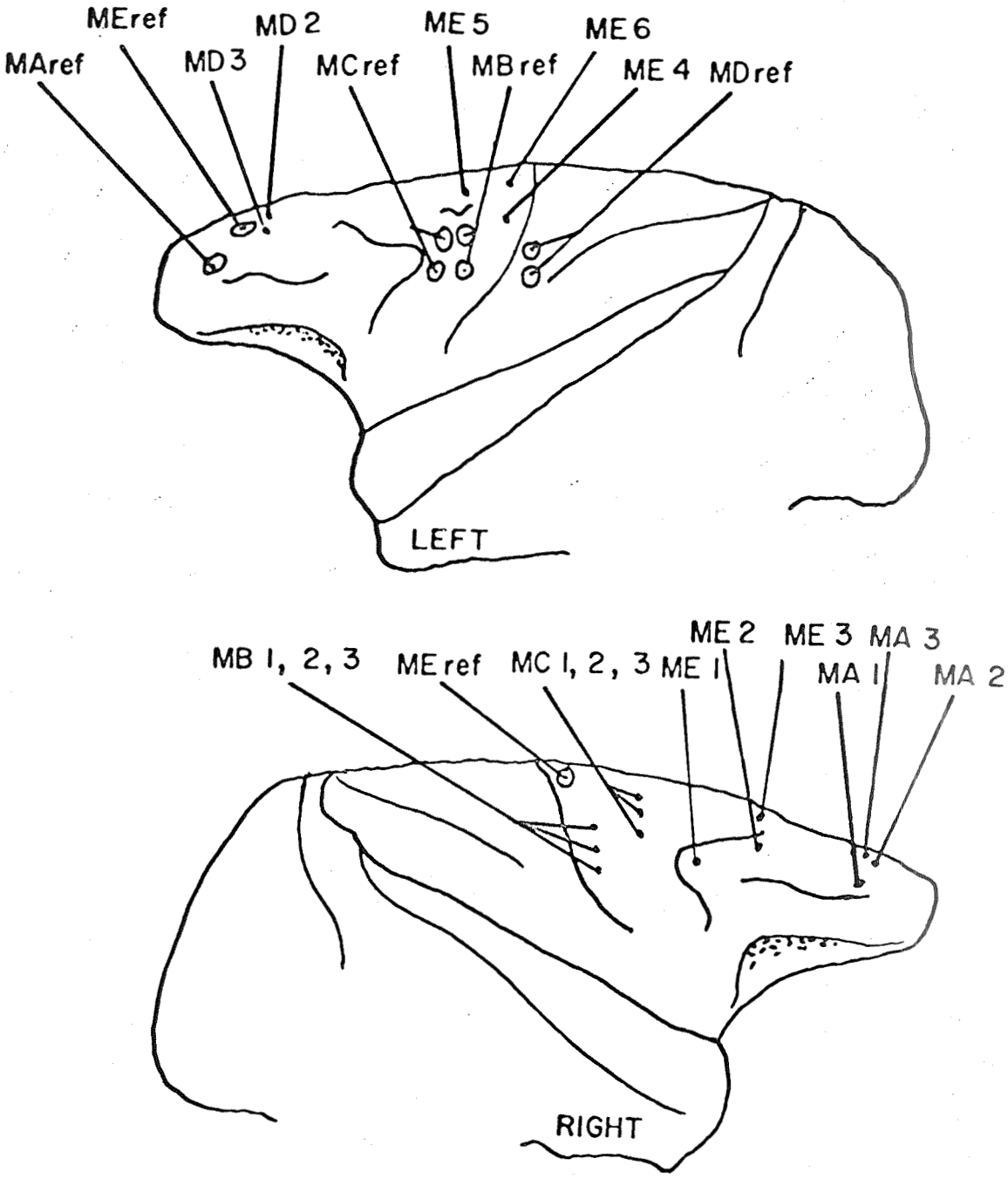


Fig. 1. Lateral surfaces of brain with points of entry of electrodes labeled. Reference pairs are indicated by a circle with a central dot. Penetrations are marked by a simple dot.

TABLE I

Penetration	Anatomic Depth (mm)	Electro-physiologic Depth (mm)	FEP	SEP	CNV	LPP
Monkey A						
1	7	6	+	+	+	
2	7	5	+	+	(+)	
3	5	6	+		+	
Monkey B						
1	2	3	+			
2	7	6	+	+	+	+
3	5.5	4				
Monkey C						
1	4	3		+	+	+
2	6.5	5		+	+	+
3	6	5		+	+	+
Monkey D						
1	3	2	+		+	
2	7	7.5	+	+	+	
3	10.5	10			+	
Monkey E						
1	11	11	+	+	+	+
2	11	7	+	+	+	+
3	7	5	+	+	+	+
4	6.5	7	+	+	+	+
5	6.5	7	+	+	+	+
6	15	15	+	+	+	+

FEP = Fast evoked potential
SEP = Slow evoked potential
CNV = Contingent negative variation
LPP = Events associated with the lever press
\+ = Presence of these potentials
( ) = CNV not found in first grey penetrated but found in wall of S. Principalis

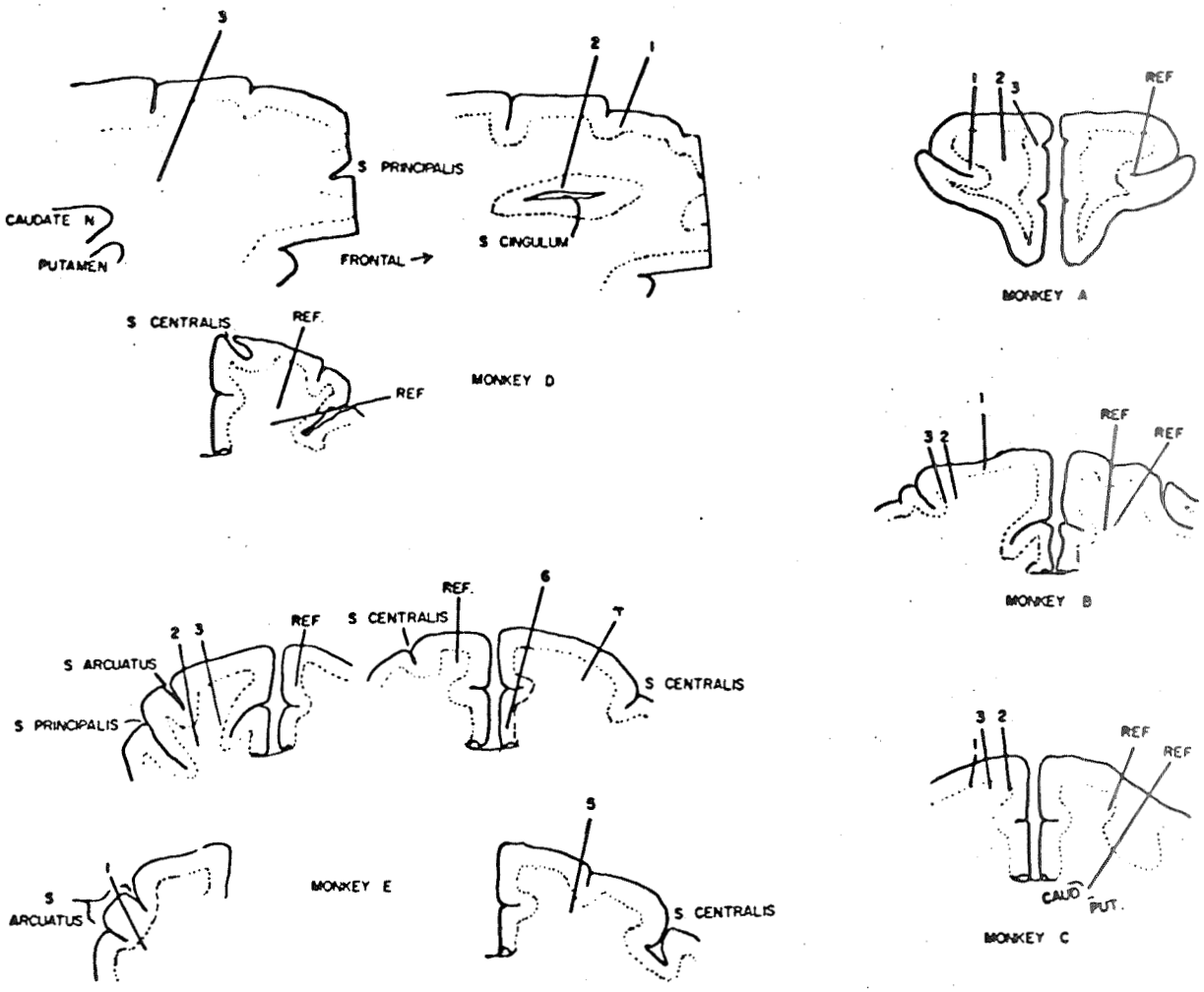


Fig. 2. Sketches of reconstructed electrode paths in brains of Monkeys A through E. The grey-white border is indicated by the dotted line. Numbers correspond to penetrations; ref. = reference electrode. All sections are coronal except the two sagittal cuts from Monkey D.

Adding the extra 3 mm to the standard computation would yield 10 mm, calculated from electrophysiological data, versus 11 mm calculated from anatomic data.

The presence of certain potentials is also presented in Table I. These are the fast evoked potential (FEP), the slow evoked potential (SEP), the contingent negative variation (CNV), and the events associated with the lever press (LPP). The appearances and distributions of each of these potentials showed considerable variability, as described below.

Evoked Potentials

Evoked potentials were recorded in most of the areas of cortex sampled. There were two general components: the fast components (with each component taking less than 100 msec to complete its rise and fall), and the slow components (taking 100 to 500 msec). All frequencies above 50 c/sec were filtered, and so activity of less than 20 msec in periodicity would be severely attenuated.

The traces in Fig. 3 are averages of 16 trials (electrode #3 referred to electrode #1). The first trace shows the absence of activity in the cerebrospinal fluid (CSF). The second trace represents activity measured when the tip electrode was 0 to 1 mm in the cortex. The subsequent traces correspond to sequentially deeper levels, progressing in 1 mm steps. The numbers to the side correspond to the number of millimeters above the surface electrode #3 is calculated to be. The fast evoked response to S_1 is a surface positive-negative-positive complex. The response to S_2 is a diminutive version of this potential. The FEP complex rides on a slower surface-positive wave that finally returns to base line 250 to 300 msec after S_1. The major positive components of these potentials change phase and reverse polarity as electrode #3 (#1 electrode now in the white matter) passes from the surface to 1 to 2 mm below the surface.

