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CHAPTER I

INTRODUCTION

Behavior can be defined as the interaction of an organism with its environment. This interaction may be divided into three neurophysiological events: (1) transduction of the environmental information into neural codes; (2) neural processing of the coded information in terms of organismic needs or wants; and (3) motor behavior to mediate the needs or satisfy the wants. These three events may be sequential or simultaneous. This dissertation focuses on the area of neural processing.

Orienting Response

To delve further into neural processing in the brain, consider the orienting response. Called the "what-is-it" response by Pavlov (1949), one of the first physiologists to investigate conscious behavior, the orienting response has since been extensively investigated in animals and in humans. If a human in a relaxed but semi-alert steady state is presented with a mild but "significant" stimulus such as speaking his name, his physiological state changes. There is a lower threshold of exteroceptive sensitivity

(e.g., 4-10 db lowering of the auditory threshold, pupillary dilation, photochemical changes in the retina thereby lowering the threshold for light intensity), an increase in the skeletal muscle tonus, an increase in electrical skin conductance, vasoconstriction in the limbs, a short delay in respiration followed by a deeper and slower respiratory rate, a decrease in the heart rate, and an alteration in the electrical activity of the brain (Lynn, 1966). Even without a physical turning of the head and/or body, the person is said to have "oriented" to the stimulus.

Consider further the pre-and post-stimulus states of the brain electrical activity. Prior to the stimulus the electroencephalogram (EEG) consists largely of high-voltage-slow waves (low frequency). Immediately after the stimulus the EEG consists of low-voltage-fast waves (high frequency). This response, termed the "activation pattern" by Rheinberger and Jasper (1937), has also been called cortical arousal or cortical desynchrony. Cortical arousal has been shown by Moruzzi and Magoun (1949), and others to result from an interaction of the reticular activating system and the thalamus with the cerebral cortex.

If the stimulus producing the orienting response is presented to a human or animal in exactly the same way on a repetitive basis, the primary orienting response diminishes and fails to occur after about 15 repetitions. The organism is said to have "habituated" to the stimulus. The EEG from most cortical areas then resembles that of the resting state. However, in the sensory cortex local desynchrony prevails. The thalamus is involved in maintaining

this localized cortical desynchrony (Sharpless and Jasper, 1956).

Thalamo-cortical Interaction

The thalamus can be divided into three areas (Ajmone-Marsan, 1965): (1) specific system; (2) nonspecific system; and (3) undetermined. Anatomical and neurophysiological evidence shows that the specific system serves as a relay station for all incoming sensory information (with the exception of olfactory stimuli) which is destined for the cerebral cortex. It also serves to distribute neural impulses to other parts of the thalamus and to other regions of the brain and may be involved in the subcortical integration of the sensory information. The nonspecific system is largely defined by its electrophysiological interaction with cortex. Thalamic portions placed in the undetermined group are of uncertain classification.

Specific System

Localized electrical stimulation of the specific thalamic nuclei elicit characteristic responses at the cortex, namely, the primary response, the repetitive response, and the augmented response.

Surface responses. Single shocks applied to a specific thalamic site produce a primary response at the surface of the sensorimotor cortex. This classic positive-negative wave was described by Adrian (1941), in connection with peripheral stimuli. The time interval between stimulus pulse and peak response amplitude of the first large positive wave is 2-4 msec (Morison

and Dempsey, 1942; Dempsey and Morison, 1943; Spencer and Brookhart, 1961a). The latency to the negative peak is of the order of 5 to 8 msec. Dempsey and Morison (1943) detail other smaller waves preceding and following the basic response, but Spencer and Brookhart (1961a) and Creutzfeldt, Watanabe and Lux (1966) identify the primary response as the positive-negative wave having the latencies mentioned above.

Following the primary evoked response, a series of spontaneous waves called the repetitive response sometimes appear. Morison and Dempsey (1943) identified these as being 8-12/sec alpha waves. Chang (1950) reported as many as 14 of these waves in a train but found the usual number to be around 4 to 5.

If repetitive electrical stimuli, 3-12/sec, are applied to a specific thalamic site, the primary response to the first pulse in each stimulus train is altered within two or three shocks. (Stimulus train is a term applied to two or more stimulus pulses in a finite series, the series duration usually not exceeding 4 or 5 secs.) The latency of the first large positive wave is increased to 5-8 msec and the negative wave latency is increased to 15-18 msec (Dempsey and Morison, 1943; Spencer and Brookhart, 1961a). In addition the amplitude of the positive wave is somewhat increased but the amplitude of the negative wave becomes substantially larger, that is, the amplitudes have been "augmented" (Morison and Dempsey, 1942; Dempsey and Morison, 1943). In cats the evoked primary and augmented responses are found in the visual, the auditory, and the somatosensory cortical projection areas as

well as in the motor cortex. Bishop, Clare and Landau (1961) found the primary response only in the sensory projection areas but observed the augmented response from wider areas. Schlag and Kuhn (1966), recording from many areas over the anterior half of the cerebral hemisphere of cats, obtained "specific" responses from sharp, focal regions in the sensorimotor cortex while the augmented responses were found to be more broadly distributed on the peri-sigmoid gyrus and adjacent cortical areas.

Intracortical responses. The alterations in primary and augmented response waveforms observed with progressive electrode penetration into the cortex have been studied with monopolar recordings. The early findings (Morison and Dempsey, 1942, 1943; Dempsey and Morison, 1943; Li and Jasper, 1953; Bishop and Clare, 1953; Amassian, Patton, Woodbury, Towe and Schlag, 1955) of a polarity reversal and other waveform changes in the cortical depths were more thoroughly studied by Li, Cullen and Jasper (1956a) and Spencer and Brookhart (1961a). Stepwise microelectrode penetration to a depth of 0.8-1.2 mm showed: (1) a decrease in latency of the order of 10-15 msec for both the positive and negative waves seen in the surface response; (2) a reduction to zero of the surface-positive wave; (3) an addition of a positive wave following the original surface-negative wave; and, (4) a 3 to 4 times increase in amplitude of the negative wave over that of the surface-positive wave. With continued sequential electrode penetration to white matter, the amplitude of the negative-positive sequence was decreased (Li, et al. 1956a), or remained the same

(Spencer and Brookhart, 1961a), and the latencies were slightly longer. Data obtained from a transcortical recording technique, reference site at the surface, substantiate the results summarized above (Schlag and Villablanca, 1967).

The results cited above provide strong support for the premise (Spencer and Brookhart, 1961a) that the primary and/or augmented responses, evoked in the sensorimotor cortex by stimulation of the specific thalamic nuclei, are generated via specific afferent terminations in layer III and in upper IV. Initial depolarization at these levels progresses upward toward the pial surface, presumably along the apical dendrites, preceded and followed by hyperpolarizing effects. These traveling wave effects are essentially completed within 40-60 msec.

The development and improvement of refined microelectrode and recording techniques during the late forties and early fifties stimulated much research on the relationships of surface and/or intracortical responses to single neuron firing. Li (1955), by simultaneous use of intracellular and extracellular microelectrodes, established that the unit spike discharge detected by extracellular means was representative of the intracellular firing event. The relationship of unitary spike discharges to cortical responses evoked by stimulation of specific thalamic nuclei, the pyramidal tract, or the cortical surface has been the object of considerable research (Li, 1956; Li, Cullen and Jasper, 1956b; Phillips, 1956; Brookhart and Zanchetti, 1956; Martin and Branch, 1958; Branch and Martin, 1958; Spencer and Brookhart, 1961b; Purpura, Shofer and

Musgrave, 1964; Torii, Endo, Shimazono, Ihara, Narukawa and Matsuda, 1965; Creutzfeldt, et al. 1966a; Humphrey, 1968a, b). A predominating feature of these studies is that the unit discharges from cells in layers III, IV and upper V are tightly clustered time-wise with the augmented response. There is less agreement in these references on the relationship between unitary responses from cells in the upper cortical layers and the augmented response. Reported results include inconsistent time-clustering, no relationship, or inhibition of spike activity.

The relationship of cortical unit activity to spontaneous electrical activity of the cortex has been studied by: Li and Jasper (1955); Li (1955); Spencer and Brookhart (1961b); Fromm and Bond (1964, 1967); Calvet, Calvet and Scherrer (1964); Creutzfeldt, et al. (1966b) and by many others. Fromm and Bond (1964) and Calvet, et al. (1964) report a decrease in the firing rate of deep cortical cells coincident with slow surface negativity. Fromm and Bond (1964) observed an increased firing rate with surface positivity. Creutzfeldt, et al. (1966b) reported an increased firing rate with surface negativity but gave no information as to the cortical level of the units monitored. There is strong evidence that the Type I spontaneous spindles (Spencer and Brookhart, 1961b) are associated with compactly clustered spike discharges of neurons in layers III and IV and that the subservient neural mechanisms are the same as, or similar to, those involved in generation of the augmented response.

Nonspecific System

Localized electrical stimulation of nonspecific thalamic nuclei produces unique responses in wide regions of cortex. These responses are related to the repetition rate of the applied stimuli. In this dissertation the primary emphasis is on the responses to low frequency stimulation, i.e., less than 15/sec. For those responses to high frequency stimulation (greater than 20/sec) which involve changes in the "steady" or DC potentials and surface shifts in potential, the reader is referred to Goldring and O'Leary (1957); Brookhart, Arduini, Mancia and Moruzzi (1958) and the thorough review by O'Leary and Goldring (1964). For more recent work relating the cortical steady potential shifts to behavioral or physiological responses, one may consult the work of Gumnit and Grossman (1961), Kawamura and Sawyer (1964), Wurtz (1966), or Rowland (1967).

Surface responses. Single stimuli applied to a nonspecific thalamic site usually do not evoke a uniquely distinct cortical response (Morison and Dempsey, 1942; Dempsey and Morison, 1942). Occasionally, such stimuli will trigger an 8-12/sec spindle burst (Jasper, 1949). Multiple stimuli applied at 3-12/sec rates evoke the classic "recruiting response" (Morison and Dempsey, 1942; Dempsey and Morison, 1942; Jasper, 1949; Starzl and Magoun, 1951; Hanberry and Jasper, 1953; Verzeano, Lindsley and Magoun, 1953; Li, 1956b; Brookhart and Zanchetti, 1956; Spencer and Brookhart, 1961a). The recruiting response is defined in terms of: (1) amplitude growth with the first several stimuli in a stimulus train; (2) polarity of response; (3) latency to peak; (4) wax-wane

properties with ongoing stimuli; and (5) topographical distribution over the cortical surface.

With supraliminal low-frequency stimuli there is usually no cortical response to the first one or two pulses. However, with each succeeding stimulus, a surface-negative wave develops (monopolar recording with respect to a remote reference) whose amplitude incrementally increases with each stimulus, reaching a maximal response amplitude by the 5th to 7th pulse in the stimulus train. The duration of this surface-negative wave is of the order of 30-50 msec. The time lapse (latency) between each stimulus pulse and the peak of the response is in the range of 15-40 msec with 20-35 msec being most common. This latency usually does not vary during the stimulus train, but it may be related to the thalamic site of stimulation and to the cortical recording site (Starzl and Magoun, 1951; Verzeano, et al, 1953; Hanberry and Jasper, 1953; Jasper, Naquet and King, 1955). If the stimulus train is continued beyond 2 or 3 secs, the recruiting response wanes and waxes in amplitude in alternating fashion. The durations of the waxing intervals are of the same order as that of spontaneous 8-12/sec spindles (Jasper, 1949).

There is some dispute in the earlier literature about the cortical locations in cats where recruiting responses were found, but there is now strong evidence that recruiting responses may be found in the primary visual, auditory and somesthetic areas, in the motor cortex and in the association areas. Furthermore, the topographical relationship between thalamic sites and cortical

response sites is more explicitly delineated (Starzl and Magoun, 1951; Hanberry and Jasper, 1953; Jasper, Naquet and King, 1955; Schlag and Villablanca, 1967; Andersen, Andersson, Junge, Lømo and Sveen, 1967; Andersen, Andersson and Lømo, 1968). Schlag and Kuhn (1966) and Schlag and Villablanca (1967) clarify the observations from the sensorimotor cortex and adjacent areas by previous authors who showed that the recruiting response may begin with a small initial positive wave followed by the large negative wave. Schlag and co-workers present data that show the "pure" surface-negative response existing in areas surrounding, or in the near vicinity of, response foci which present the small-positive large-negative recruiting response. They further differentiate between the recruiting responses obtained in the anterior and in the posterior cortical regions.

Intracortical responses. The relationships between surface and intracortical recruiting responses, spontaneous spindle activity and single unit activity have received considerable study (Li and Jasper, 1953; Verzeano, et al. 1953; Li, 1956a, b; Spencer and Brookhart, 1961a, b; Li, et al. 1956b; Purpura, Shofer and Musgrave, 1964; Fromm and Bond, 1964, 1967; Calvet, et al. 1964; Torii, et al. 1965; Creutzfeldt, et al. 1966a, b; Schlag and Kuhn, 1966; Humphrey, 1968a, b). These authors report little change from the recruiting response characteristics of the cortical surface with electrode penetration through the initial cortical layers. As the intracortical exploring electrode approaches the 0.5-0.8 mm region, the monopolar records show a slight increase in amplitude

which very quickly changes to either a low amplitude positive-negative wave or else no response. With continued electrode penetration the response almost immediately becomes a large-amplitude positive wave which then diminishes somewhat in amplitude with electrode progression into white matter. These authors do not report any significant change in latency of the response as a function of cortical depth. This "constant" latency is in contrast to that observed for the augmented response of the specific system.