Fig. 4 shows the activity recorded from three electrode pairs along the probe on a single trial. The top trace corresponds to electrode #4 referred to electrode #1, with #4 approximately 2 mm above the surface of the cortex. The second trace represents electrode #3 referred to #1, with #3 in the outer 1 mm of cortex. The third trace is derived from electrode #2 referred to electrode #1, with #2 descending 2 to 3 mm into the gray. Electrode #1 is in the subjacent white matter. On line and in the averages, very little activity could be found between electrodes in the white matter, either locally or

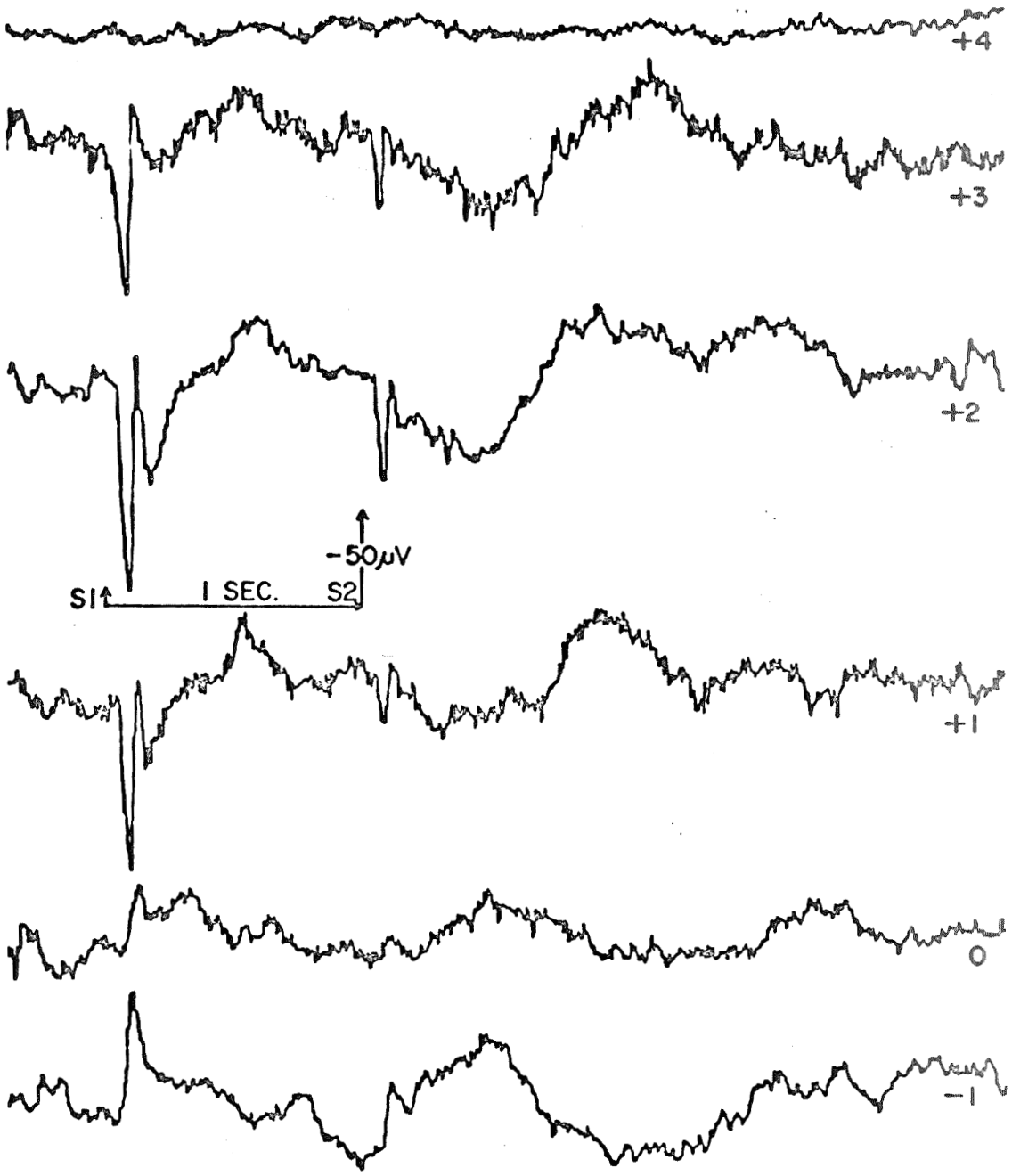


Fig. 3. Averages of 16 trials of the activity between electrodes 3 and 1. In the first trace both electrodes are in the epicortical CSF. In the second trace electrode 1 is in the cortex and electrode 3 is still in the CSF. Each subsequent trace corresponds to a run 1mm deeper in the cortex than the preceding one. The numbers to the right correspond to the distance above the cortical surface electrode 3 is calculated to be. Negativity of electrode 3 is up.

between widely separated areas. There were exceptions, which were post-S_2 events, presumed to be related to the lever press. The most intense activity still appeared to be in the gray matter with a spread of the field into the subjacent white matter. The possible explanations of this activity in the white matter will be discussed later. Thus, the white matter was considered an inactive reference as far as potentials measured in evoked-potential and CNV experiments were concerned. With this presumption in mind, the activity seen in Figs. 3 and 4 shows that there is a reversal of polarity of major potentials associated with S_1 and S_2 between the epicortical CSF and 1 to 2 mm deep into the cortex. These two figures illustrate a simple penetration of gray to white. In those cases where the electrode penetrated gray-white-gray, a somewhat more complex analysis of the events at various levels was required. First, a cross section along the electrode tract was reconstructed from multiple frozen sections. The level of entry of each electrode was calculated from the electrophysiological data. Electrode #1 was presumed to enter when significant activity started between #1 and the other three electrodes on the probe. Electrode #2 was presumed to enter when the activity between #2 and #1 began to differ from the activity between #3 (or #4) and #1. Similarly, #3 was presumed to enter when the activity between #3 and #1 began to differ from the activity between #4 and #1. Another system of estimating the entry of #2 into the cortex, found to give results identical to the above, was to observe the first level at which there was activity between #2 and #3. At each level at which an electrode entered the cortex, the position of the other electrodes could be located on the anatomically calculated cross section. Finally, the deepest level was used to reconstruct the position of each electrode at that level.

When one electrode of a pair was in an electrically inactive substance (the CSF, for instance), the activity was presumed to originate from the other electrode. When multiple pairs of electrodes with one lead in common showed the same activity, that activity was presumed to originate at the common electrode. Using the anatomical reconstruction of the run to locate the electrodes at each level of a run and comparing the activity of different pairs at each level, the electrical activity at each level was computed.

Fast evoked-potential complexes were found in all but one of the premotor runs. In six of eight penetrations, the first positive wave inverted its polarity as the electrode passed through the outer millimeter of cortex. In one of the other cases, the penetration was not deep enough to be sure of inversion, and in the last case the potential inversion was not clearly localized, the probe paralleling the surface in the wall of a sulcus for several millimeters. The potential did, however, invert in the depths.

Fast complexes in the precentral runs were less common, being present in only five of the nine runs, and were less prominent. The first component of the potentials appeared to invert on passing through the outer 1 mm of cortex in all but one run. In the exception, the inversion was complete at a depth of 2.5 mm (where cortex was 3.2 mm thick).

Slow evoked-potential components were present in six of nine premotor runs. The magnitudes ranged from 20 to 80 μV. Three of the six were surface-positive waves of 300 to 500 msec duration. There were two surface-negative waves of 100 msec duration. Both 500 msec and 100 msec positive waves were seen in layer II of the superior wall of sulcus principalis. The penetration was not extended to the pia, but the potential arose between 2.5 and 0.5 mm from the surface.

Six of the nine precentral runs demonstrated slow evoked components. These ranged from 5 to 40 μV and were surface positive. All were 300 msec or longer in duration, and one had a superimposed 100 msec wave. In three cases there was a surface-positive swing associated with both S_2 and R that could not be differentiated, and in the other three the response to S_1 was greater than that to S_2.

Contingent Negative Variation

The CNV took three forms. First, in two instances there was a negative shift, measurable over the surface, with no intracortical shifts. Second, in nine cases there was a negative shift in the CSF and sometimes in the outer 1 mm of cortex, but with a relatively positive shift in the next 1 or 2 mm of gray matter. Third, in one run there was only a relatively positive shift in the second millimeter of gray, with no superficial negativity. Twice, the penetration showed a superficial negativity but was not carried deep enough to establish the presence or absence of positivity. Once, the probe stopped before layer I of the superior wall of sulcus principalis and measured a negative shift at that level. No discernible shift was seen in two instances. Both reference electrodes that were in gray measured negative shifts.

In addition to evoked potentials, Figs. 3 and 4 demonstrate the presence and polarity reversal of the CNV. This example is typical of runs showing clear CNVs and clear evoked potentials in that they both are seen to invert in the outer millimeter of cortex. The level of polarity reversals of evoked-potential complexes and CNVs correlated well.

Premotor runs manifested more CNV-related potentials than precentral runs. Virtually every penetration contained either a negative-surface shift

or a negative-positive dipole or positive-depth shift. The only area of frontal cortex that yielded no shift was the lateral aspect of the superior frontal gyrus (SFG), approximately 40 mm anterior to the interaural line. The run just medial to this yielded only a positive shift of 10 μV, whereas a third run in the same monkey, medial to both of these, yielded a 10 μV surface-negative/ 20 μV depth (2mm)-positive dipole. As the lateral run continued through white matter into the superior wall of sulcus principalis, a negative shift (with respect to the white matter) became apparent.

The precentral runs yielded a more variable picture. In two of the runs, there were no discernible shifts; in one, there was a highly variable, comparatively low voltage shift; in three, only a superficial negativity; and in the last three, the complete negative-positive dipole. The last three were from Monkey E, which was less experienced than the other two. The range of voltages was 8 to 20 μV in runs showing negative shifts and was 10 to 20 μV in runs showing positive shifts.

Lever-Press-Associated Events

Lever-press-associated data were extracted by averaging forward or backward or both from correct lever presses. Activity was averaged only from those runs that showed, on stimulus-locked averages, very significant activity after S_2 that continued through cortex into white matter. Fig. 5 shows why these special averages were computed. The figure is drawn from the superimposition of the average activity from one electrode pair on the probe (#1 referred to #2, solid line) and the average of short, square-wave code pulses given at the time of correct responses (dotted line). There is an obvious similarity between the electrophysiological data and the electromechanical data, with a 160 msec lag of the electromechanical data.

In the motor cortex on the runs showing little or no CNV, such surface-positive potentials as those illustrated in Fig. 6 were seen commonly in experienced monkeys. In the first trace on the left, the average of 16 trials with both electrodes (#2 referred to #1) still in the epidural CSF shows no significant activity. The second trace corresponds to the average with the tip 1 mm into the cortex. Each subsequent trace represents the average of 8 to 16 trials at 1 mm steps through the cortex. The numbers correspond to the number of millimeters above (+) or below (−) where surface electrode #2 is calculated to be. The traces to the right correspond to the averages from a single set of trials with the tip in the white matter and electrodes #4, #3, and #2 located +2 mm, 0 mm, and -2 mm above the surface. These traces show the surface-positive potential becoming a surface-negative

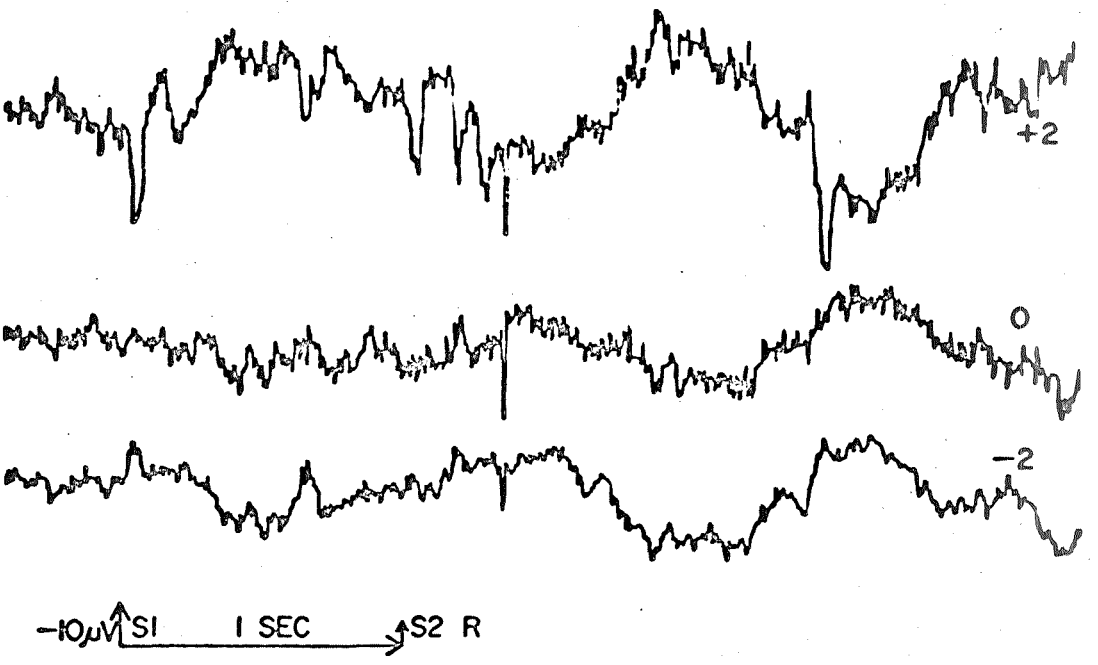


Fig. 4. Each trace is the activity recorded on a single trial. Top: electrode 4 versus 1. Middle: electrode 3 versus 1. Lower: electrode 2 versus 1. Electrode 1 is in the white matter below 4, 3 and 2, and is used as a reference. The numbers to the right correspond to the distance (in mm) above the cortical surface the active electrode is calculated to be.

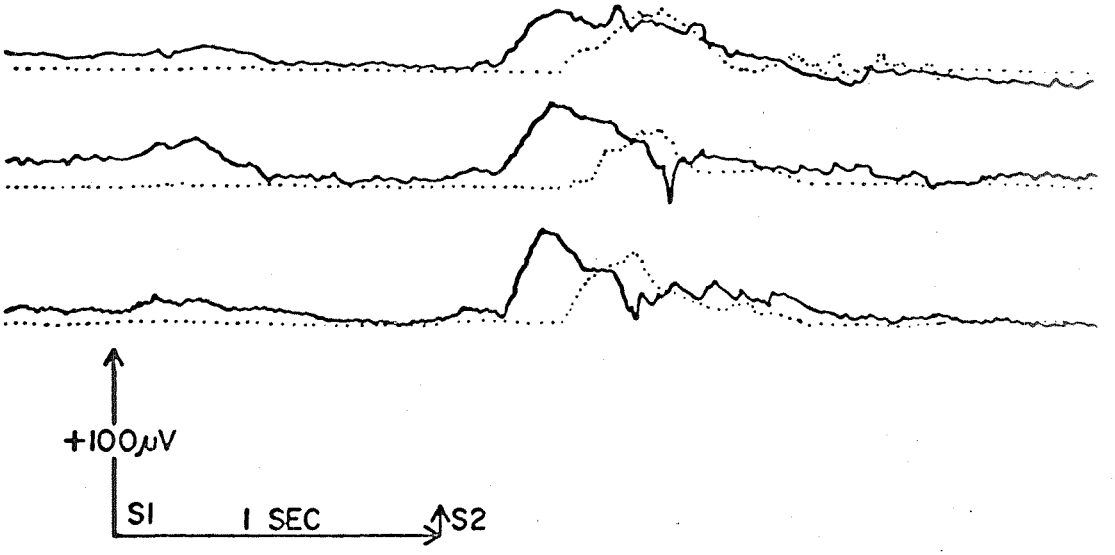


Fig. 5. Solid line is from electrode 2 referred to 1. Dotted line is from the output of a code channel monitoring correct lever presses. Each pair of curves comes from the average of 16 trials at one of three progressively deeper levels. In each case electrode 1 is in the grey and electrode 3 is in the epicortical CSF. There is an obvious similarity in the rise time of the ECG data and the electromechanogram data. Negativity of electrode 1 is up.

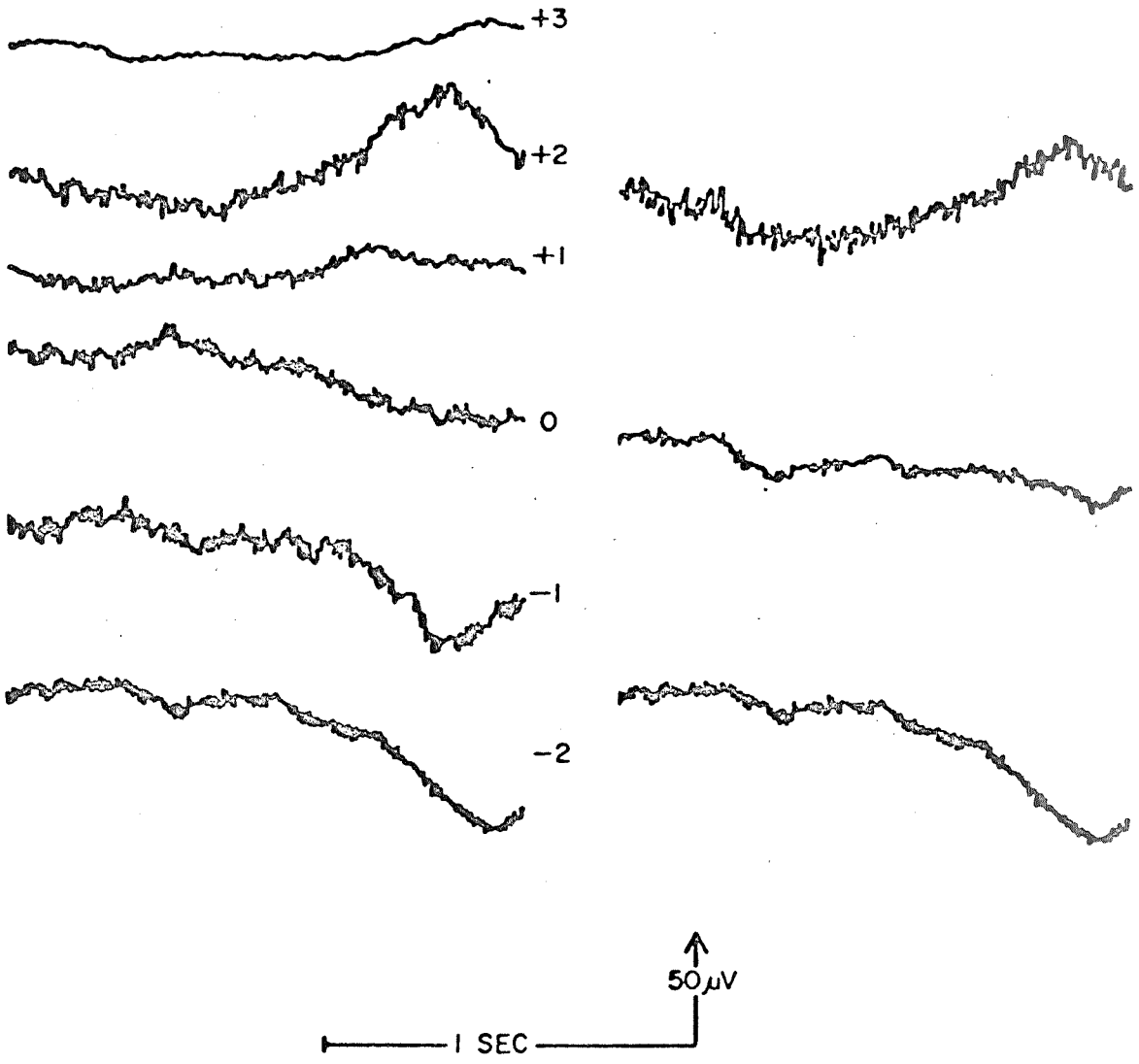


Fig. 6. Traces to the left are averages from one pair of electrodes (2 referred to 1), starting above the surface of the cortex and continuing into the cortex and subjacent white matter. The numbers between the traces correspond to the number of mm above the surface of the cortex the active electrode is placed. The traces to the right are averages from three different pairs (4-1, 3-1 and 2-1) on one set of trials with the deepest electrode (1) in the white matter and the other electrodes positioned according to the numbers between traces.

potential (relative to the subjacent white matter) as the electrode position varies from CSF to deep gray.

When the tip was referred to a distant area of white matter, the negative potential persisted to the end of the run. However, the most intense negativity is clearly nearer the more superficial electrodes than the tip, since the more superficial electrodes reflect a negativity with respect to the deeper electrode. It is possible that the reference electrodes were, in fact, recording positive shifts when the probe was at the deeper levels, or that the white matter was generating a slow-potential variation, or that there was a spread of the known negative field in the gray into the subjacent white matter. The last seems the most likely explanation, since it does not require the assumption that the white matter possesses slow-potential generators or that reference electrodes in two monkeys were near strong positive sources.

In the runs through motor cortex showing good CNVs, the termination of the CNV and the onset of the positive wave were intermingled with the slow components of the evoked response to S_2, so that the potential just described could not be distinguished from other concurrent activity. Various aspects of the activity after the lever press were easily distinguishable. There was a complex of surface-positive-negative-positive waves terminating 200 msec after the lever press. The traces in Fig. 7 are from penetrations 5 and 6 on Monkey E. Each trace represents one set of trials with electrode #4 at +2 mm, electrode #3 at 0 mm, and electrode #2 at -2 mm with respect to the surface, each referred to the tip in the subjacent white. The complex does not deteriorate or invert in run 5 (first three traces) but does deteriorate in the cortex in run 6. Also seen in these traces are some late slow potentials (surface-positive-negative) which invert in the outer 1 mm of cortex.

Premotor-cortex activity clearly associated with the lever press was confined to the post-lever-press period. Fig. 8 illustrates a small, fast potential (arrow) and a large, slow potential. The first trace is electrode #3 to electrode #1, and the second is electrode #2 to electrode #1. Electrode #1 is in the white matter, electrode #2 is very near the bottom of sulcus arcuatus, and electrode #3 is in the gray of the wall of sulcus arcuatus. Fig. 9 shows how one can superimpose the stimulus-locked (solid line) and lever-press-locked (dotted line) data and achieve a fairly good fit at the ends with differences in the time course and amplitudes of various components. Inversions of the large, slow, lever-press potential were seen to be locked to the inversions of the fast lever-press potential. The fast potential bears a remarkable similarity to the click-evoked potential in time course, polarity, and levels of