The region of polarity change has been called the equipotential level, inversion layer, or the inversion zone. The inversion zone has been observed by many investigators and has been reported at levels from 0.2 mm to 2 mm beneath the cortical surface. The region at which the inversion zone has been most frequently encountered is from 0.5 mm to 0.8 mm. It has been observed with less frequency at 1.2-1.5 mm (Li, et al. 1956b; Spencer and Brookhart, 1961a). The variation in depth at which the inversion zone is encountered within the same preparation and from one preparation to another remains an unexplained puzzle. Interpretation of the inversion zone phenomenon by most authors has been in terms of a depolarization sink in the superficial cortical layers.

The striking similarities between the spontaneous 8-12/sec spindle waves and the surface recruiting response was noted by early investigators (Morison and Dempsey, 1942; Dempsey and Morison, 1942; Jasper, 1949) and repeatedly supported by subsequent authors.

Spencer and Brookhart (1961b) provided convincing evidence that common or similar neural mechanisms subserve both the spontaneous spindle waves and the recruiting response by showing that Type II spindles exhibited surface and intracortical properties of wave duration, amplitude, polarity, and depth of polarity inversion nearly identical with those which defined the recruiting response.

No clear relationship between unitary spike activity, non-specific thalamic stimulation, spontaneous spindles, or other on-going electrical activity is discernible from reports in the literature, and there are even contradictory findings. Li and Jasper (1953) found increased firing of posterior sigmoid cells during the "waxing" phase of spontaneous alpha spindles and found decreased firing or silence during the "wane" period. Li (1956a) could not elicit spike activity from cells in layers III and IV by stimulating the central median nucleus. Li, et al.(1956b) reported spike activity 1.42 mm beneath the surface of the posterior sigmoid gyrus which increased during the waning phase of the recruiting response as compared with the spike activity during the waxing phase. Spencer and Brookhart (1961a) observed unitary silence from layer IV cells of the posterior sigmoid gyrus during the recruiting response but emphasized the irregularity and diversity of unitary activity with the Type II recruiting-like waves. They also noted the "indirect" activation effect of recruiting on the neurons of layers III, IV and V. Calvet, et al. (1964), using spontaneous cortical activity in cats, found no relationship between the Type A surface-negative waves (30 msec

duration) and the unit activity at 600 μ. However, the surface-positive Type B1 and B2 waves (60 msec and 300 msec durations, respectively) coincided with increased unit firing. The Type C surface-negative wave (250 msec duration) was coincident with unitary silence for the cells at 1000 μ.

Torii, et al. (1965) compared the unit activity of cells, without regard to their depth, from the association, the motor and the sensory cortical areas in response to low frequency non-specific thalamic stimulation. In the association cortex, 82% of 98 cells showed synchrony of firing with the cortical response, 8% exhibited an increased firing rate, and the remainder showed no change. In the somatosensory cortex only 9% of 140 cells showed synchronized firing patterns, 50% exhibited an increased firing rate while 3% revealed a decrease. In the motor cortex, 53% of 256 cells showed firing synchrony, 21% exhibited an increased firing rate, and 20% showed no change. Of the three cortical areas studied the motor cortex had the larger percentage of cells showing a decrease in firing rate. This last result provides neurophysiological support for the behavioral "arrest reaction" observed by Jasper (1949).

Creutzfeldt, et al. (1966b) presented results showing a close correspondence between the intracellular potential, the synchrony of unit spike activity coincident with the surface-negative recruiting activity, and the surface response. However, they supplied no information on the depth of the monitored cells beneath the surface of the motor cortex. Fromm and Bond (1964,

1967) observed single neurons firing more rapidly during surface-positive waves and slower during negative ones. The "reversed" relationship reported by Creutzfeldt, et al.(1966a) was also observed by Fromm and Bond (1964, 1967) when the standing potential of the surface was much more negative than usual. These seemingly contradictory findings will become more understandable after consideration of the research findings reported in the subsequent chapters.

Interaction at the Cortex

The specific and nonspecific thalamic afferent effects at the cortex have been shown to exhibit both independent and interdependent properties.

Independent properties. The notion of independence of neural effects received early support by Dempsey and Morison (1943) who noted no effect of the recruiting response on the specific stimulus-evoked repetitive response. Jasper (1949) noted no effect of thalamic reticular stimulation on the primary potentials evoked by tactile, visual or auditory stimuli. Jasper and Marsan (1950) postulated separate neuronal elements for termination of specific and nonspecific systems at the cortex. The work of Spencer and Brookhart (1961a, b) and Torii, et al.(1965) further elucidated independent cortical effects. These independent cortical effects are supported by the evidence for independent thalamocortical pathways (Jasper and Marsan, 1950; Hanberry and Jasper, 1953; Hanberry, Marsan and Dilworth, 1954). These latter findings

were reconfirmed and considerably extended by Skinner and Lindsley (1967) and Velasco, et al. (1968).

Interdependent properties. The literature also reveals evidence of the interaction of these two thalamo-cortical systems at the cortex.

An alerting sensory stimulus was reported (Jasper, Naquet and King, 1955) to block the recruiting response. Li (1956a) found that a nonspecific thalamic "conditioning" shock appearing before a specific thalamic shock would alter in a cyclical fashion the number of spike discharges from single units in the lower part of layer III and in layer IV of the posterior sigmoid gyrus as a function of the time interval between the shock pairs. Brookhart and Zanchetti (1956) showed that the relayed medullary pyramidal volley, evoked by surface stimulation of the motor cortex, increased in magnitude and duration with the growth of the augmented response. However, there was no pyramidal volley during recruitment even though there was no detectable change in responsiveness of the efferent neural system. The pyramidal volley responsiveness was altered by normal spontaneous spindling in a predictable way.

By observing and analyzing the spontaneous spindle activities with respect to depth from the cortical surface, Spencer and Brookhart (1961b) concluded that a normal spindle burst was composed of Type I (augmenting-like) spindles in the early portion while the later portion of the burst consisted of Type II (recruiting-like) spindles. Torii, et al. (1965) reported apparent convergence, or synchrony of unit firing with specific or nonspecific

thalamic stimuli, as follows: 75% of 63 cells in the association cortex; 41% of 176 cells in the motor area; and 8% of 178 cells in the somatosensory cortex. These authors also reported an amplitude facilitation, by antecedent nonspecific thalamic stimulation, of neuronal discharges evoked by specific thalamic stimulation. Maximum facilitatory effects occurred when the paired-stimulus spacing interval was in the 20-30 msec range. Therefore, evidence reported in the literature supports the notion of a modulatory role on cortical function by nonspecific thalamic nuclei (Jasper, 1960; Ajmone-Marsan, 1965; Krupp and Monnier, 1966).

Purpose of Dissertation

The previous findings notwithstanding, the functional mechanisms by which nonspecific thalamic afferents influence cortical function and their precise location in the cortical layers remain undefined. Therefore, the purpose of this dissertation was to determine the laminar site(s) of functional action at the cortex of nonspecific thalamic afferents evoked by low frequency electrical stimulation.

The cortical recruiting response was selected as the indication of the nonspecific thalamic activity. The laminar site of functional action was assumed to be determined if the principle sources and sinks of the ions generating the recruiting response were located with respect to the pial surface. Since either ion movement or the physical separation of different charge densities in a conducting media give rise to potential fields, the measurement

problem becomes one of resolving the spatial properties of potential normal to the pial surface. Previous studies employed single microelectrodes progressing in sequential steps into the cortex. The well-known variability of cortical function with time is inherent in such data and complicates the interpretation of the results. For this study, a triple barreled microelectrode probe was developed such that its three tips were each separated by about 100 μ along the axis of the probe. Thus, simultaneous measurement of potential between the two pairs of tips would facilitate the determination of the spatial gradient of potential in the region monitored. With sequential transcortical steps a complete profile of the gradient of potential normal to the pial surface could be determined. The divergence, derived from the spatial gradient of potential profile, could then be interpreted in terms of ion sources and sinks. These techniques and analytical methods were employed to accomplish the purpose stated above.

CHAPTER II

MATERIALS AND METHODS

Experiments were conducted on 8 rats and 19 cats to develop the experimental techniques and procedures for the dissertation protocol. This protocol was used in 12 additional experiments on adult cats to acquire the data which form the basis for this dissertation. The description of the analytical method which sets the requirements for the experimental data is followed by a description of the animal preparation and of the experimental procedures.

Method of Analysis

Volume conductor theory has been applied to the study of nerve action and neuronal potentials since Lorente de Nó (1947). The review article by Freeman (1963) is a clear, concise presentation of the basic concepts of volume conductor theory and shows an application of these concepts to the analysis of primary olfactory cortical potentials. For a recent application of these principles to cortical potentials produced by pyramidal tract neurons, see Humphrey (1968a and b). Since these principles are amply described in the neurophysiological literature and in electrical engineering references (e.g., Kraus, 1953), they are briefly

summarized below.

In a conductive medium the existence of a potential difference between any two points implies a three-dimensional field of potential. This three-dimensional field of potential results from a field of current or from differences in densities of spatially separated ions, either having the same or unlike charge. Thus, electrical potential waveforms in the brain are manifestations of ionic currents, or spatial ion densities, whose distributions are determined both by the structural configuration and the functional operation of active neurons, and by the properties of the extracellular fluid.

Ions in the brain are not ordinarily subjected to magnetic fields which would produce rotational forces. Thus, the movement, or tendency for movement, of ions in the conductive medium of the brain is in the direction defined by the spatial gradient of the potential field. There is considerable evidence in the neuroanatomical and neurophysiological literature to support the idea that the primary direction of extracellular ion movement is parallel to the apical dendrites. Hence, the maximum value of the spatial gradient of potential is most probably parallel to the apical dendrites (see footnote 1 in Calvet, et al. (1964). Since the gradient of potential is defined as the rate of change of potential with respect to distance, measurement of the potential difference between closely spaced electrode pairs, as with the tri-probe used in the research reported herein, yields an approximation to the potential gradient.

In a homogeneous conducting medium, current is proportional to the potential gradient. Thus, the gradient of potential obtained from the experimental data is proportional to the ionic current. Sources or sinks of ions can be evaluated by determining the spatial locations where the maximal rate of change of current occurs. Horizontal components, or current components orthogonal to the apical dendrites, are assumed to be negligibly small. Thus, the linear derivative of the potential gradient with respect to cortical depth (called the divergence) provides this evaluation. The divergence is zero where there are no sources or sinks. Non-zero values of divergence indicate sources or sinks of ions. In this dissertation the negative values of divergence correspond to sinks for positive ions while positive values of divergence correspond to sources of positive ions. Stated differently, a sink is a region where ion densities are being diminished whereas a source is a region where ion densities are being increased.

The assumption of a homogeneous conductive medium in the cortex must be examined. The proportionality factor relating potential gradient and current is the specific resistivity. If the specific resistivity changes significantly as a function of transcortical distance, or as a function of time, then an error in both the existence and the location of ionic sources and sinks may result (Freeman, 1963). In the extreme case of two contiguous media with greatly different conductive properties, the naive application of the divergence concept would erroneously reveal a source or sink. The degree to which this kind of error might appear

in the cortex is related to the rate of change of specific resistivity with respect to depth. The studies to date support the idea that the cortex does not contain laminar regions where the rate of change of resistivity with depth is significantly different from zero (Li, Bak and Parker, 1968). Considering the existence of other errors in the data reported herein (i.e., inaccuracy of depth from the pial surface, track misalignment from parallelism with the apical dendrites, and the spatial resolution of the potential gradient), the assumption of a homogeneous conductive media in the cortex is reasonable. This assumption has been made by others (Towe, 1966; Humphrey, 1968) for lack of more definitive information.

This analytical method requires the measurement of potential between closely spaced points in the cortex which define a line parallel to the apical dendrites. The tri-probe was developed to accomplish this requirement. The fabrication and characteristics of the tri-probes used in this research are described in Appendix II. The preparation of the animal and the experimental procedures employed to gather the data are discussed below.

Animal Preparation

The Laboratory Animal Facility at the University of Oklahoma Medical Center provided health care for the 12 cats for a minimum of one week prior to their use to insure their good health.

Anesthesia

Cats (2.4 to 4.6 kg) were anesthetized with alpha chloralose solution of 5% concentration at a dose of 50-55 mg/kg (Strobel and

Wollman, 1969; Clifford and Soma, 1969). This solution was prepared by mixing 1 gm alpha chloralose with 5 gm of ethyl carbamate (urethane) in 20 cc of 0.9 per cent saline. The presence of the urethane and immersion in a hot water bath, about 60° C, facilitated solution of the crystalline alpha chloralose. The dissolved mixture was divided into 5 cc test tubes which were then sealed and refrigerated. Mixtures refrigerated longer than 12 days were discarded to avoid the toxic effects of β-chloralose. Just prior to use, the precipitate in the cold mixture was redissolved by immersion in a hot water bath and agitation.

The preferred route of injection was intravenous. With cooperative cats, both forelimbs were clipped to expose the skin over the cephalic veins. Intravenous injection was accomplished by having an assistant restrain movement of the cat. Onset of anesthesia occurred within 3 to 10 secs from the start of the injection. Noncooperative cats received about 40 per cent of the total dose intraperitoneally. The remaining volume was administered intravenously after the cat became manageable. Time for induction to manageable levels varied from 15 to 45 minutes.

The scalp region was closely clipped. Additional clipping of forelimbs and hindlimbs bilaterally exposed the skin overlying the cephalic and femoral veins for intravenous injection of sustaining anesthesia during the prolonged experiments.

Surgery

The anesthetized cat was placed in the Narishige SN-2 heavy duty stereotaxic instrument and a temperature probe (Yellow Springs Instrument) inserted about 5 cm into the rectum. Manual control of DC power to a heating pad beneath the preparation maintained a body temperature of at least 37° C. The scalp region was scrubbed with 70 per cent ethanol and a midline incision was made from the nasal bone to the occipital ridge. The overlying skin and connective tissue were reflected bilaterally. For the larger cats another incision was usually made from the anterior end of the midline incision, extending 1 to 2 cm laterally to the right to facilitate the muscle reflection.

The periosteum over the right hemisphere was scraped free of the calvarium from the nasal bone posteriorly to the attachment of the temporalis muscle to the occipital ridge. Points of primary attachment were usually left intact. Muscle and tissue were reflected to expose the calvarium homolaterally to the right.

Bleeding was minimized by clamping with hemostats and the application of cotton pellets or Gelfoam. The pellets and Gelfoam were either dry, moistened with saline, or soaked with thrombin solution, 100 units/cc.

The area over the frontal bone anterior to the coronal suture was prepared for the reference site by cleaning first with saline and then with acetone. A 10 mm length of nylon tubing, about 6 mm inside diameter and truncated at the end in contact with bone, was positioned over the frontal bone so as to include

a portion of the sagittal suture. Dental acrylic cement and liquid catalyst were applied to the exterior junction of the tubing with the bone to obtain a good mechanical bond and a liquid seal.

Near the posterior end of the suprasylvian gyrus, which was hazily visible through the calvarium, a crescent-shaped slot was cut in the calvarium with a small dental burr. This cut was extended under optical magnification down to the dura, care being taken to prevent damage to the dura. The calvarium was carefully removed with the rongeuers to expose the dura over a substantial portion of the suprasylvian gyrus. The exposed area ranged from the posterior end of the gyrus anteriorly to the coronal suture and laterally to the supraslvian sulcus. Except for the anterior end, the medial border of the exposed dura was about 4 mm medial to the lateral sulcus. At the anterior end, the medial border was less than 1 mm from the sagittal suture for a distance extending 12-15 mm posteriorly from the coronal suture. This medial inset provided physical clearance for subsequent positioning of the stimulating probe in the nonspecific thalamic nuclei.

During removal of the calvarium, bone bleeding was controlled by packing the margins with Lukens bone wax. Excess wax squeezed between the bone margin and the dura was carefully removed. Thrombin was <u>not</u> used once the process of calvarium removal was initiated.

A 26 gauge needle, mounted on the barrel of a 1 cc tuberculin syringe, served as a microscalpel to aid in the removal of dura. An incision site was located midway between the lateral and

the suprasylvian sulci over that portion of the suprasylvian gyrus having minimal vascularization in the pia. Under optical magnification the dura was impaled with a shallow scooping motion of the needle, lifted free of pia and was slit for 1 to 3 mm. Cerebrospinal fluid spilling over the dura surface was evidence that the slit had completely penetrated the dura. The edge of the slit was grasped with a fine-pointed pair of jeweler's forceps and lifted free of the pia. Iridectomy scissors were used to extend the slit anteriorly and posteriorly along the middle of the gyrus and thence to all corners. Flaps of the dura were reflected back over the bone margins, thereby exposing the pia and the underlying cortex. Strips and pieces of Gelfoam, slightly moistened with 0.9 per cent saline, were used to bond the dura flaps to the bone margins and provide stasis for the slight oozing of blood from portions of the dura.

Fluffed pieces of cotton saturated with warm saline were carefully placed on the pial surface. A patch of mylar film placed over the cotton coverings prevented evaporative water loss and cortical cooling. In so far as was experimentally feasible, these coverings were maintained during the subsequent experimental procedures.

Experimental Procedures

This section describes the protocol followed in acquiring the data presented in Chapter III, RESULTS. These procedures utilized instrumentation which is more fully described in Appendix I.

A brief description is included in the discussion below for the convenience of the reader. The design and fabrication of the triple-barreled microelectrode probe (tri-probe), used to detect the intracortical brain potentials, is described in Appendix II. Information about the wick electrodes, used for the electrical reference and for monitoring the electrical activity from the pia, and the coaxial stimulating probe are included in Appendix II.

Preliminary Procedures

All data channels were direct (DC) coupled. Four monopolar, high-input impedance preamplifiers provided a gain of 10 for each of the four electrode signals. These four signals consisted of the pial surface activity and the brain potentials detected by the three tri-probe tips. Operational amplifiers provided secondary amplification of about 10. Gain trimming adjustments were incorporated and the preamplifier and the secondary amplifier combined gain was set at 100 $\pm$ 1 per cent. The secondary amplifier low impedance outputs were supplied to three functional elements: (1) Beckman Type 482 main pen-driver amplifiers; (2) Tektronix Type 502 Oscilloscope; and (3) two differential amplifiers. The differential amplifier outputs, representing the bipolar differences in potential between adjacent pairs of tri-probe tips, were connected to two Type 482 pen-driver amplifiers. Power was supplied continuously, week by week, to the preamplifiers and the secondary amplifiers to minimize thermal drift. After a 30 minute warmup of the Beckman Type R Dynograph Recorder, centering and balance

adjustments were progressively made on the pen-driver amplifiers, the secondary amplifiers, and the preamplifiers until pen deflections from their graph midlines were minimal for an input sensitivity of 200 μv/cm.

The silver silver-chloride electrode potentials were measured by immersing the tri-probe tips and the wicks of the two wick electrodes in 0.9 per cent saline and alternately switching the preamplifier inputs between a direct short and the electrodes. One of the wick electrodes was arbitrarily selected as reference for the other four electrodes. The electrodes met the criterion for acceptability if the potentials with respect to the reference were less than 1 mv.

The reference site tubing fixed on frontal bone was filled with 0.9 per cent saline and the reference wick was immersed in it. Cortical protective coverings were removed sufficiently to allow placement of the pial wick electrode in light contact with the pia. The preferred starting site was the middle portion of the suprasylvian gyrus in the region of anterior + 4 mm to + 9 mm (Horsley-Clark stereotaxic coordinates).

Spontaneous cortical-surface electrical activity was recorded on two channels. One channel was direct coupled while the other was capacitatively coupled, on a temporary basis, with a time constant of 0.03 secs. These recordings contained qualitative information about the depth of anesthesia, the physiological state of the cortex, and the quantitative performance of the instrumentation system. Gross sensory stimuli, e.g., hand clap, mechanical tap

of the earbar, flicking of the footpad, or manual stroking of a limb were used to verify the general responsiveness of the nervous system. The sharp stimuli produced short, evoked potential responses (Buser and Bignall, 1967) while the stroking stimuli caused alterations in the frequency and amplitude of the ongoing electrical patterns. Upon successful completion of this general test, the capacitor-coupled channel was restored to direct coupling.

Acquisition of Recruiting Responses

The coaxial stimulating probe was positioned on muscle exposed by the surgical procedures. Pulses of 0.5 msec duration were applied at a 1/sec rate with successively higher voltage levels until a slight muscle twitch was observed. This event usually required about 5 volts of stimulus intensity. This procedure verified proper functioning of the stimulating system.

The stimulating probe was then positioned in one of four nonspecific thalamic sites, namely: (1) center median nucleus (A +7, L 2.5, V +2 to -1); (2) caudal end of central lateral nucleus or rostral end of center median (A +8, L 3 or 3.5, V +5 to -0.5); (3) central lateral nucleus (A +9.5, L 2.5, V +5 to +1); or (4) central lateral nucleus (A +10.5, L 2, V +3 to +1.5). These sites, and other references to brain structures in stereotaxic coordinates in this dissertation, utilize the Jasper-Marsan atlas as reproduced in Sheer (1961). The vertical coordinate values listed above have been adjusted for the stimulating probe track of $+16^{o}$, tilted anteriorly from the vertical and lying in the

sagittal plane. This angular tilt resulted from a compromise between the need for physical space above the cortex for the electrode holders and their carriers, and the need to increase the probability of hit for the selected nuclei. Small variations in anterior or lateral positions were sometimes necessary to avoid vascular trauma in the pia or to prevent common hole tracking resulting from brain compliance and probe flexure.

With the stimulating probe located at the dorsal end of the selected thalamic track, stimulating pulse trains of 2 to 3 secs duration were applied. Pulses of 0.5 msec duration were applied at a rate of 5/sec. Pulse amplitudes were selected at successively greater values in the range from 3 to 11 volts. Continuous graphical recordings were made at paper speeds of 2½, 5, or 10 mm/sec while the oscilloscope display of pial activity was used to identify the recruiting responses and was photographed at appropriate times. The horizontal sweep of the oscilloscope, 10 msec/cm, was triggered by each stimulus event. Thus, any electrical event timelocked to the stimulus appeared stationary in the display. The presence of recruiting responses was evaluated by the following criteria: (1) the peak amplitude of the response was in excess of 0.2 mv; (2) the polarity of the pial response was negative with respect to the reference site; (3) the latency was in the range of 20-40 msec; (4) the duration of the response was of the order of 40 msec; (5) the pial response amplitude developed incrementally after a few pulses; and (6) the recruiting

response peak amplitude remained fairly constant during most of the stimulus train. If these criteria were not met, the stimulating probe was lowered 0.5-0.8 mm, and the stimulating procedure was repeated.

Maximal Response Site

When recruiting responses were obtained, the stimulus amplitude was adjusted to produce responses of about 1 mv peak amplitude. The pial wick was then used as an exploring electrode. It was systematically positioned in 1½ mm increments along the midline of the gyrus both anterior and posterior to the initial site. At each cortical location, the average response amplitude to constant intensity stimulation was visually estimated from the oscilloscope display. From these data a location showing the maximal response amplitude, henceforward called the maximal site, was selected for transcortical exploration. The pial wick was positioned at the maximal site, and the stimulus intensity was adjusted to produce recruiting response peak amplitudes in the range of 0.5-1.2 mv.

The tri-probe was visually aligned for perpendicular entry into the middle portion of the suprasylvian gyrus at the maximal site and moved into contact with the thin layer of fluid covering the pia. The pial wick electrode was carefully positioned as close as possible to the tri-probe. The standing potential of the pia was then recorded. DC centering adjustments in the preamplifiers were made to compensate for the pial standing potential, thus

returning the pens to graph midline. The channel sensitivities were increased and a short recording made of the spontaneous electrical activity at the maximal site. These data again permitted qualitative evaluation of the state of the preparation.

Excess fluid was carefully removed from the pial surface and an adjustment made in the coarse vertical control of the triprobe carrier to alternately make and break contact with the pia. Contact separation caused the recording pens to be driven to the extreme limit of travel. Consistent contact with the pia produced continuous midscale recording of ongoing electrical activity. Once the pial surface was located with respect to stereotaxic coordinates, exposed portions of the surface were protectively covered.

Preliminary Data

The next step was to make a preliminary recording of the pial surface recruiting responses to the standard stimulus, consisting of a train of 0.5 msec pulses presented at a rate of 5/sec for a total of 12-13 pulses. The pulse train duration was determined by a timing circuit (see Appendix I). The stimulus intensity was set at the value previously established to elicit recruiting responses of 0.5-1.2 mv peak amplitude.

Paper speed was shifted from the usual 5 mm/sec to 250 mm/sec. The stimulus was initiated about 1 sec after the speed change. About 2 secs following completion of the stimulus train, the paper speed was returned to 5 mm/sec.

The record was examined with respect to the 6 criteria for

recruiting responses previously presented. If the fully developed recruiting response peak amplitudes did not meet the 0.5-1.2 mv criterion, then appropriate adjustment in the stimulus intensity was made, and the trial run was repeated. The recorded outputs of the two differential amplifiers were checked for zero amplitude response.

Transcortical Exploration of Recruiting Responses

While recording ongoing brain activity, sensitivity adjustments were made to produce 2 to 3 cm peak-to-peak pen excursions in each monopolar channel. Adjustments of the preamplifier centering controls positioned the midpoint of the pen excursions on or near the midline of each channel's graph. The following data was entered on the graphical record: (1) stimulating probe site; (2) vernier reading of the stimulating probe vertical carrier; (3) stimulus pulse width and repetition rate; (4) identification number of the tri-probe; (5) identification of data source for each of the 6 recorded channels, i.e., pia, tri-probe tips, differential channels; (6) vernier reading of the micrometer controlling the vertical position of the tri-probe; and (7) sensitivity of each data channel.

The four preamplifier inputs were switched to zero potential to allow a measure of the absolute potential of the pial surface. The four inputs were switched to the recording electrodes, the paper speed momentarily set at maximum speed and the stimulus applied, as in the trial data run. Subsequent to the data run the

tri-probe was driven an incremental distance into cortex, usually 100, 150 or 200 μ. The specific value selected was based on the axial separation distance between the three tri-probe tips. For a given tri-probe transcortical track, the incremental change in depth was constant. To facilitate tri-probe penetration into the cortex, vibration was applied to the carrier micrometer head. This vibration was produced by rapidly rotating the fluted handle of a small screw driver in contact with the micrometer head. The forementioned procedures for sensitivity adjustments of pen excursions, midpoint centering, and zero potential check were repeated for this new position.

Application of the next stimulus train was delayed for at least 60 seconds following completion of the preceding stimulus. This intertrial interval was based partly on reports in the literature (e.g., Krauthamer and Albe-Fessard, 1965) but largely derived from tests conducted during the development of the research techniques and procedures. The intertrial interval was usually between 90 and 120 secs because of the extremely delicate trimming adjustments required by the preamplifier centering controls. The paper speed was temporarily increased and the stimulus-response train was recorded. The adjustment of tri-probe incremental penetration was made and the entire procedural sequence repeated until 2000 to 2200 μ had been traversed, thus completing the maximal site transcortical exploration.