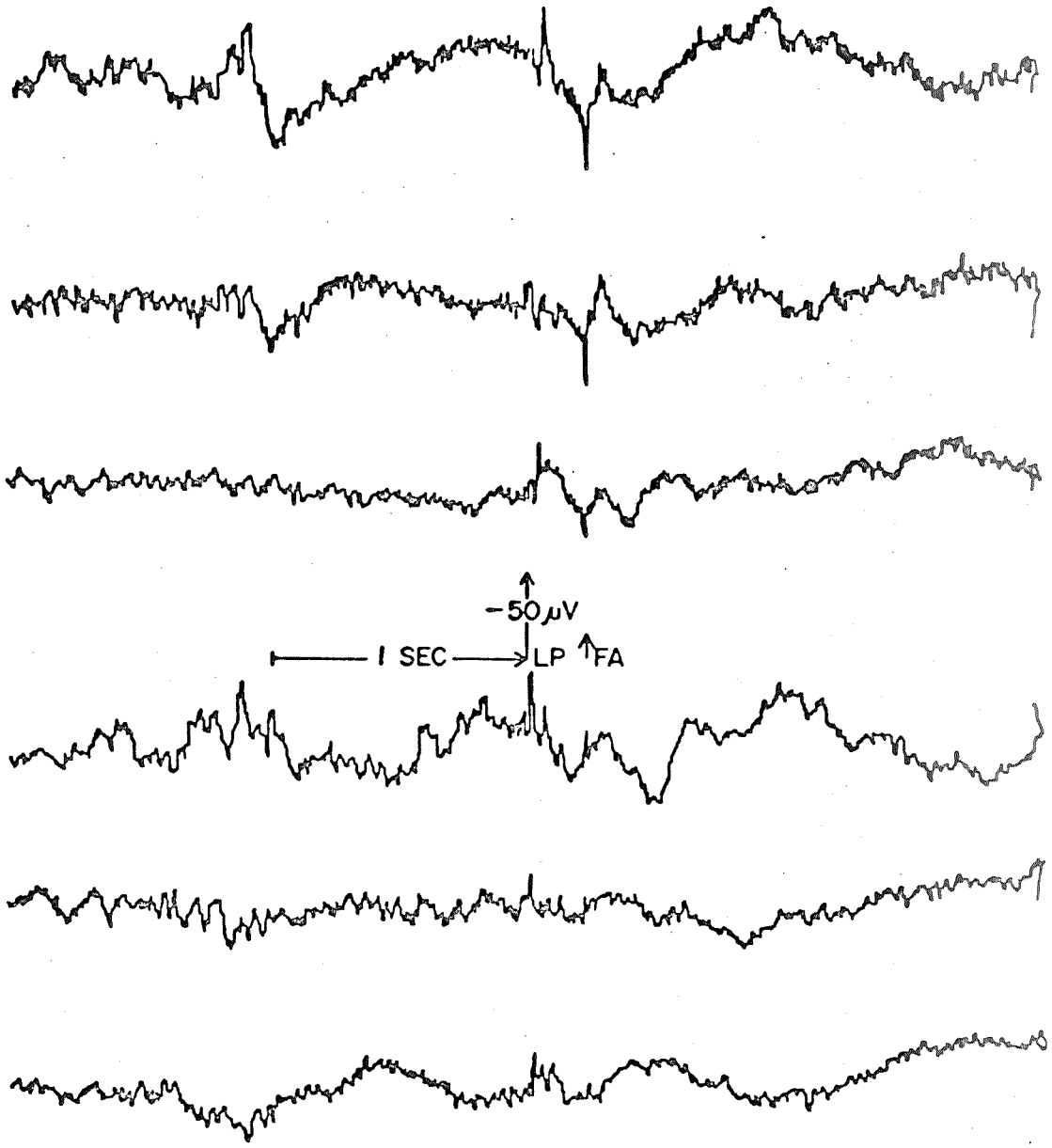


Fig. 7. Upper three traces: run 5, Monkey E, each an average of 16 trials with the reference electrode (1) in the white matter and the active (4,3 and 2) +2, 0 and -2 mm above the surface. Lower three traces are the analogous averages from run 6, Monkey E. FA = feeder artifact; LP = leverpress.

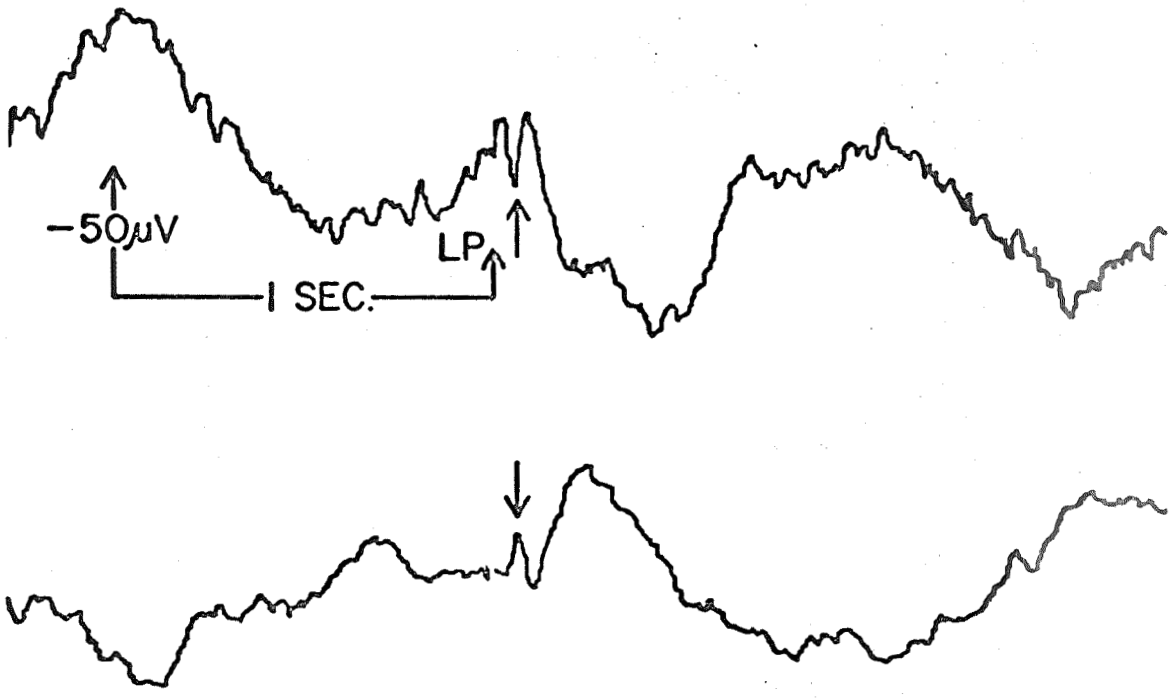
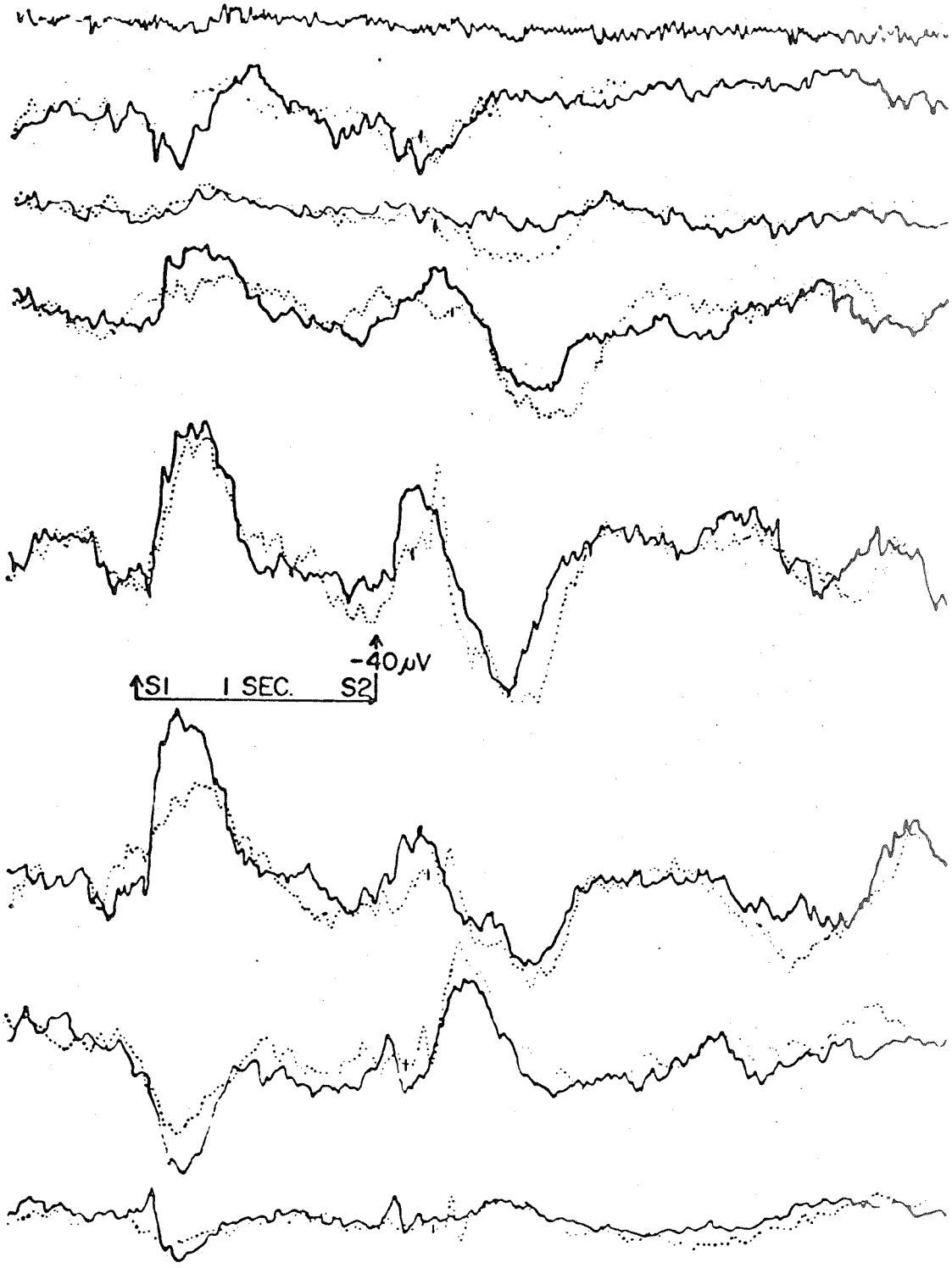


Fig. 8. Averages of a set of trials. Upper trace is the activity between electrodes 3 and 1. Electrode 3 is in the wall of sulcus arcuatus; electrode 1 is in the white matter below the base of sulcus arcuatus. The lower trace is the activity between electrodes 2 and 1. Electrode 2 is in the grey at the base of sulcus arcuatus. Positivity at 1 is up. LP = leverpress artifact. Arrow indicates fast potential presumed to be evoked by the "click" of the microswitch closure.

Fig. 9. Solid line: averages locked to stimulus. Dotted line: averages locked to leverpress. Traces are drawn from averages of sets of trials of run 2, Monkey E. Electrode 3 is referred to electrode 1. The first trace is activity before penetration of the cortex by electrode 1. In the last trace electrode 1 is in the white matter below the base of sulcus arcuatus and electrode 3 is in the deep grey of the base of the sulcus.



inversion in Monkey E, penetrations 1 through 3. There was an audible click associated with the closure of the microswitch. One can easily speculate that this sound evoked the fast potential.

Summary of Electrophysiologic Findings

In summary, evoked potentials were found over wide areas of cortex and were absent in neighboring areas. Both the slow evoked potentials and the fast evoked potentials had greater amplitude in the premotor runs.

The CNVs were found to be distributed primarily in premotor areas. Table II summarizes amplitude data for CNV runs showing CNVs or the intracortical positive-potential correlate or both.

Lever-press-associated potentials of three types were studied. In the motor region of overtrained monkeys, there was a surface-positive wave peaking before the lever press, arising after S_2. There were rapid complexes and slow waves after the lever press in motor and premotor areas. The rapid complexes in the premotor areas bore a remarkable resemblance to click-evoked potentials.

All superficial stimulus-related potentials recorded either reversed polarity or became unrecognizable as the electrode passed through the outer 1 mm of cortex, except in one case when the inversion was complete 2.5 mm into the cortex.

DISCUSSION

The origins of slow-potential (SP) shifts can be assumed to be vascular, glial, or neuronal. The first of these is considered seriously for slow-potential shifts with a long period (10 sec or more), but is not likely to play much of a role in the shorter-duration SP shifts recorded in the present study. There is much evidence which indicates that glia interact with neurons in a functional manner and that they have specialized bioelectric connections. Slow depolarizations of glial-membrane potentials have been demonstrated during thalamic stimulation (Karahashi and Goldring, 1966) and have been shown to correlate well with surface-negative SP shifts (Castellucci and Goldring, 1970). Glial-mediated shifts are characterized, however, by the absence of any reversal of polarity in a laminagraphic analysis (Castellucci and Goldring, 1970), so their role in the generation of the SP shifts reported in the present study

Table II. Mean Number of Penetrations ± SD (in uV)

	Premotor	Precentral
FEP	45 ± 23	25 ± 20
	n = 7	n = 5
SEP	45 ± 21	24 ± 17
	n = 6	n = 6
CNV -shift	21 ± 16	13 ± 7
	n = 8	n = 8
CNV +shift	17 ± 8	15 ± 10
	n = 7	n = 4

FEP = Fast evoked potential
SEP = Slow evoked potential
CNV = Contingent negative variation
\- = Negative
\+ = Positive
n = Number of penetrations showing such a shift

must not be a major one.

The neuronal model for surface slow potentials presumes that neurons generate surface potentials by one of several means: Goldring et al. (1958) suggested depolarization of the cell bodies (and presumably dendrites); Sugaya et al. (1964) invoked superficial postsynaptic depolarization; and others (Li and Chow, 1962; Pollen and Sie, 1964) have noted that somatic hyperpolarization is associated with surface-negative shifts. By presuming alterations in neuronal activations, one can explain slow-potential shifts associated with increasing levels of consciousness (increased input from subcortical sources, pre- and postsynaptic depolarization, and compensatory somatic hyperpolarization, all yielding a surface-negative shift) as well as potential shifts occurring secondary to hypoxia and hypoglycemia (lack of energy input to maintain membrane potential results in depolarization and the consequent negative shift described by O'Leary, 1963) and the slow-potential shifts associated with substances that produce neuronal irritability (e.g., depolarization and negative shifts caused by hypercapnic acidosis).

The above considerations, plus the known properties of the neuronal membrane, lead us to the conclusion that the SP shifts recorded in the type of stimulus-response paradigm used in the present study are generated primarily by neural activity. Specifically, surface-negative, depth-positive potentials correspond to somatic IPSPs or repolarization; a surface-negative potential alone corresponds to dendritic depolarization; and surface-positive, depth-negative potentials correspond to somatic EPSPs, synchronized corticofugal or afferent volleys of action potentials, and dendritic repolarization. Dendritic IPSPs have not been demonstrated.

The fast evoked potentials predominantly found in premotor cortex in the present study fit the Creutzfeldt model of axosomatic input generating the primary positive-negative complex, with or without axodendritic activity coincident with the primary negative wave. The slow, surface-positive waves underlying the fast components probably represent spread to the dendrites of IPSPs. Their low voltage, variable latency and form, and the overshadowing CNV made it difficult to be sure if the slow component inverted or merely faded on penetration of the outer 1 mm of cortex. The usual finding of S_1's fast evoked potential being greater than S_2's evoked potential came as no surprise, since Walter, in his first article on the CNV (Walter et al., 1964), noted such a relationship. It suggests, however, that the cortex was less susceptible to EPSPs late in the S_1-S_2 interval or that the fast evoked-potential information was less likely to be forwarded to

frontal areas. McAdam (1968) noted that the latency of later components of shock-evoked potentials superimposed on S_1-S_2 intervals had shorter latencies and lower amplitudes, findings prompting the conclusion that the CNV correlated with increased excitability.

Discovery that the CNV had a superficial negative component and an intracortical positive component with respect to subjacent white matter suggests that in the areas where both were present there was simultaneous depolarization superficially and hyperpolarization in the depths. Olds et al. (1969) described rats in a similar conditioning situation as being in ". . . a state of intense readiness." The occurrence of areas of isolated hyperpolarization in the depth or superficial depolarization can be attributed to differential function of different areas of cortex. The absence of shifts in motor cortex of well-trained monkeys (Borda, 1970) is best explained as a learning effect. Early in conditioning, the entire frontal lobe is primed, including the motor cortex, with excitatory and inhibitory influences in a dynamic balance until the arrival of S_2, when, through disinhibition or axosomatic excitation or both, the organism discharges the tension in the form of a lever press, decision, or release from uncertainty. After hundreds of trials, the organism develops more discrete inputs to the motor cortex, probably axosomatic excitatory inputs, that enable that region of cortex to be preserved from the intense expenditure of energy in expectancy. The premotor regions, however, continue to receive, process, and transmit information relevant to stimuli and the response.

The picture of frontal-lobe function that emerges from this study of the CNV is that of a region where stimuli are filtered, drive-related stimuli are selected, information is stored (possibly elsewhere in the brain), and reaction patterns are evaluated for effectiveness. Knott and Irwin's (1968) theory that intention to respond, stress, and anxiety are all subserved by the same mechanism fits well with the concept that the CNV represents an intensified balance between excitatory superficial inputs and inhibitory somatic inputs. There is certainly a maximum amount of postsynaptic inhibitory input that any cell body can respond to, and this is balanced by maximal excitatory input to the dendrites. An important conclusion is that stress is modulated by the same tense balance of inhibition and excitation as anxiety and intention.

The only depth-negative CNV measured among the 18 penetrations was deep in the wall of sulcus principalis. The negative shift ended very abruptly with the onset of S_2, and a late negativity of about 30 μV eventually developed (Fig. 10). An explanation for this area's excitation is that it is the source of the axosomatic inhibition found in other areas of cortex and perhaps reinforces the behavioral set as the reward is appreciated. It is suggested that

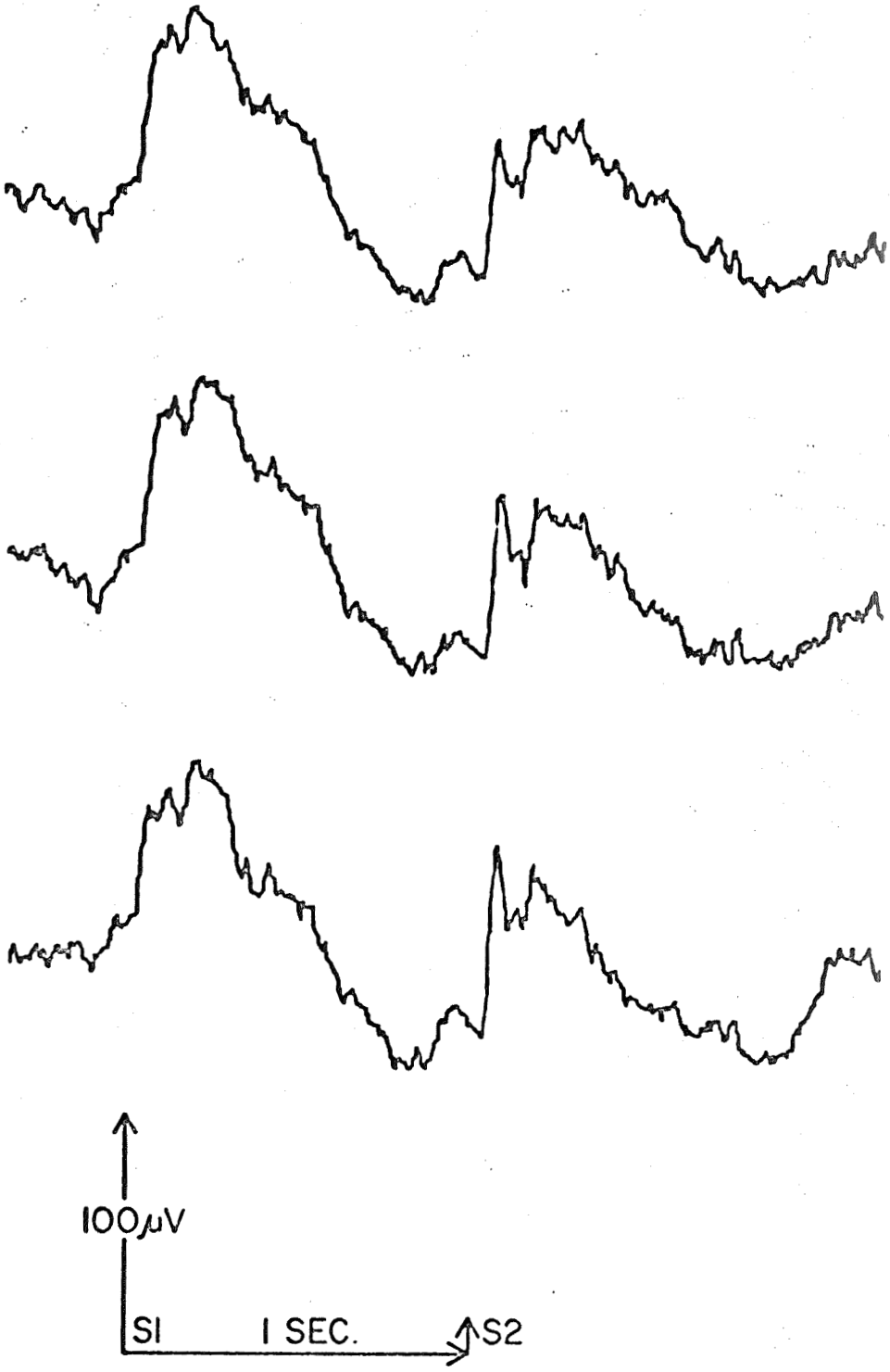


Fig. 10. Average activity from electrode pairs 4-1, 3-1, and 2-1. Electrode 4 is in the grey-white border of lateral superior frontal gyrus (SFG); electrode 3 is in the subjacent white; electrode 2 is in the grey-white border of the superior wall of sulcus principalis; and electrode 1 is 1.5 mm from the surface of the superior wall of sulcus principalis. Positive activity at electrode 1 results in an upward deflection of the trace.

this area of cortex, along with its subcortical connections, should be the focus of further exploration into the cerebral mechanisms that control the CNV.

SUMMARY

Research conducted during the past year has contributed greatly to our understanding of the origin and physiological significance of cerebral slow potentials. Of primary interest was elucidation of the origin of the contingent negative variation (CNV), a slow potential which appears to be related specifically to the mechanisms underlying attention and alertness.

Results summarized in previous reports to NASA suggested that the CNV is not a unitary potential but rather a composite of several distinct potentials of different time course and topographic distribution. The recent work confirms this hypothesis and extends it to the intracortical level. The homogeneous wave with widespread areal distribution one records with scalp electrodes loses this appearance when recorded with the smaller, intracortical probe. The classic surface-negative wave is seen to be composed of many small patches of negativity, each of which may take any of three forms: 1) a surface-negative/depth-positive dipole across the cortex, 2) only superficial negativity, or 3) only positivity in the deeper layers of the cortex. This suggests that the source of the CNV is not, as is generally assumed, a widespread partial depolarization of the apical dendritic feltwork, but rather consists of multiple discrete generators scattered throughout the cortical mantle of the frontal lobe. Each of these generators is viewed as being the locus of a phasic increase in "neural tension," an increase in superficial dendritic excitation and somatic inhibition.

Yet to be answered are the questions of what subcortical systems predominate in the elicitation of these SP shifts, and whether specific cortical regions can be identified where the electrical activity consistently reflects mobilization of these systems. Once these questions are answered, techniques can be developed whereby the integrity of the neural systems underlying attentiveness may be assessed.

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THE EFFECT OF ALTERED DRIVE STATES ON THE CONTINGENT NEGATIVE VARIATION (CNV) IN RHESUS MONKEYS[1]

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A phenomenon that appeared to be the first known electrophysiological correlate of a specific mental process was recorded from human subjects during presentations of associated stimuli by Walter *et al.* (1964a). This "prolonged vertex negative wave" was called the "contingent negative variation (CNV): an electro-cortical sign of sensori-motor association in man".

Considerable work has been done in the past 5 years in attempts to determine the physiological and psychological variables that affect this phenomenon. Motivation (Irwin *et al.* 1966; Walter 1966; Cant and Bickford 1967; Rebert *et al.* 1967), expectancy (Walter *et al.* 1964b), attention or alertness (Walter 1966; Hillyard and Galambos 1967; Low *et al.* 1967; Tecce and Scheff 1969), and conation (Low *et al.* 1966b) have been suggested as being involved in elicitation or maintenance or both of the CNV. Rebert *et al.* (1967), in assessing the role of motivational variables in the production of the CNV, concluded that this slow potential may reflect "cerebral changes related to the general drive state of the organism".

Low *et al.* (1965) demonstrated an electrical event in rhesus monkeys analogous to the CNV; this led to the use of these animals as subjects in the present study of the relationship between drive state and CNV wave form. Although the concept of drive is complex and physiologically imprecise, it was assumed for the purposes of the present study that deprivation of food would produce an increased hunger drive and higher motivation to respond for appropriate reinforcement.