The tri-probe was withdrawn from the cortex and relocated either anterior or posterior to the maximal site but still in the

middle of the convexity of the suprasylvian gyrus. The transverse increment of movement was either 0.5, 1.0 or 1.5 mm, with 0.5 mm being the most frequently used increment. Then the complete process of establishing the pial surface in stereotaxic coordinates and conducting a stepwise transcortical exploration was repeated.

The preferred procedure of bracketing the maximal response site with two or more transversely-spaced transcortical tracks was not feasible during most experiments. Several factors contributed to this limitation: deterioration of the preparation, equipment malfunction and experimenter fatigue. In the few experiments where some bracketing was possible, a second procedure was also performed. This procedure was to relocate the stimulating probe in another thalamic site, to search for recruiting responses, to determine a maximal site, and to conduct one or more transcortical explorations of the cortex as previously described.

Corrective Procedures

During the course of some experiments complications with the preparation arose which required corrective action or altered procedures. The most vexing complication was cortical movement. Movement of the cortex in the vertical direction was most noticeable in conjunction with skeletal muscle activity (jaw, limb or body movements), coughing spasms, or deep respiratory activities. Skeletal muscle movements (including myoclonic body jerks) were one of the indicators that anesthesia was becoming "light." The corrective procedure was to administer a sustaining dose of 5 per cent alpha

chloralose intravenously, usually 10-20 per cent of the initial dose. Sustaining doses were never administered during a transcortical exploration. Frequently, 0.5-3 cc of 0.9 per cent saline would be added to the anesthetic dose as a partial compensation for the loss of body fluids during the course of the experiment.

When coughing occurred, recording operations were stopped. Normally, the coughing would cease after a few minutes. Then the last set of stimulating conditions would be repeated, and the cortical responses would be compared with those preceding the coughing event. If the responses were deemed similar, transcortical recording was continued. If the responses were greatly different the tri-probe was withdrawn from the cortex, the pial surface reestablished with respect to stereotaxic coordinates, and the tri-probe driven to the laminar depth reached at the start of the coughing event. The transcortical recording was then continued as before.

Cortical movement due to heart beat was never a significant problem. Cortical movement caused by respiratory action was usually associated with low blood pressure. Blood pressure was not measured, but manifestations of low blood pressure were: (1) a blanching of the cortex from its normal reddish pink to lighter shades of pinkish-white; (2) cephalic or femoral veins collapsed or reduced in size; and (3) a reduction in the brain volume (shrinking of the brain and a settling down into the cranial vault). The primary corrective measure was to increase blood volume through intravenous injection of 0.9 per cent saline, 10 per cent glucose

solution, or Ringer's solution.

Occasionally during the course of an experiment one or more of the recording electrodes would exhibit appreciable increases in electrode potentials. This event, usually manifested by offset potentials greater than 5 mv, was verified by electrode potential measurements with the electrodes immersed in saline. This trouble was due usually to the cotton wick element of the reference electrode. Corrective measures were to flush out the wick pipettes with 0.9 per cent saline or to replace them. The silver silver-chloride wire electrodes seldom caused these apparent changes in electrode potentials. When they were at fault, simple replacement solved the problem.

On a few occasions during the tri-probe penetration into the cortex, the recruiting response amplitudes were rather erratic from one stimulating train to the next. If the 6 criteria set forth on page 29 were not met, then appropriate adjustments in stimulus amplitude were made, and the stimulus was repeated for that particular level. If the recruiting response amplitudes were below the 0.2 mv minimum, a simple repetition of the same stimulus parameters would usually elicit acceptable responses. In one or two severe cases of persistent erratic responses, the track was abandoned. The stimulating probe was then relocated to another thalamic site, and the transcortical recording procedures were repeated.

Termination of Experiments

With the tri-probe positioned intracortically and the pial wick adjacent to the tri-probe point of entry, a lethal overdose of sodium pentobarbital was administered intraperitoneally or intravenously. Electrical potential changes were recorded as death ensued. After death the electrodes were placed in a saline pool and the potentials between them remeasured. Zero potential inputs to the preamplifiers, the secondary amplifiers, and the driver amplifiers were recorded to determine the extent, if any, of the electrical and the mechanical drift in each channel.

The tri-probe was stored with its tip immersed in chemical cleaning solution. Normally, digestion of all tissue, either inside or outside of the tips, was complete within 48 hours. A series of rinsing operations in distilled water were performed to prepare the tri-probe for subsequent use.

The calvarium was removed from the frontal sinus region posteriorly to the tentorium and bilaterally almost to the ear-bars. Four marker wires were stereotaxically positioned so that the posterior pair determined a coronal plane 1 to 2 mm caudal to the most caudal extent of travel of the stimulating probe. The anterior pair similarly determined a coronal plane 1 to 2 mm anterior to the most rostral site of entry of the stimulating probe. A 1½ to 2 cm section of the brain, including the marker wires, was dissected free. A short marker wire was manually inserted in an anterior-posterior direction through the left thalamus. The brain

section was placed in 10 per cent formalin for fixation.

Histology

After seven or more days in formalin, the brain section was removed, rinsed in water, and the left hemisphere marker wire removed. Using the corona plane marker wires as guides, knife cuts were made to trim off the rough tissue and to establish a coronal plane of reference. Peripheral cortical gyri were trimmed bilaterally and dorsally to obtain a blocked rectangular section suitable for mounting on a freezing microtome. The section was mounted on the freezing plate rostral face down for slicing from caudal to rostral direction. Slices of fifty micron thickness were removed until the "shadow" of the lower end of the track left by the stimulating probe became visible. Slices, one hundred micron in thickness, were removed until the lowest extent of the track was included in a slice. The slice was placed in water over a black background and appropriately lighted to accentuate cytoarchitectonic structure. The location of the probe site with respect to well defined landmarks, cellular groups, and white matter pathways was compared with the atlas of Jasper-Marsan (Sheer, 1961) to determine the coordinate locations of the thalamic sites of the stimulating probe.

The above methods were employed to produce the results reported in the next chapter.

CHAPTER III

EXPERIMENTAL RESULTS

Twelve cats comprised the group of experimental animals used to obtain the data for this dissertation. The results of six experiments were discarded without analysis. Two animals developed cerebral edema and did not provide viable preparations. The other four were rejected on the basis of two or more of the following conditions: (1) low amplitude electrical signals; (2) appreciable vertical movement of the cortex; (3) blocked tri-probe tip; or (4) improper experimental techniques. Unmanageable cortical movement was the major problem in three of these four experiments.

Cortical Recruiting Responses

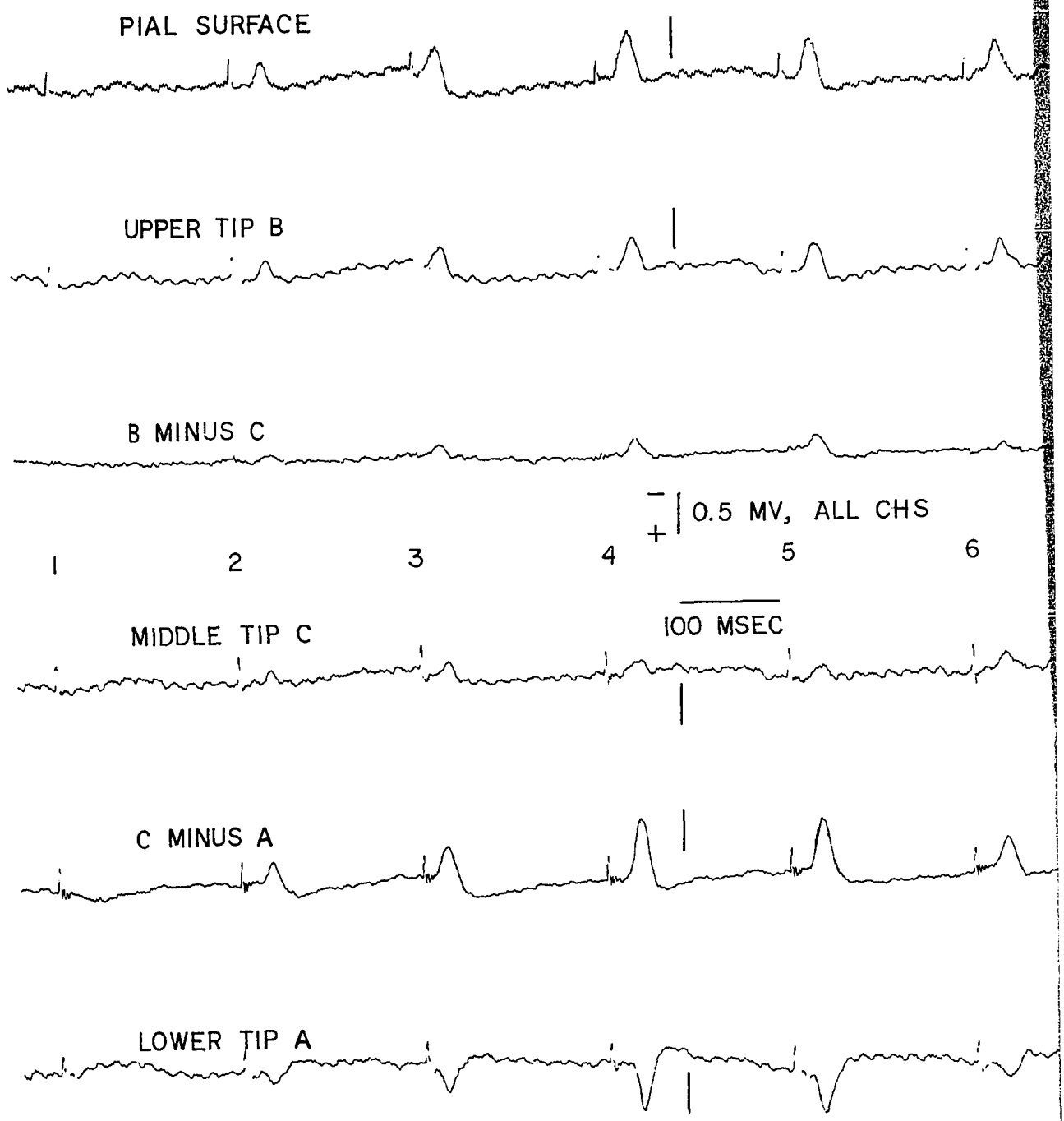
As outlined in Chapter II, a train of responses was obtained at each level of a transcortical exploration of the cortex. The transcortical penetration of the tri-probe was called a track. From the 6 experiments providing the basis for the dissertation, 31 tracks were explored. Twenty-one met the criteria for usability. Of the 10 discarded, 3 were rejected because of a partially blocked or broken tip of the tri-probe. The remaining 7 were discarded on the basis of low amplitude or erratic recruiting responses, disruptive cortical movement, or improper experimental technique.

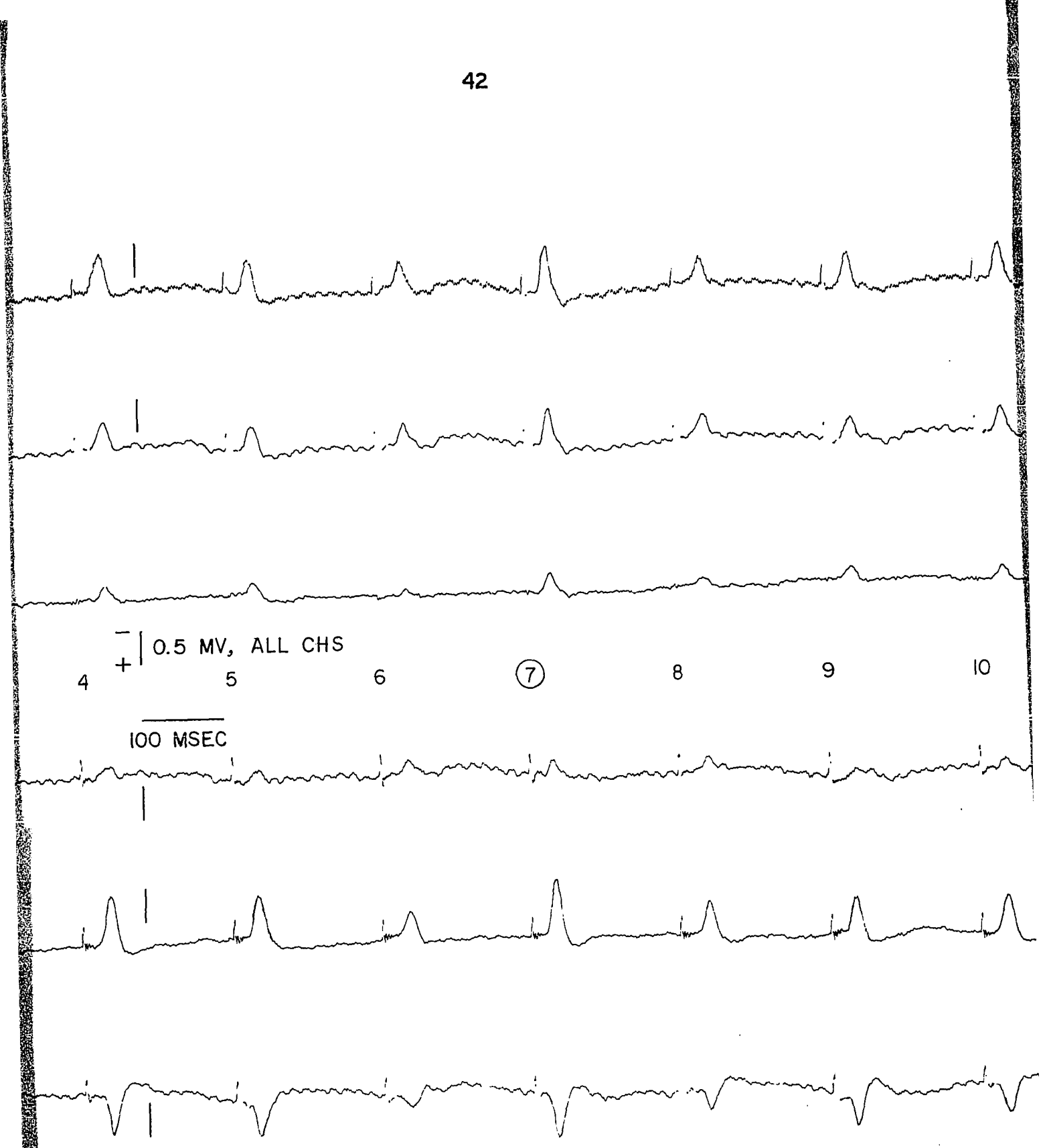
Over 250 response trains from the 21 tracks were examined to abstract the data used in the analysis. Seventeen of these tracks were obtained by use of identical protocol. Variations in this protocol formed the basis for the other 4 tracks.

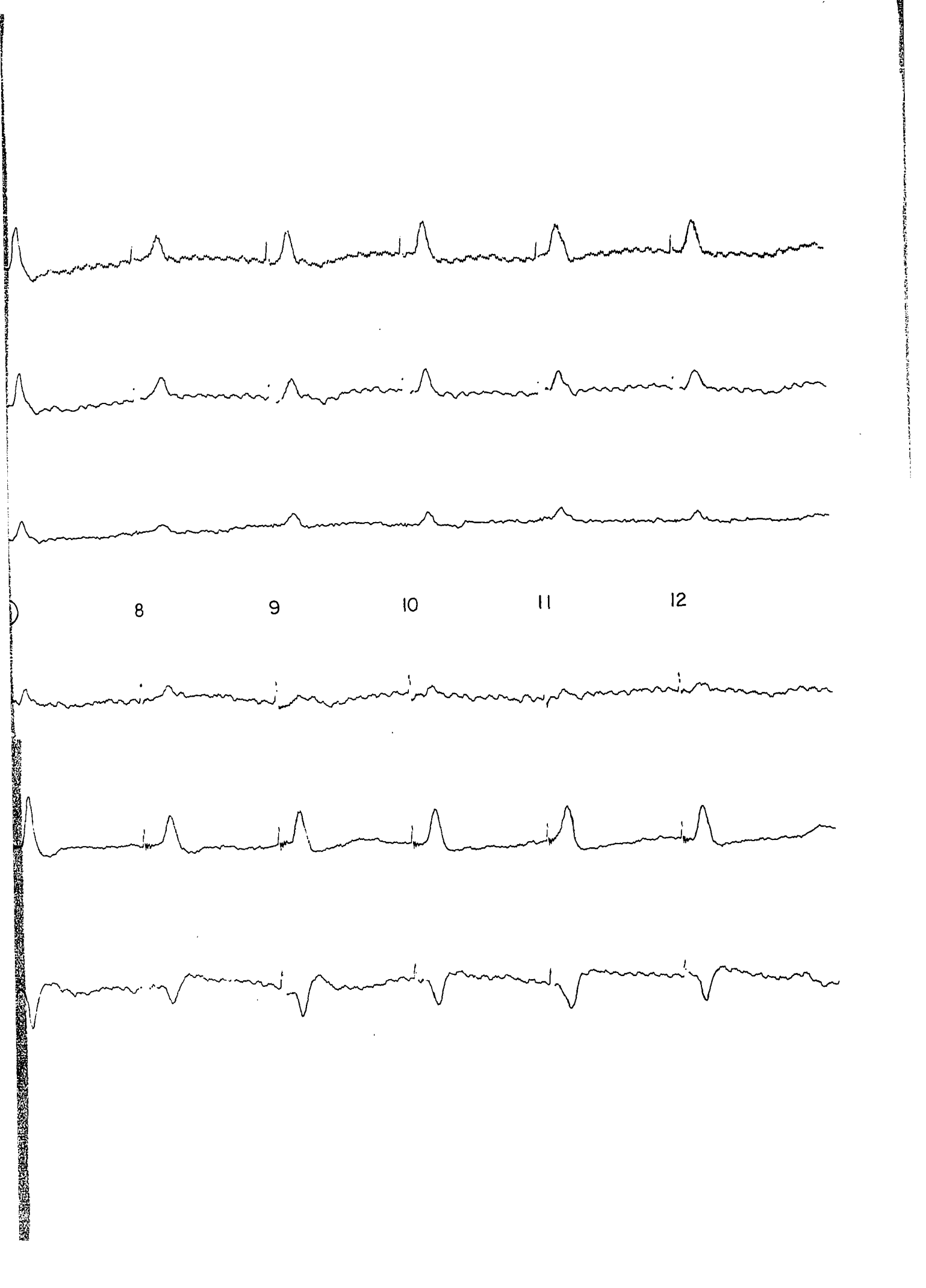
Many investigators have utilized single electrodes in recording the recruiting responses sequentially in transcortical tracks. In general they report that the response is monophasic negative at the surface. At deeper levels the amplitude of the response diminishes to zero, or low-amplitude biphasic, then becomes monophasic positive with increasing depth. The region of polarity inversion has been called the inversion zone and is said to represent an equipotential surface with respect to a remote reference. The classic properties of the recruiting response and the inversion zone are identifiable in the tracing shown in Figure 1. This figure shows a complete 12-pulse stimulus-response train with the lowest tip, A, of the tri-probe located at 350 μ beneath the pia. The spacing between the middle tip, C, and the lower tip, A, was 70 μ. The spacing between the upper tip, B, and the middle tip, C, was 59 μ. The pial surface wick and tri-probe tips B, C and A were monopolar with respect to the frontal bone reference. Note that the tip A is beneath the inversion zone while C is just slightly above. The differential traces, B minus C and C minus A, illustrate the time variant differences in potential between the two pairs of tri-probe tips. Note the direct relationship between response amplitude at the surface and in the depth, including the differential responses. One can also observe

Figure 1. Cortical recruiting responses: a complete stimulus-response train.

This record was selected to illustrate the salient features of the cortical recruiting response simultaneously at the pial surface and in the cortical depth. Tip A of tri-probe 18A was 350 μ beneath the pia. The tri-probe was located at a maximal response site, A +9mm, mid-suprasylvian gyrus. Pulses of 0.5 msec duration were applied at a rate of 5/sec to a coaxial stimulating probe positioned in the central lateral nucleus (A +11, L 3, V +4). The pial wick and tri-probe tips B, C and A are monopolar with respect to the frontal bone. BC tip spacing was 59 μ and the CA spacing was 70 μ. Polarity was negative for an upward pen deflection on all six channels. Experiment 6071-24.


PIAL SURFACE
UPPER TIP B
B MINUS C
0.5 MV, ALL CHS
1
2
3
4
5
6
100 MSEC
MIDDLE TIP C
C MINUS A
LOWER TIP A


0.5 MV, ALL CHS
4
5
6
7
8
9
10
100 MSEC


8
9
10
11
12

that there is some pulse-to-pulse variation in response amplitude. These relationships are important in the subsequent analysis. From each response train only one recruiting response event was selected for analysis. The rationale for this approach is developed in the next section, Method of Analysis. In Figure 1 the response following stimulus pulse 7 was chosen for further analysis.

To illustrate in greater detail the data obtained from a transcortical track at a maximal site, Figures 2 through 7 are presented. These figures, taken from original records in this study, present segments of response trains selected from 6 representative levels of a 13-level track. Figure 2 illustrates the data obtained when the tri-probe was located at the pial surface. The similarity of responses between the pia, B, C and A traces is apparent. It may also be noted that the B-C and C-A differential responses are zero. In Figure 3, tip A was at 450 μ. The zero amplitude response of tip A indicates it's location to be at the inversion zone. The differential response in C-A exists over a physical distance of 129 μ. With continued tri-probe penetration into the cortex (Figures 4-6), tips C and B also pass through the inversion zone. For tip A at 1050 μ, Figure 6, all tri-probe tips are beneath the inversion zone since monophasic positive recruiting responses are seen in the three monopolar tri-probe traces. Observe the differential responses present in B-C. With tip A at 1350 μ (Figure 7) there are no differential responses in the traces of B-C and C-A. The recruiting responses on all

Figure 2. Cortical recruiting responses: pial surface. A segment selected from a stimulus-response train to illustrate the surface and transcortical responses germane to the selection of the response data for analysis of one transcortical track. The individual response selected for analysis follows the circled stimulus pulse number. The following information applies to Figures 2 through 9 and to Table I. Tri-probe 12B was located at a maximal response site, A +6mm, mid-suprasylvian gyrus. The BC spacing was 123 μ and the CA spacing was 129 μ. Pial wick and the tri-probe tips B, C and A were monopolar with respect to the frontal bone. The difference potentials between the tip pairs BC and CA are represented respectively by the traces B-C and C-A. Pulses of 0.5 msec duration were applied to a coaxial stimulating probe positioned in the ventral part of the lateral dorsal nucleus (A +10, L 3, V +5.6). Polarity was negative for an upward pen deflection on all six channels. Vertical calibration was 0.5 mv and the horizontal time calibration was 100 msec. Depth-beneath-pia information in the titles identifies the depth of tip A. Experiment 6452-27.

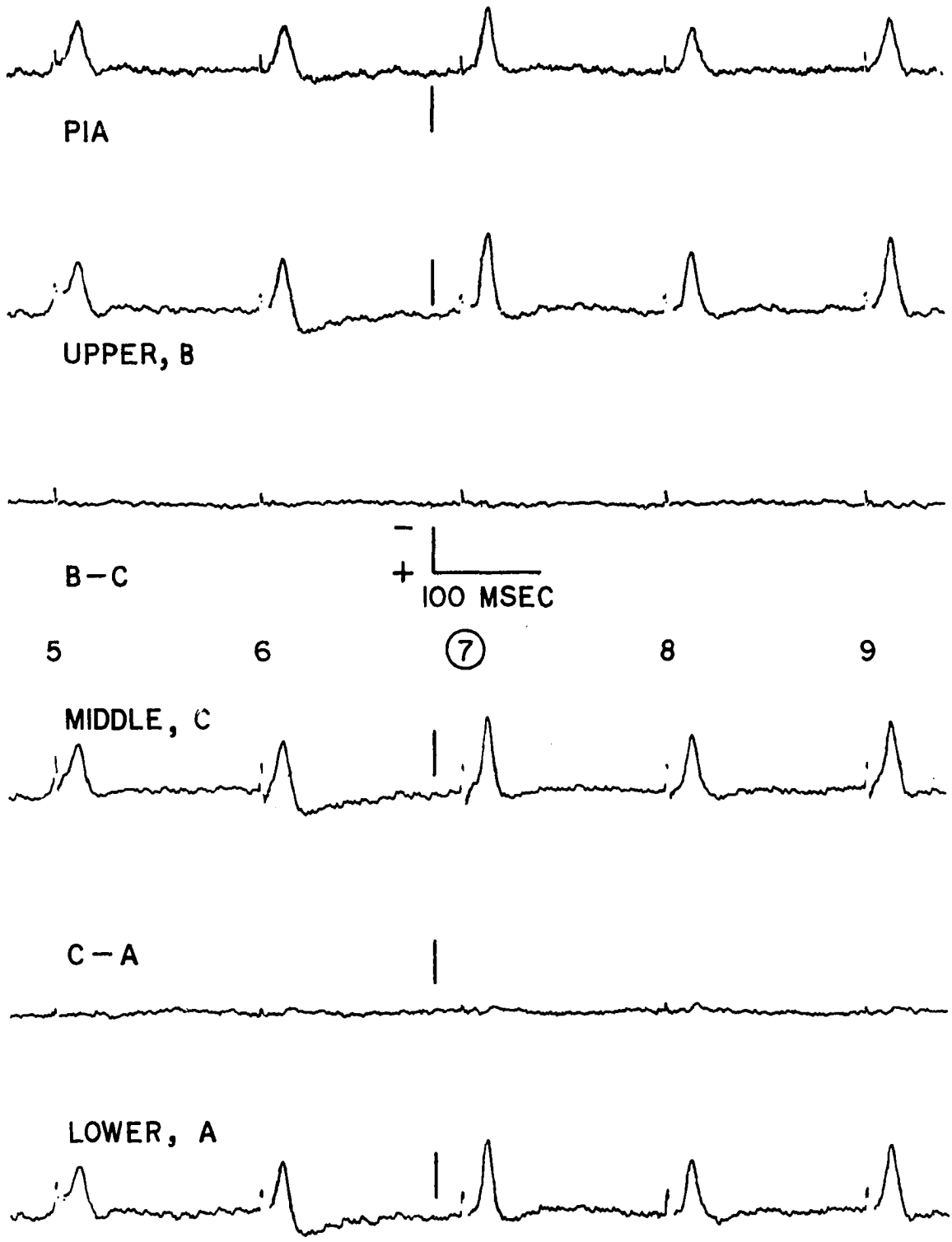

PIA
UPPER, B
B–C
–
+
100 MSEC
5
6
7
8
9
MIDDLE, C
C–A
LOWER, A

Figure 3. Cortical recruiting responses: 450 μ.
The near-zero response amplitude in the trace of tip A indicates that it was very close to the inversion zone. Observe the differential response present in the trace C-A. See the legend of Figure 2 for more complete information.