Previous reports concerning the distribution of the CNV in man (Walter *et al.* 1964b; Low *et al.* 1966a) have been limited to the relatively early stages of training. The present study extends this analysis to infrahuman primates and additional stages of training.

METHODS

Four female rhesus monkeys (*Macaca mulatta*), naive to operant conditioning and weighing 3–4 kg, served as subjects. Each was placed in a restraining chair (Foringer) for a period of 1–2 h at the same time every day. Water was provided *ad libitum*.

Conditioning

Each subject was first trained to press a lever in order to receive banana-flavored nutrient pellets (300 mg, CIBA); all other food was withheld until this response was well established. A discrimination task was then introduced; only lever-presses which occurred during the presentation of a 2500 c/sec tone were reinforced. Initial training was considered complete when the tone could be shortened to 4 sec and the subject's responses remained primarily confined to its presentation. As each subject reached this criterion (4–10 days), training was interrupted and normal diet was reinstated for a period of 1 week, after which elec-

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trodes were implanted epidurally and subcortically.

Electrodes

Wire of 90% platinum/10% iridium alloy was used for all epidural and subcortical (reference) electrodes. These were insulated, except for the recording surface, with Epoxylite and then "platinized" (Schwan 1963) by plating platinum-black from a solution of platinum chloride. The epidural electrodes consisted of 0.016″ diameter wire wound in coils of two complete turns 3.5 mm in diameter. Subcortical reference electrodes were straight wires with a 1.5–2.0 mm uninsulated tip. The electrodes were soldered to the terminals of a subminiature connector (Continental) which was anchored to the skull with stainless steel screws and dental acrylic (Shur-Weld).

The implantation procedure was done under aseptic conditions: subjects were anesthetized by intravenous sodium pentobarbital. In three subjects (M-1, M-3 and M-4), surface electrodes were implanted 30 and 15 mm anterior and 5 mm posterior to the inter-aural line, 5–8 mm from the midline over the left hemisphere. These were designated left frontal, left central and left posterior, respectively, and corresponded to left superior frontal or middle frontal gyrus, an area just anterior to the motor strip, and the inferior parietal gyrus. In each subject, the three electrodes were all referred to a single, subcortical (white matter) reference located 3 mm anterior to the inter-aural line and 7 mm from the midline, inserted to a depth of 7 mm below the surface. The fourth subject (M-5) had transcortical paired electrodes, as a better control for eye movement artifacts (see Wurtz 1965), at locations analogous to those above designated frontal and central.

Testing procedure

After recovery from the operation, each subject was placed on a food-deprivation schedule and begun on a final discrimination task. The tones gradually were shortened to 1 sec and were now preceded by a "click" (S_1), the interval between the two being 1.1 sec. By pressing the lever during the tone (S_2), the subject could receive a food pellet, but such a response now terminated the tone so that only one pellet was available on each trial. Additionally, lever-presses in the click-tone interval aborted the sequence so that no tone followed. Thus, the subject had to wait for the tone onset before a response would produce reinforcement, and the paradigm now approximated that used in previous studies of the CNV in man. Solid-state programming equipment (BRS-Foringer) controlled the stimulus-presentation and reinforcement contingencies.

Recording procedure

Recordings were begun as the above discrimination task was introduced; up to 100 trials were presented aperiodically in daily sessions of 45 min to 1 h, with a minimum inter-trial interval of 8 sec. Capacitor-coupled amplifiers (Brush Instruments) amplified brain electrical activity, using time constants of at least 2.3 sec and filters which attenuated frequencies above 50 c/sec. System noise level was determined to be under 3 μV peak-to-peak. The amplified signals were taped with an FM recorder (Precision Instruments, PI-400) at 18 cm/sec for later analysis with a LINC-8 digital computer. The programming device placed a code on tape 0.7 sec before the click in each trial.

Trials were "averaged" in blocks of 10 using a technique previously described (Borda and Frost 1968). A 4 sec epoch of EEG, beginning with each trial's code onset, was digitized at a rate of 64 points/sec and stored on digital magnetic tape. Amplitude histograms were then constructed for the sample of 10 voltages occurring at each of the 256 points in the 4 sec epochs, and the median voltage and variance were calculated for each histogram. Plots of these variances and median voltages were then written out on a plotter (Houston Instruments). To reduce the possibility of averages reflecting movement artifact, any block showing a variance which was higher during the S_1–S_2 interval than during the pre-stimulus period was excluded from further analysis. Blocks showing an over-all variance that deviated to a great extent from those typically recorded were also eliminated.

As an objective measure of the relative size of any negative shifts (CNVs) occurring in the S_1–S_2 interval, the area between an extension of the pre-stimulus baseline and the portion of the averaged signal between the evoked potentials was measured. This has been previously suggested as an alter-

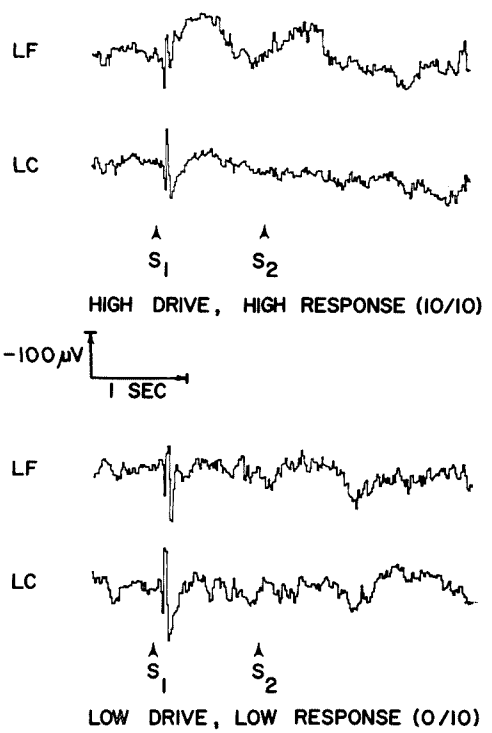

Fig. 1
Brain electrical activity recorded early in training (subject M-1) during different drive states. Traces are averages (median plots) of 10 trials. S_1 = click, S_2 = tone onset; LF = left frontal recording site, LC = left central.

native to peak amplitude or amplitude at a given latency after the first evoked potential (Low and McSherry 1968). Such areas were computer-calculated by first obtaining the average voltage of the pre-stimulus interval, then summing all voltages more negative than this occurring during a 1 sec period after the first evoked potential.

Three subjects were tested under the condition of high drive (total deprivation of food except for what was obtained in the test sessions) for 2–3 weeks, then under low drive (normal diet) for 1–2 weeks, and once again under high drive for 1 week to control for training effects. Recording sessions were suspended for 1 week at the beginning of each new test condition. The fourth subject (M-5), with transcortical electrodes, was tested under the high-drive condition alone for a period of 3 weeks.

To eliminate the possibility that any slow potentials recorded under these conditions resulted wholly or in part from events surrounding the mere performance of a specific motor response, recordings were obtained from one subject (M-5) freely responding without cue or reinforcement. A short code pulse was placed on tape coincident with the subject's response; averages were then obtained of the 4 sec epoch preceding the response by playing the tape backward, triggering at the code, and averaging as described above.

RESULTS

Histological examination of the reference electrode tracks indicated that the subcortical electrodes in two subjects (M-3 and M-4) passed through white matter and entered a convolution of cortex. These cannot, then, be considered truly "inactive", and allowances for this must be made in interpreting the results from these subjects.

Neither positive nor negative shifts were noted at any time during the S_1–S_2 interval in recordings obtained from the posterior electrode site. CNVs were recorded at frontal and central electrode sites in all subjects. The average electrical activity typically accompanying the stimulus–response paradigm in the early stages of conditioning under high- and low-drive conditions is shown in Fig. 1.

Effect of drive state

Mean CNV areas (calculated from median plots as described above) for three subjects under

TABLE I

Mean CNV areas: different drive states

		High drive	Low drive	Significance
M-1	LF	1424.9 (13)	657.6 (15)	S
	LC	404.8 (19)	401.1 (20)	NS
M-3	LF	1609.4 (24)	574.8 (21)	S
	LC	280.6 (24)	487.6 (21)	S
M-4	LF	1421.9 (23)	724.3 (14)	S
	LC	289.0 (22)	327.5 (15)	NS

Note: Numbers in parentheses indicate number of blocks of 10 trials for which mean was calculated. Areas are proportional to $\mu V \cdot$ sec at a ratio of approximately 25:1. Significance (S) or non-significance (NS) determined with Mann-Whitney U test (at 0.05 level). LF = left frontal recording site, LC = left central.

the two drive conditions are given in Table I. The numbers of blocks of 10 trials from which the mean areas were calculated are indicated in parenthesis. Statistical significance of the differences in the means of these areas was determined by using the Mann-Whitney U test (Siegel 1956) with a significance level of 0.05. Clearly, CNVs from frontal sites were larger under the high-drive condition. CNVs were recorded initially from central sites as well, but these apparently were unrelated to the drive condition. Although recordings from frontal areas are usually suspected of contamination from potentials generated by vertical rotation of the eyes, a comparison of recordings from these subjects and the one with transcortical frontal electrodes (M-5) ruled this out. The negative shifts recorded from the frontal sites of M-5 were of almost identical wave form and amplitude as those recorded from the other subjects at a comparable stage of conditioning.

Effect of performance level

The imposed drive condition apparently was not the only factor affecting the CNV. When blocks were separated only on the basis of percentage of correct responses, regardless of diet condition, significant differences in CNV areas were also obtained. Mean areas separated in this manner are listed in Table II. The only way of excluding the effect of performance would be to compare blocks of trials which had identical percentages of correct responses but which were recorded under different diet conditions. Unfortunately, too few such blocks occurred to permit a statistically valid comparison, since the subjects' response levels usually paralleled the drive conditions. By separating trials recorded during a single session according to the presence or absence of a correct response, it was possible, however, to exclude the effect of drive. The average activity accompanying these individually selected trials is shown in Fig. 2, where it is apparent that a larger negative shift is associated with the "response" condition even when, as must be assumed, the effects of diet are held constant.

TABLE II

Mean CNV areas: different response levels

		High response	Low response	Significance
M-1	LF	1136.4 (20)	646.8 (5)	S
	LC	405.1 (28)	467.2 (8)	NS
M-3	LF	1616.7 (22)	528.0 (20)	S
	LC	287.4 (22)	439.7 (21)	S
M-4	LF	1605.7 (20)	631.2 (17)	S
	LC	246.2 (20)	347.1 (18)	NS
M-5	LF	1161.7 (10)	580.2 (5)	S
	LC	915.9 (10)	483.2 (5)	S

Note: Blocks included in high response column were those in which the subject responded on at least 7 of the 10 trials; low response includes those in which responses were made on no more than 3 of the 10 trials. For definitions of abbreviations, see Table I.

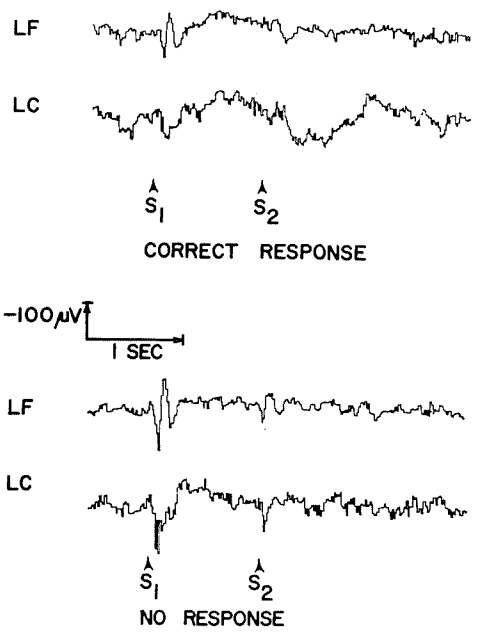

Fig. 2

Brain electrical activity recorded early in training from subject with transcortical electrode placements (M-5). Traces are averages (median plots) of specially selected trials. One block of 10 trials contained only those on which a "correct" (reinforced) response occurred; the other, those on which no responses occurred. For abbreviations, see Fig. 1.