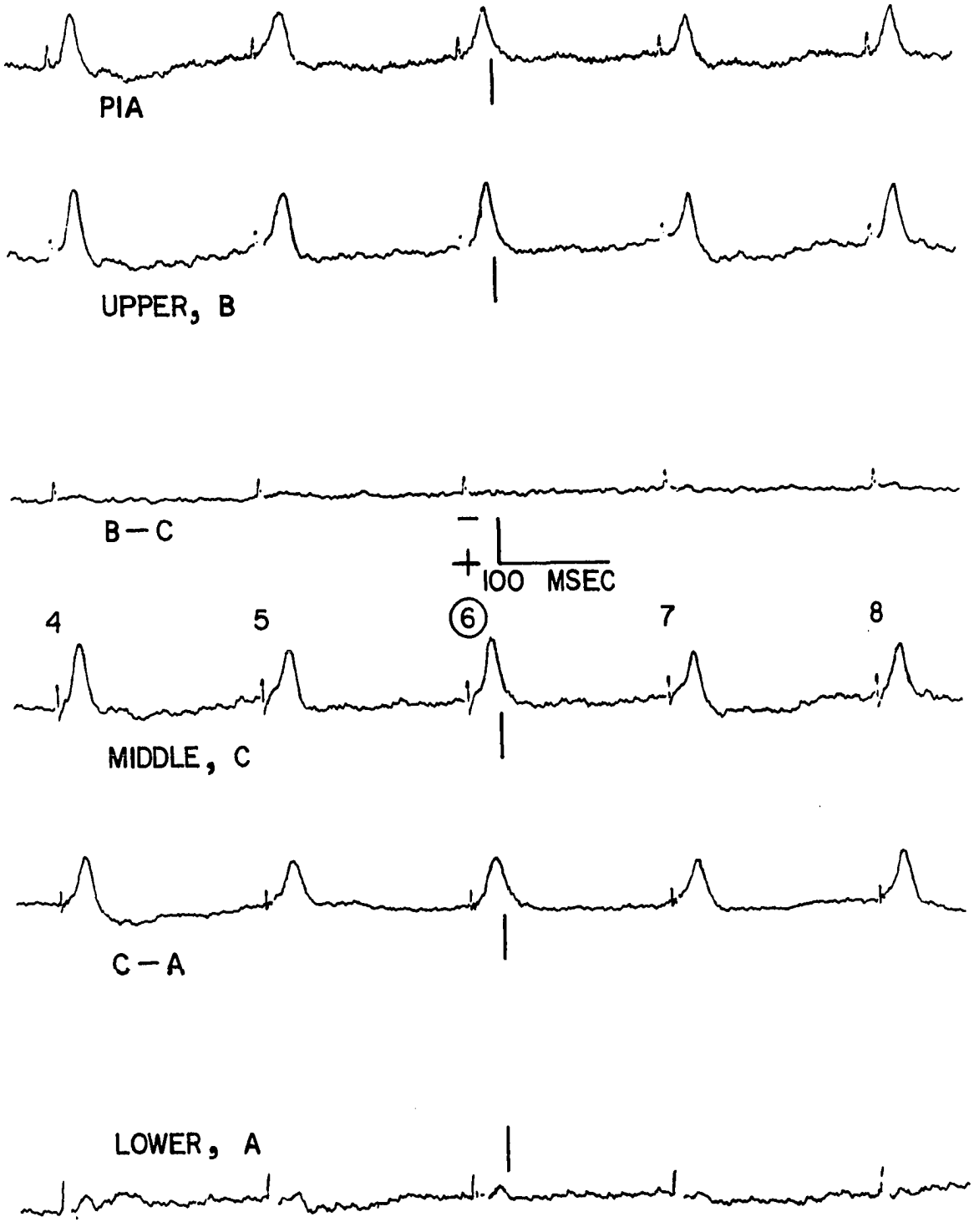

PIA
UPPER, B
B—C
100 MSEC
4
5
6
7
8
MIDDLE, C
C—A
LOWER, A

Figure 4. Cortical recruiting responses: 750 μ. Tri-probe tip A was slightly below the inversion zone while C was a little above the inversion zone. See the legend of Figure 2 for more complete information.

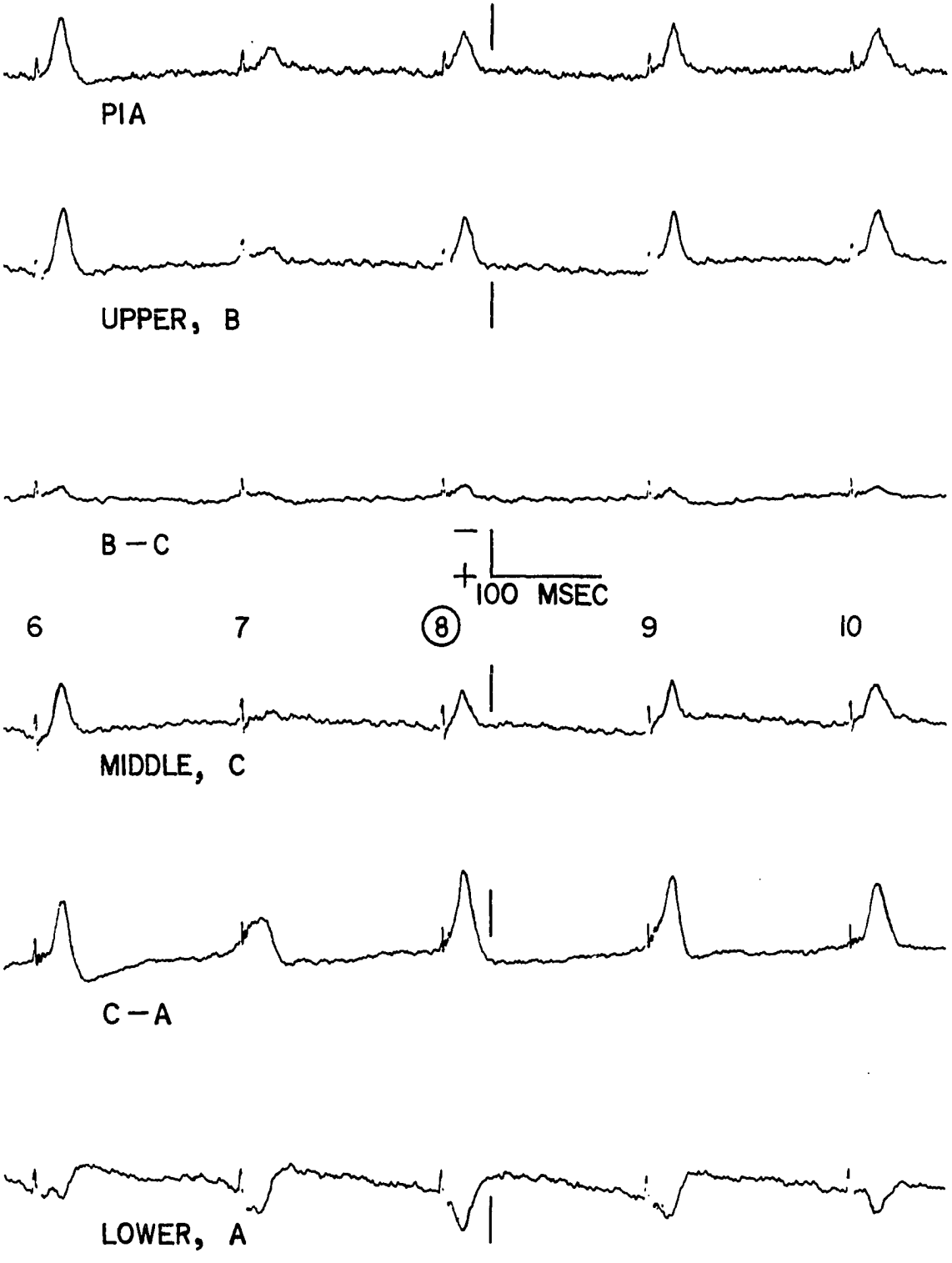

PIA
UPPER, B
B — C
100 MSEC
6
7
8
9
10
MIDDLE, C
C — A
LOWER, A

Figure 5. Cortical recruiting responses: 900 μ. The upper tip B was at the inversion zone. Observe the decreased differential response in C-A and the large differential response in the trace B-C. See the legend of Figure 2 for more complete information.

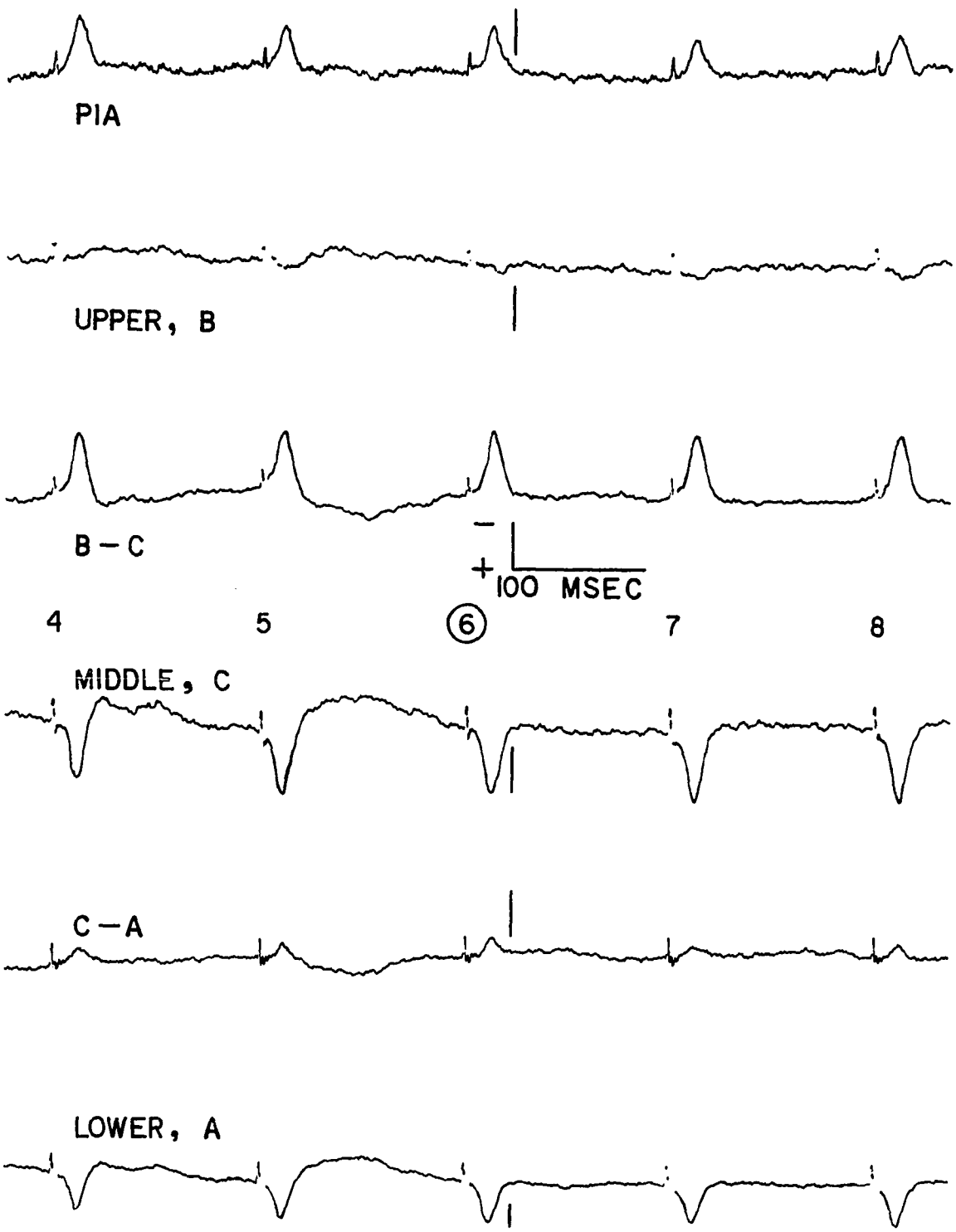

PIA
UPPER, B
B – C
–
+
100 MSEC
4
5
6
7
8
MIDDLE, C
C – A
LOWER, A

Figure 6. Cortical recruiting responses: 1050 μ. All three tri-probe tips were beneath the inversion zone. The differential response B-C has decreased and the C-A differential response is almost zero. See the legend of Figure 2 for more complete information.

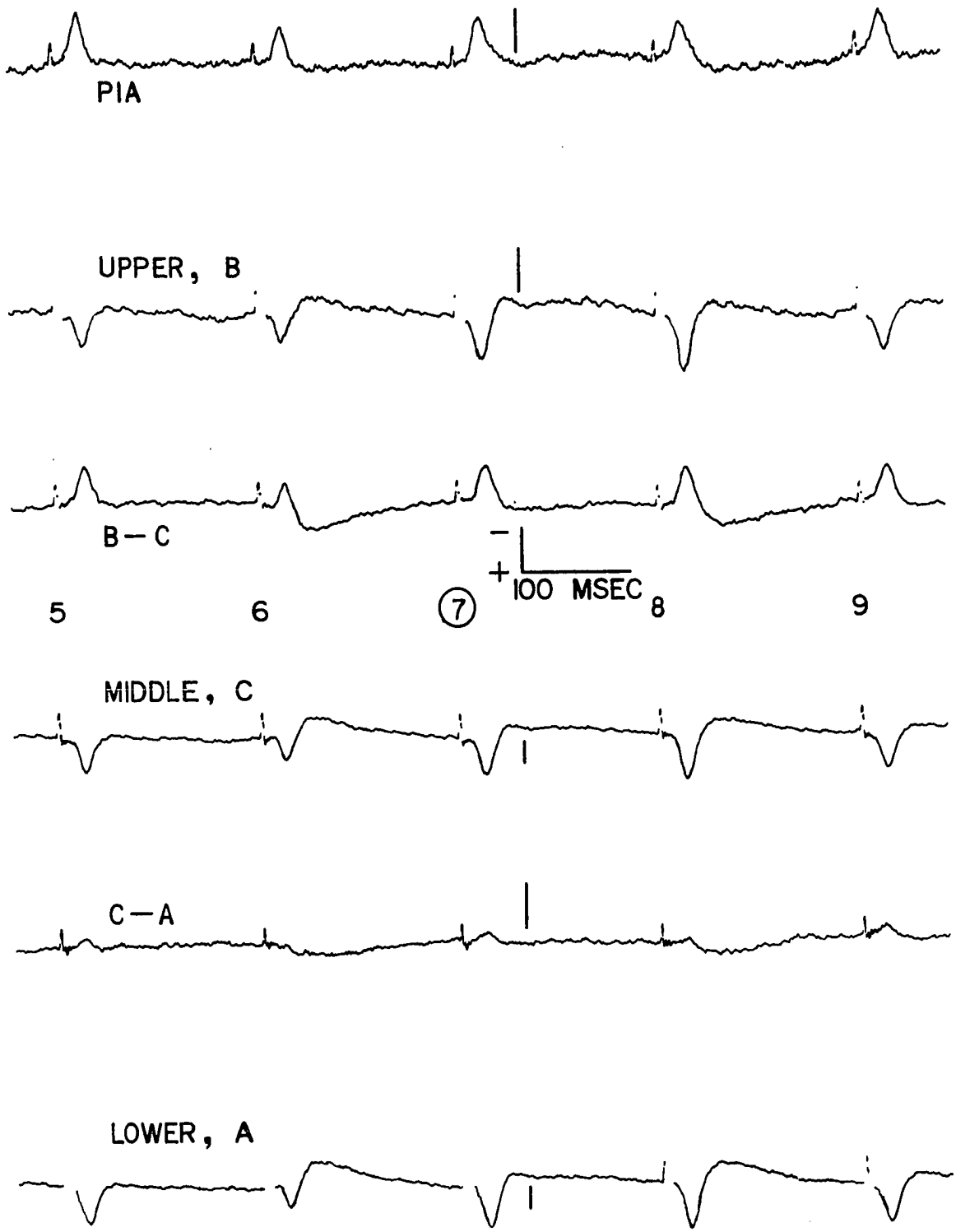
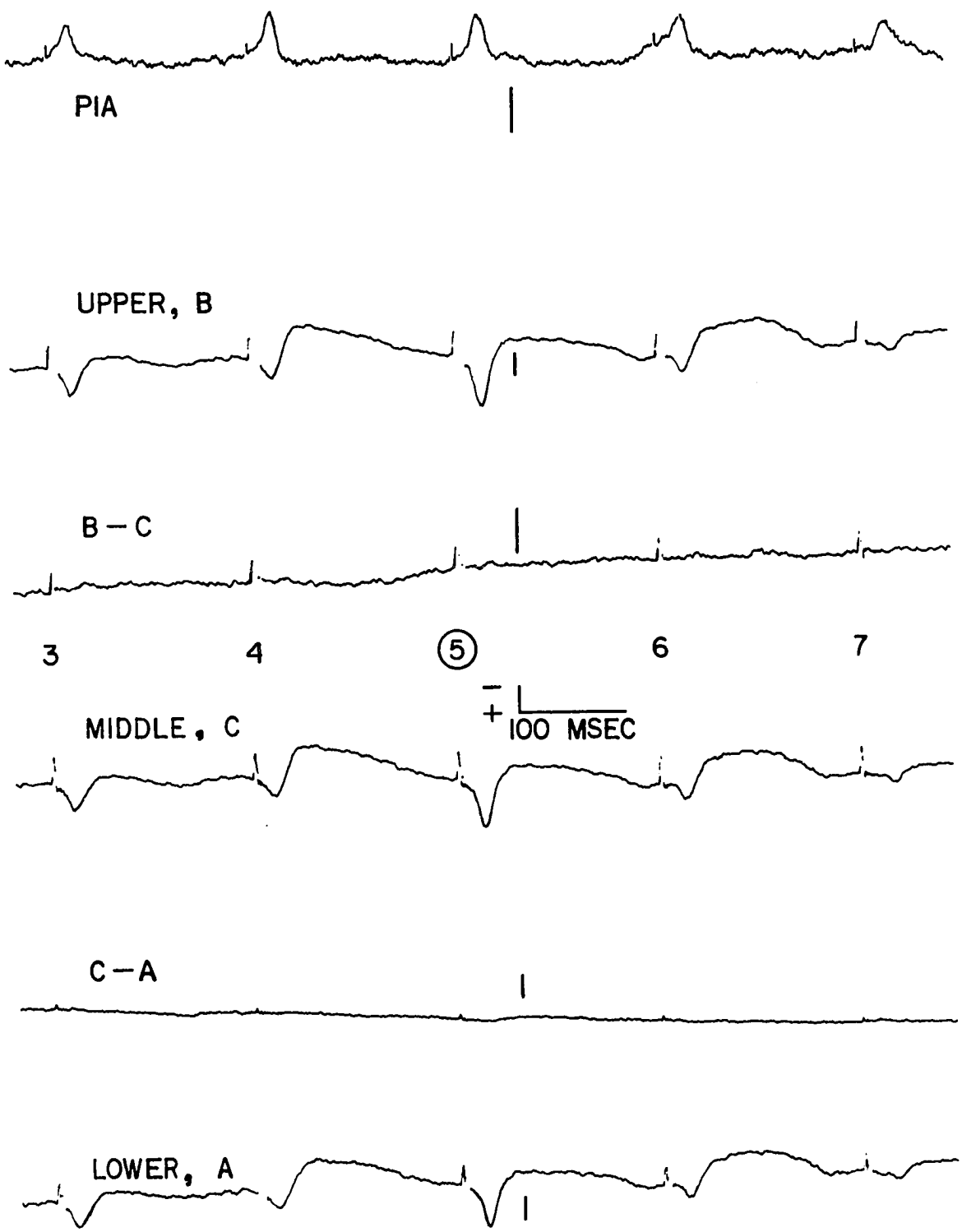

PIA
UPPER, B
B—C
−
+
100 MSEC
5
6
7
8
9
MIDDLE, C
C—A
LOWER, A

Figure 7. Cortical recruiting responses: 1350 μ.
The tri-probe tips were well below the inversion zone and the differential responses are zero. This figure is typical of the responses observed with further tri-probe progression through the cortex to the white matter. See the legend of Figure 2 for more complete information.


PIA
UPPER, B
B – C
3
4
5
6
7
MIDDLE, C
100 MSEC
C – A
LOWER, A

channels remained essentially the same as shown in Figure 7 as the tri-probe was sequentially lowered to the white matter. In these figures the recruiting response following the circled stimulus pulse was selected for further analysis. In all of these data there was no detectable time latency (within ±4 msec) between simultaneous response peaks, regardless of depth.

Selection of Data for Analysis

The first step was to select the responses for use in the analytical procedures. A statistical approach could have been used whereby most of the responses in the train for each trans-cortical level would be amalgamated into a single statistical equivalent response. This procedure would mask the individual variations of the recruiting response (e.g., alternation phenomenon and wax-wane properties) and introduce unwanted parameters of noise and erratic responses. Thus, the approach used was to select only one cortical response from each train. The criteria for this selection were: (1) the amplitude of the cortical recruiting response would be fully developed; (2) the most representative response occurring earliest in the train would be used; (3) only those responses relatively free of noise, cortical movement and spurious artifacts were to be considered; and (4) in the presence of mild cortical movement due to respiration, only those responses occurring during the lowest excursion of the cortex were considered. Further conditions associated with the taking of data were: (1) the amplitude was measured between the onset of the

response and the peak of the response wave; (2) post-response, or after-potential, variations were ignored; and (3) the amplitude data were taken only from the pial surface trace and the differential traces, B-C and C-A.

Table 1 contains a sample tabulation of the data obtained from the 13-step track from which the 6 segments shown in Figures 2 through 7 were selected. (See Appendix III for explanation of symbols and abbreviations). The lower 5 steps were excluded from the table because the differential channels indicated zero amplitude responses. Column 3 of Table 1 lists the depth, S_A, of triprobe tip A from the pial surface. The selected recruiting response amplitudes from the differential trace, B-C, are recorded in column 6 while those from the differential trace, C-A, are recorded in column 8.

Analytical Procedures

The procedures used to analyze the data are based on the principles of electric fields in conducting media, as discussed in Materials and Methods. Briefly, the spatial gradient of potential as a function of depth beneath the pia was determined. Evaluation of the rate of change of the potential gradient as a function of depth yielded the divergence. The divergence function, when not equal to zero, was interpreted in terms of sources and sinks of ionic current.

As previously mentioned, there were pulse-to-pulse amplitude variations in the response trains. Figure 1 clearly shows

TABLE I

SAMPLE TABULATION: DATA AND ANALYTICAL RESULTS

1 DATA PAGE	2 PULSE NO. IN TRAIN	3 S_A μ	4 ΔV_{PIA} mv	5 $\frac{\overline{\Delta V}}{\Delta V}\Big\|_{pia}$	6 ΔV_{B-C} mv	7 G_{BC} $\mu v/\mu$	8 ΔV_{C-A} mv	9 G_{CA} $\mu v/\mu$	10 D $nv/\mu/\mu$	11 S_A' μ
70225	7	00			0.00	0.00	0.00	0.00	0.00	00
70229	7	150	-0.50	0.925	0.00	0.00	-0.15	-1.02	- 8.08	24
70233	7	300	-0.55	0.841	0.00	0.00	-0.30	-1.93	-15.35	174
70243	6	450	-0.50	0.925	0.00	0.00	-0.50	-3.52	-27.90	324
70248	9	600	-0.40	1.156	-0.03	-0.23	-0.37	-3.24	-23.87	474
70253	8	750	-0.35	1.321	-0.15	-1.59	-0.70	-7.14	-44.05	624
70259	6	900	-0.45	1.028	-0.65	-5.34	-0.20	-1.54	+30.18	774
70263	7	1050	-0.50	0.925	-0.35	-2.59	-0.10	-0.65	+15.41	924
70268	7	1200	-0.45	1.028	-0.15	-1.23	-0.05	-0.31	+ 7.34	1074
70273	5	1350			0.00	0.00	0.00	0.00	0.00	1224

$\Sigma \Delta V = -3.70$

$\overline{\Delta V} = -0.463$

that the larger the pial response amplitude, the larger the response amplitudes in the traces B, C, A and in the differential traces B-C and C-A. These transcortical amplitude relationships can also be seen in Figures 2 through 7. Thus, in order to make valid comparisons of the differential responses at the different transcortical levels, a differential response amplitude correction was devised based on the pial response amplitude. First, a mean value of pial response amplitude was computed (bottom of Table 1, column 4). The population sample of pial response amplitudes used in this computation was limited to those cortical levels which exhibited differential responses. As shown in Table 1, for example, the differential response amplitudes at 00 and 1350 μ were 0.00. Thus, the corresponding pial response amplitudes were omitted from the population sample used in the computation of the mean. The equalization ratio, or correction factor, (for adjusting the differential response amplitudes for the track) was based on the ratio of the mean pial response to the individual pial response at each level. The results of this calculation are shown in column 5 of Table 1.

The next step was to compute the component of the spatial gradient of potential, G_{BC} and G_{CA}, parallel to the transcortical track. These calculations were based on the following relationships:

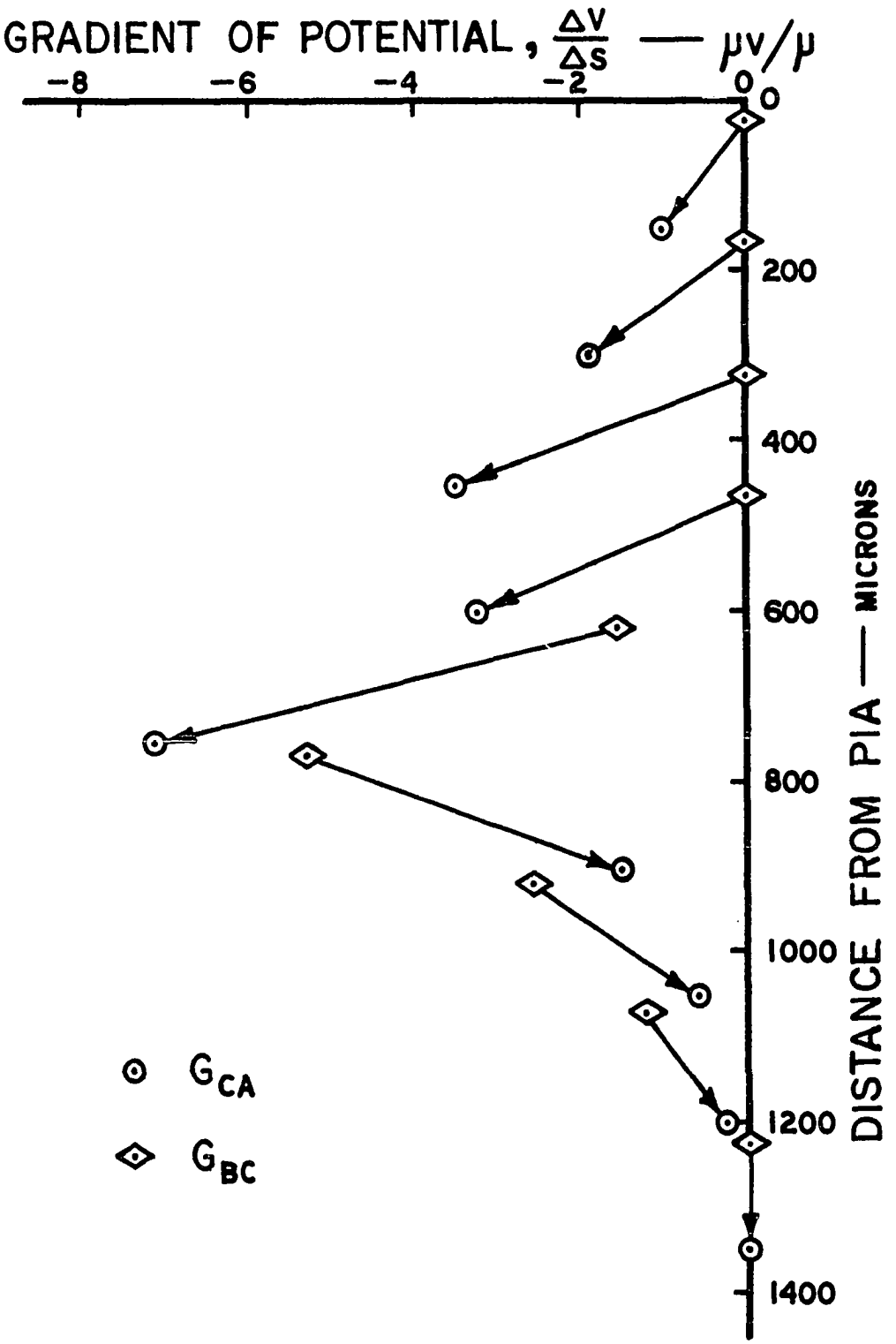
$$G_{BC} = \frac{\left(\Delta V_{B\text{-}C}\right)\left(\frac{\overline{\Delta V}}{\Delta V}\Big|_{pia}\right)}{S_{BC}}$$