CNV area as a function of training

Comparison of frontal CNVs recorded during high-drive conditions early and late in training indicated that this potential changed very little in amplitude or wave form with time. Those re-

corded from the rolandic regions, however, showed a decline in amplitude (area) with time even though performance levels remained high. Late in training, central CNVs were more frequently seen at the beginning of a food-deprivation condition but rarely at amplitudes as large as those recorded in earlier sessions.

Comparison of frontal and central CNVs

The data presented above suggest that the potentials recorded from frontal and central sites occur independently; this was further supported by comparison of relative amplitudes of these potentials in various 10-trial blocks. All possible combinations were found: frontal negative shifts could be larger than central, or central larger than frontal; large shifts might be present at both sites, or shifts totally absent at both.

Control for movement artifact and "pre-motor" potentials

The elimination of blocks of trials that evidenced high signal variance, as described in the Methods section, should have removed trials containing artifacts owing to the subjects' movements in the restraining chair. Analysis of records obtained from subject M-5 during a period of uncued, unreinforced responses further indicated that very little, if any, consistent artifact was associated with the lever-pressing response itself. The average electrical activity preceding 10 such responses is shown in Fig. 3. Clearly, no negative potentials of magnitude approximating the 25–100 μV shifts recorded in this subject are in evidence. There is, if anything, a slight *positive* deflection of the baseline during the 1 sec interval preceding the response in the traces from the frontal leads.

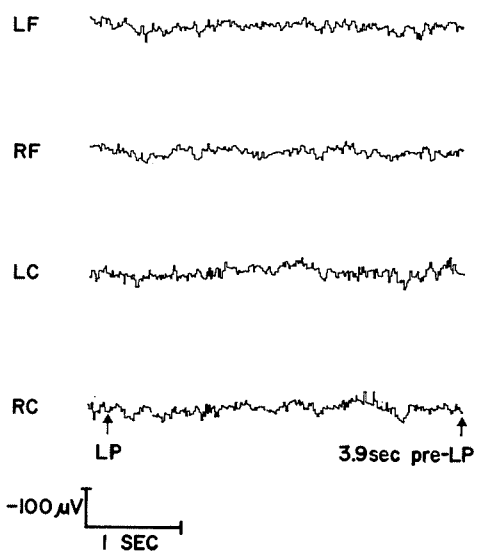

Fig. 3

Brain electrical activity (subject M-5) preceding spontaneous, unreinforced responses. Averages of ten 4 sec epochs were obtained by playing tape backward and triggering at the lever-press (LP). LF = left frontal transcortical electrode pair, RF = right frontal, LC = left central, RC = right central.

DISCUSSION

Whereas the present results do not refute the hypothesis that the CNV reflects "cerebral changes related to the general drive state of the organism", it would appear that "drive" is an inadequate term to explain the complex physiological processes that must underlie the production of this potential. The early attempts at describing the psychological state with which the CNV was associated emphasized either the signal-detection or motor-response aspects of the conditioning task. The terms expectancy and conation illustrate these attempts, and both must still be considered applicable if not totally adequate.

It was assumed in the present study that gross alterations of food intake would be associated with different drive levels. An organism's motivation to respond at any given instant is, however, affected by multiple drives and many subtle variables. Statistical theories of learning, like that of Estes (1959), avoid mention of these subtle influences and speak only of the net *probability* of an organism's responding in a given situation. In the present instance, then, food deprivation could be said to alter the subjects' *probability* of detecting, or attending to, the cues and subsequently making a correct response. This suggests that attention is the common process underlying correct performance and the presence of a CNV, a conclusion which was reached by Tecce and Scheff (1969) in a study of the effect of distracting stimuli on the CNV.

The CNV recorded from the human scalp usually has been described as a single potential maxi-

mal either at the vertex or over the frontal regions (Walter *et al.* 1964b, 1965; Low *et al.* 1966a). Cant and Bickford (1967), however, found it necessary to postulate a non-unitary process underlying the CNV to explain discrepancies in recordings from frontal and central areas; the present findings indicate that such might be the case. Although there may be differences in the topographic distributions of CNVs recorded in man and monkey, it is possible that the CNV as previously recorded in man represents the summation of at least two potentials of disparate origin. As mentioned above, examination of individual 10-trial blocks suggested that the relative amplitudes of central and frontal CNVs were unrelated. This finding is obscured in the data presented in Tables I and II since these are averages of many such blocks. These figures do show, however, that one of the subjects (M-3) had, on the average, central CNVs which were significantly larger during the low-drive condition than during high drive. This may have been due to the fact that this subject's reference electrode was placed within a convolution of cortex, and may mean that under high drive a larger area of cortical surface was shifting negatively so that the reference electrode was, in this instance, also active. The other subject with a misplaced reference (M-4) also showed this trend, but not to a statistically significant level.

In addition to the CNV, slow, surface-negative potentials distributed over the central regions of man's scalp have been reported to occur incidental to sensory stimulation or preceding voluntary responses. The vertex non-specific response, elicited by stimuli of any modality, has been reported to increase in amplitude during tasks requiring a decision (Davis 1964). Slow potentials that precede uncued motor responses also have been recorded from the vertex or over rolandic cortex (Kornhuber and Deecke 1965; Gilden *et al.* 1966). The motor potential (MP) consists of a surface-negative wave that precedes muscle movement by up to 1 sec and is distributed over the motor strip contralateral to the muscles used in the response (Vaughan *et al.* 1968). The *Bereitschaftspotential* or readiness potential (RP) described by Kornhuber and Deecke (1965) likewise precedes uncued responses but has been reported to be bilaterally symmetrical over pre- and postcentral regions (Deecke *et al.* 1969). The RP also has been shown to increase in amplitude with increased levels of motivation (McAdam and Seales 1969). Recorded from the frontal regions, however, the RP has a surface-positive polarity (Deecke *et al.* 1969) and must, therefore, have a distribution in man differing from that of the CNV.

The surface-negative potentials recorded from the frontal regions in the present study might appear to be related to the RP or MP since they invariably preceded correct responses but, as can be seen in Tables I and II, these potentials were usually much larger than those recorded just anterior to the motor cortex. Such a distribution precludes the possibility of their arising from activation of units in the motor strip. Although it is possible that the distribution of the RP differs in man and monkey, it seems more probable that the frontal dominant potentials are analogous to the CNV in man. Conversely, the negative potentials recorded at the central electrodes sites are topographically similar to the RP and MP, but since they could be present even in the absence of a response (see Fig. 1), they would seem to be related more to the vertex non-specific response. The lack of any significant negative potentials time-locked to the response itself (Fig. 3) is further proof that both frontal and central shifts were unrelated to the MP or RP. It is tempting to speculate that the slight positive shift in the frontal traces preceding spontaneous responses might be the monkey equivalent of a *Bereitschaftspotential*, but too few trials were averaged to make any such conclusion. These results are included only as they lend further support to the argument that the negative potentials recorded from frontal and central electrode sites were not simply response-related.

Activation of different subcortical systems was suggested by Chiorini (1969) as the basis for the two distinct types of slow potentials he observed in cat cortex during classical conditioning. He attributed equal bilateral potentials to a diffuse system such as the midbrain reticular formation; less widespread, bilateral potentials of unequal amplitude were thought to result from activation of a more "localized" system. The two independent surface-negative potentials described in the present study must also arise from activation of different subcortical systems. The topographic

distribution and time of appearance in conditioning indicate that the more central of these potentials may arise from a diffuse system such as Chiorini proposed. A more localized system, arising in the anterior one-third of the intralaminar system of the thalamus and projecting to the orbitofrontal cortex, has been traced in the rat brain by Scheibel and Scheibel (1966, 1967). The frontal-dominant potential present in primates may well arise from activation of a medial thalamus-to-frontal cortex system such as this, a system which may subserve a basic mechanism of selective attention.

SUMMARY

The contingent negative variation (CNV), a vertex-negative slow potential that occurs in the interval between two stimuli when the second is made the cue for a motor response, has been linked in the literature with psychological concepts such as drive, motivation, expectancy, attention, preparation set and conation.

The CNV was studied in rhesus monkeys using epidural and subcortical platinized-platinum electrodes and long time constant, capacitor-coupled amplifiers. The study was designed to determine the effects of different levels of drive, as determined by normal diet or total deprivation of food, on the amplitude of the CNV in subjects trained to respond for a food reward. Recordings made over several months from four subjects led to the following conclusions:

1. At least two independent negative slow potentials are present during the course of conditioning a task of this type; one is present over the frontal regions and the other over more central areas. The two potentials may sum to produce the CNV typically recorded at the scalp.

2. The central-dominant potential decreases in amplitude with overtraining, appearing most commonly after periods of food deprivation. The frontal-dominant potential reaches its maximal amplitude early in training and continues to be present as long as the subject maintains a high level of performance.

3. The CNV is not related in any simple manner to drive. It is suggested that food deprivation acts to increase the probability of the subject's detecting the cues and subsequently making a correct response, and that the frontal-dominant potential may, therefore, be the electrical sign of activation of a basic mechanism of selective attention subserved by non-specific thalamo-cortical pathways.

RÉSUMÉ

L'EFFET D'ÉTATS PULSIONNELS ALTÉRÉS SUR LA VARIATION CONTINGENTE NÉGATIVE (CNV) CHEZ LES SINGES RHÉSUS

La variation contingente négative (CNV), potentiel lent négatif au niveau du vertex qui survient dans l'intervalle entre deux stimuli quand le second sert de consigne à une réponse motrice, a été reliée dans la littérature à des concepts psychologiques tels que pulsion, motivation, attente, attention, préparation à l'action et cognition.

La CNV est étudiée chez les singes rhésus à l'aide d'électrodes épidurales et souscorticales au platinum-platiné et d'amplificateurs à longues constantes de temps à couplage de capacité. Le but de cette étude est de déterminer les effets de différents niveaux pulsionnels créés par une diète normale ou une privation totale de nourriture, sur l'amplitude de la CNV de sujets entraînés à répondre pour obtenir une gratification alimentaire. Ces enregistrements poursuivis sur plusieurs mois chez quatre sujets ont conduit aux conclusions suivantes:

1. Deux potentiels lents négatifs indépendants au moins existent au cours du conditionnement d'une tâche de ce type; l'un s'observe au niveau des régions frontales et l'autre au niveau d'aires plus centrales. Ces deux potentiels peuvent s'additionner pour produire la CNV enregistrée classiquement au niveau du scalp.

2. Le potentiel qui domine au niveau des régions centrales diminue d'amplitude avec le surapprentissage, apparaissant plus habituellement après des périodes de privation de nourriture. Le potentiel à dominance frontale atteint son maximum d'amplitude précocément au cours de l'apprentissage et continue à exister aussi longtemps que le sujet maintient un niveau élevé de performance.

3. La CNV n'est pas liée de façon simple à la pulsion. Les auteurs font l'hypothèse que la privation de nourriture agit de façon à accroître la probabilité pour le sujet de détecter les

consignes et par conséquent de faire une réponse correcte; le potentiel à dominance frontale peut ainsi être le signe électrique de l'activation d'un mécanisme de base d'attention sélective soustendue par les voies thalamo-corticales non spécifiques.

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TECHNICAL CONTRIBUTION

A HEAD-HOLDING DEVICE FOR REPEATED MICRO-ELECTRODE STUDIES IN MONKEYS DURING OPERANT RESPONDING[1]

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The recording of single-cell activity from the brains of conscious animals has provided significant information about the neural bases of sensation, higher integrative functions, and observable behavior (Hubel 1959; Jasper *et al.* 1960; Buchwald *et al.* 1966). The recording techniques employed by most researchers have been based upon the use of either semi-permanently implanted, fixed-position electrodes (Strumwasser 1959) or a combination electrode holder and driver unit attached to the skull (Ricci *et al.* 1957; Hubel 1959; Davis and Tallow 1966; Evarts 1968).

Our research goals required a micro-electrode recording system that would: (a) be adaptable for use in large (rhesus, cynomolgus) monkeys; (b) allow repeated experimental sessions with return of the animal to its home cage between recording periods; (c) provide a means for rapid replacement of damaged glass micro-electrodes; (d) hold the animal's head rigidly in relation to the micro-electrode carrier and driver while leaving its limbs free to perform a variety of tasks; (e) provide a means for controlling brain pulsations during the recording session as well as a means of reducing the flow of spinal fluid from the subarachnoid space between recording sessions.

Since previously described methods were deficient in one or more of these criteria, we have developed a new device to meet our needs.

METHOD

Four 8-32, flat-head, stainless-steel stove bolts are surgically implanted into the animal's skull, with the head of the bolt between the dura mater and the inner surface of the skull. The threads protrude approximately $\frac{1}{2}$ in., and a Delrin foot pedestal (Fig. 1, *A*) is screwed down over each one to hold it firmly in place. These bolts serve to support 4 stainless-steel rods ($\frac{1}{4}$ in. in diameter and 3 in. in length) (Fig. 1, *B*) which are tapped on one end so they will screw down snugly on the skull bolts. The other end of each rod slips into a swivel assembly (Fig. 1, *C*) that will clamp it rigidly in place and also provide a means of compensating for minor variations in the angle at which the rod extends from the animal's head. The swivel assembly clamps around a ring (Fig. 1, *D*) that is $5\frac{1}{4}$ in. in

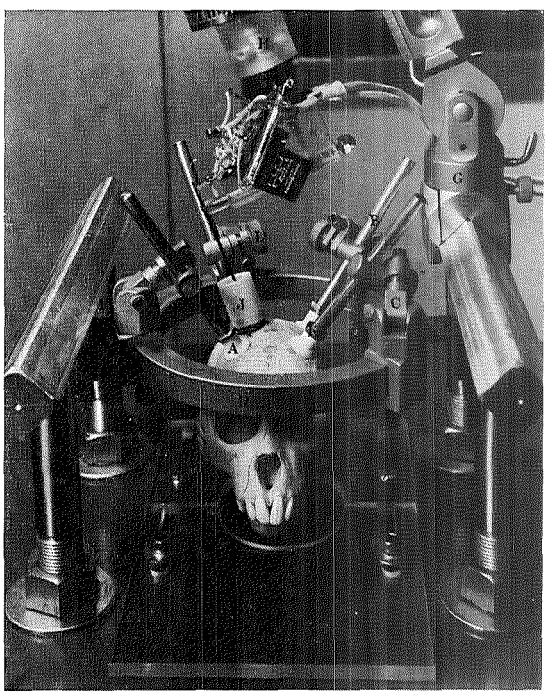

Fig. 1
Position of animal's head in head-holding device. *A*: Delrin foot pedestal. *B*: stainless-steel support rod. *C*: swivel assembly. *D*: support ring. *E*: "ear bars". *F*: support frame. *G*: Kopf electrode carrier. *H*: micro-electrode driver. *I*: access chamber. *J*: Delrin extension tube. *K*: stainless-steel "take-up" screw. *L*: thumb "take-up" screw.

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diameter and ½ in. in cross section. This heavy metal ring has two ⅜ in. square "ear bars" (Fig. 1, *E*) that protrude at a 90° angle on either side. These bars fit into a rigid frame (Fig. 1, *F*) that supports this device and also provides a slide track for a standard Kopf electrode carrier (Fig. 1, *G*). The Kopf carrier is used to hold a hydraulic micro-electrode driver and pre-amplifier (Fig. 1, *H*) as well as to adjust the position and angle of the electrode to enter the desired location of the brain. To provide a place through which the micro-electrode can be inserted into the brain, a ½ in. trephine hole is made over the desired location and tapped with a conventional 9/16 in. bottom-cutting tap. A short length of stainless-steel tubing is screwed into the hole to provide an access chamber (Fig. 1, *I*). During the experimental sessions, a hollow ¾ in. length of Delrin tube (Fig. 1, *J*) is screwed down over this access chamber and is filled with half sterile saline and half molten wax after the electrode is put into position. In this way the electrode is held rigidly in place but will slide vertically, and the brain palpitations are also reduced to a minimum. Between experimental sessions, the access chamber is completely filled with sterile saline and covered with a protective Delrin cap.

The entire head-restraining assembly attaches to a specially designed chair (Fig. 2) that consists of a heavy outer shell constructed from ½ in. cold rolled steel and an inner plastic module that supports the animal's body. To minimize the effects of body movements, the inner module is suspended by springs and mechanically damped by four shock absorbers. The entire apparatus weighs approximately 250 lbs., and is mounted on rolling casters that will lock in place.

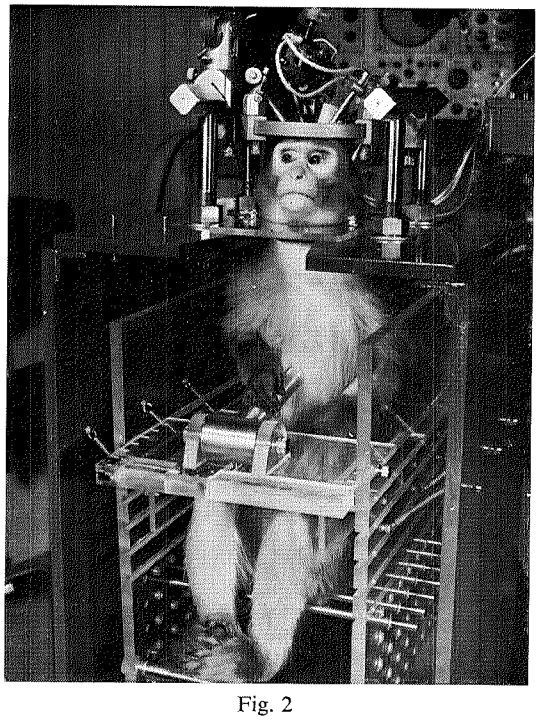

Fig. 2
Animal seated in support module before a recording session. The pellet dispenser and wire from the operant-response bar have not been put in place.

PROCEDURE

The animal is prepared in two operations approximately one week apart. Each operation is performed under sterile conditions with the animal's head fixed in a stereotaxic instrument. Sodium pentobarbital, administered intravenously, is used as an anesthetic. In the first operation, the four 8-32 skull bolts are implanted. The frontal set is located at A-P zero and 1.5 cm off the midline, and the posterior set is at A-P−2.8 cm and 1.5 cm off the midline. A 5/16 in. button is trephined midway between each set, and a side-cutting dental drill is used to make a trench which connects the buttonhole and the place where the skull bolts are to exit. The bottom of the side-cutting drill has been polished smooth to prevent injury to the dura mater. The heads of the 8-32 bolts, which have been milled flat, are inserted through the trephined hole and slid in place. The Delrin foot pedestals are then screwed down on the skull bolt to hold it firmly but with care to avoid excessive tightening of the foot pedestal, which can result in erosion of the bone. After all four skull bolts are in place, the trenches and burr holes are filled with Shur-Weld dental acrylic and the skin and subcutaneous tissue closed separately around the pedestals. These bolts will remain rigidly in place indefinitely with no apparent discomfort to the animal. Infection has not been a problem; however, during the first 3 post-operative days, a wide-spectrum antibiotic is administered systemically, and then a topical antibiotic (Panalog) is applied for an additional 4 days and at the time the sutures are removed.

Before the date of the initial recording session, the animal is again anesthetized and placed in the stereotaxic device. A ½ in. button is trephined over the area where the micro-electrode recordings are to be made. This hole is threaded with a 9/16 in. bottom-cutting tap that has 18 threads to the inch. The dura mater is then carefully removed or cut in a "V"-shaped flap and left in place. If there is any bleeding from the cut edge of the dura after several minutes, it is stopped with a very fine tipped battery cauterizer. Next, the stainless-steel access chamber is screwed in place. In practice, it has been found that the chamber will be held tightly by the threads alone; however, on occasion, either dental acrylic or a Delrin retaining ring has been found useful in securing the chamber to the skull. To complete this phase of the procedure, a Teflon protective cap is screwed down on the access chamber. A 4-40 set screw in one edge of the top of this cap serves to allow saline to be injected into the chamber, and it can be tightened to the edge of the metal access chamber to hold the cap firmly in place while the animal is in its home cage. Also, having the chamber filled and sealed with sterile saline maintains intracranial pressure and prevents excessive leakage of the spinal fluid from the subarachnoid space.

During the recording sessions, the animal is seated in the support module with its head held only by the plastic restraining collar. Animals that have been trained on

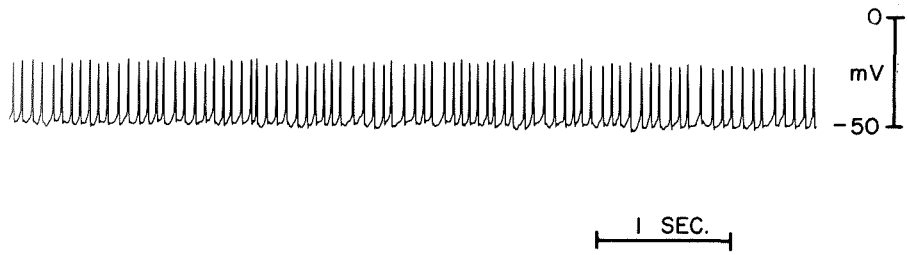

Fig. 3
Sample of an intracellular recording of a neuron in the pre-frontal area of the brain (recorded in co-operation with James D. Frost Jr.).

positive reinforcement usually remain sufficiently still so that the rods that connect the skull bolts to the swivel device can be screwed in place with no difficulty. An 8-32 stainless-steel nut (Fig. 1, *K*) that has been screwed part way down the skull bolt is then tightened against the $\frac{1}{4}$ in. rod to remove any slack in the threads. Intractable animals may be given Fluothane gas anesthesia during this procedure. The swivels have been designed so that one thumb screw (Fig 1, *L*) will tighten each rod and prevent its movement in any direction. When the animal's head has been secured, the Teflon cap is removed and a $\frac{5}{8}$ in. $\times$ 1 in. Delrin extension tube is added to the access chamber. Once the micro-electrode is lowered into position, the extension tube is filled with half saline and half molten 58°C histological wax. In this way, the electrode is held rigidly but can easily be inserted by advancing the driver mechanism. Also, the pulsations of the brain are reduced to a minimum. In the event that an electrode tip becomes damaged, it can be replaced in a matter of a few minutes.

The best recordings can be obtained during the first 3–4 days after the dura is cut. After that time, vascular proliferation and fibroblastic tissue make it difficult to insert the delicate glass electrodes.

DISCUSSION

This system has been used successfully to record single-unit potentials for relatively long periods of time as well as intracellular potentials for up to 20 sec (Fig. 3). The animals are fully conscious, and their limbs are free to perform a variety of mechanical tasks.

SUMMARY

A technique is described for the rigid fixation of a fully conscious, freely responding monkey during micro-electrode recordings from single cortical units.

RESUME

SYSTEME DE MAINTIEN DE LA TETE POUR ETUDES REPETEES PAR MICRO-ELECTRODES CHEZ LE SINGE, AU COURS DE REPONSES OPERANTES

L'auteur décrit une technique de fixation rigide d'un singe pleinement conscient, répondant librement au cours d'enregistrements par micro-électrodes d'unités corticales isolées.

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