$$G_{CA} = \frac{(\Delta V_{C-A})\left(\frac{\overline{\Delta V}}{\Delta V}\Big|_{pia}\right)}{S_{CA}}$$

where S_{BC} and S_{CA} represent the spacing, or separation distance, between tri-probe tip pairs BC and CA, respectively. For the data of Table 1 the S_{BC} was 123 μ while the S_{CA} was 129 μ. Observe that this calculation also equalizes the transcortical differential response amplitudes. The results of these computations are shown in columns 7 and 9 of Table 1. For convenience in plotting the gradient of potential as a function of depth from pia, the G_{CA} value was assumed to occur at S_A beneath the pia (the depth of tri-probe tip A). Also, G_{BC} was assumed to occur at the location of the middle tip, C, beneath the pia. Figure 8 presents a plot of the results recorded in columns 3, 7 and 9 of Table 1. Those points coincident in time at each depth setting are connected by an arrow, arbitrarily directed from G_{BC} to G_{CA} to add clarity. It can be seen from Figure 8 that the gradient of potential along the transcortical track becomes increasingly more negative with tri-probe penetration until a depth of about 750 μ is reached. At lower levels the potential gradient diminishes in negative amplitude, becoming zero at about 1200 μ and remaining at that value throughout the deeper levels of cortex. The failure of the points

Figure 8. The spatial gradient of potential along a transcortical track.

This figure was plotted from the results recorded in columns 3, 7 and 9 of Table 1. See the legend of Figure 2 for more complete information pertaining to this track.

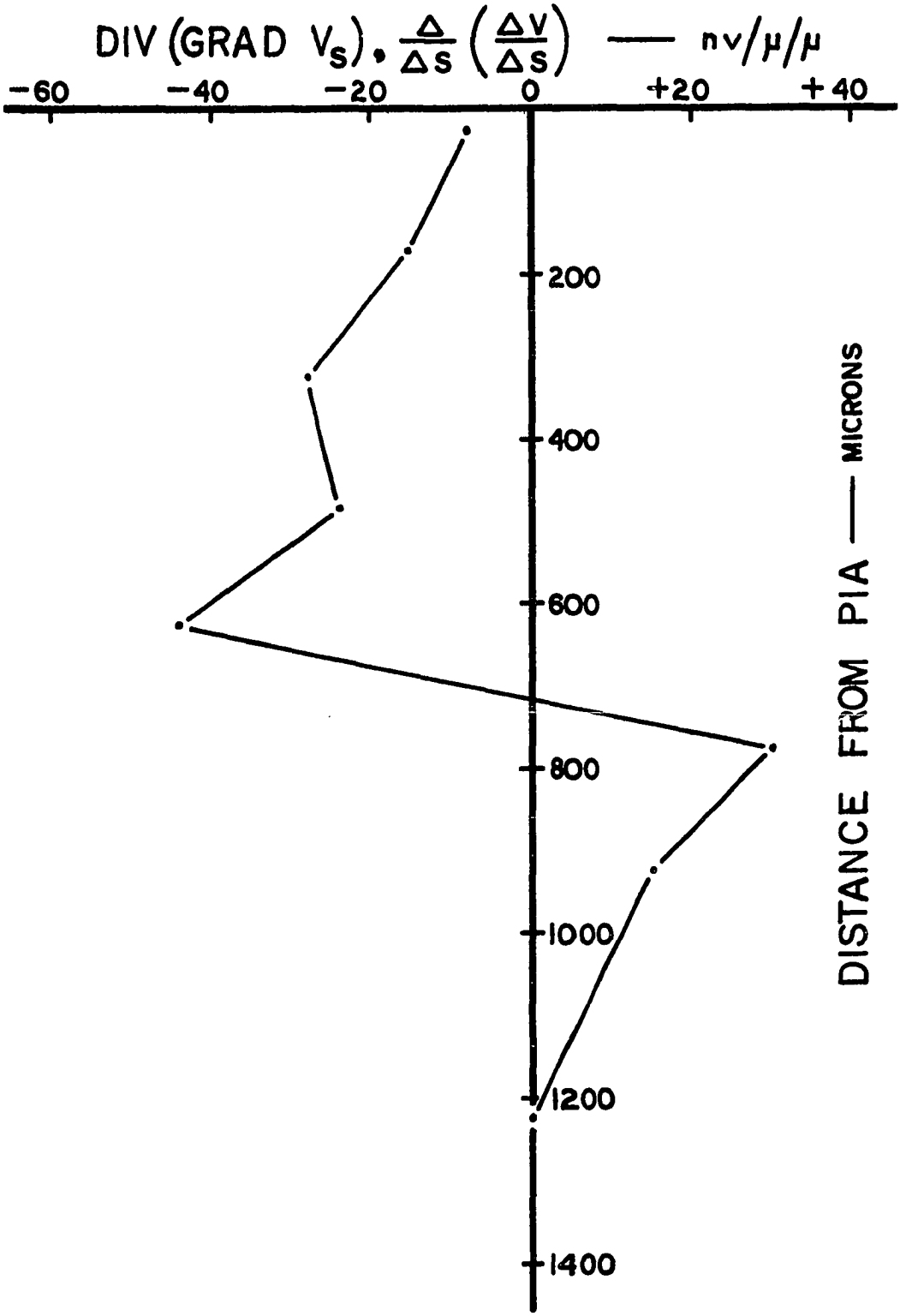

GRADIENT OF POTENTIAL, $\frac{\Delta V}{\Delta S}$ — μv/μ
-8
-6
-4
-2
0
0
200
400
600
800
1000
1200
1400
DISTANCE FROM PIA — MICRONS
G_{CA}
G_{BC}

to describe a smooth, continuous function is believed to be due to imperfections of the experimental techniques, i.e., tissue trauma caused by tri-probe passage, distortion of electric fields due to the presence of the tri-probe, 'stiction' between the neuronal tissue and the probe with cortical penetration, and the moderately large tip spacings.

As mentioned earlier, the divergence is the rate of change of the gradient with respect to cortical depth. Hence, an evaluation of the slope of the gradient function determines the divergence. Reflecting on the nature of the data plotted in Figure 8, the conclusion was made that the best approximation to the slope of the gradient was, in fact, the slope of the arrows connecting the time-coincident gradient values for each depth setting. Thus, a divergence function was obtained by subtracting the gradient values of time-coincident data points and dividing that result by the mean spacing of the two pairs of tri-probe tips (126 μ for the data of Figure 8). The results of this calculation are recorded in column 10 of Table 1. Each calculated value of divergence was assumed to occur at a distance beneath the pia equal to the depth of tip A, S_A, minus the mean tip spacing (126 μ for these data). The results of this adjustment of "effective" cortical depth, S'_A, are shown in column 11 of Table 1. The results shown in columns 10 and 11 are plotted in Figure 9. The important features of the divergence function in Figure 9 are: (1) the negative value of divergence in superficial cortex with an area-weighted mean depth of about 550 μ; (2) a zero-value crossover just below 700 μ; (3) the positive value

Figure 9. Transcortical divergence of the recruiting response peak amplitude: example 1.

This graph was plotted from the calculated results recorded in columns 10 and 11 of Table 1. See the legend of Figure 2 for more complete information.


DIV (GRAD V_s) • $\frac{\Delta}{\Delta s}\left(\frac{\Delta V}{\Delta s}\right)$ — nv/μ/μ
-60
-40
-20
0
+20
+40
200
400
600
800
1000
1200
1400
DISTANCE FROM PIA — MICRONS

of divergence from about 700 μ to 1200 μ with an area-weighted mean depth just below 800 μ; and (4) the zero value of divergence below 1200 μ. The area-weighted mean depth for a lobe is that depth which equally divides the area of the lobe. The convention of signs is such that a negative value of divergence corresponds to a sink, or a region of diminishing positive ion density, while a positive value corresponds to a source, or a region of increasing positive ion density. Zero values of divergence indicate regions where ion densities are neither increasing nor decreasing (neither source nor sink).

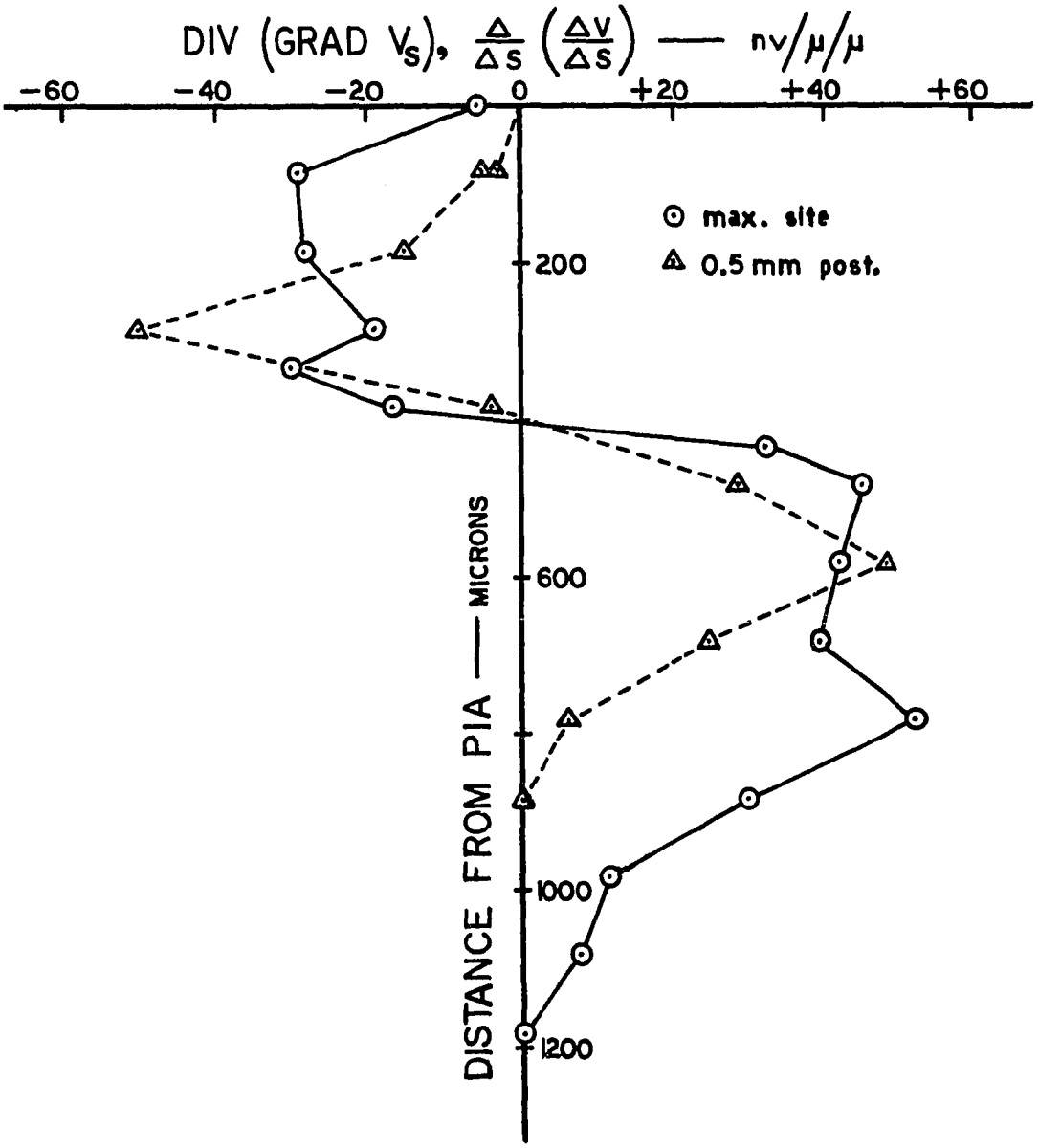
Analytical Results

The preceding two sections presented examples of original data and the procedures by which those data were analyzed to obtain a divergence function for each transcortical track. This section presents additional examples of the divergence functions, a tabular summary of findings, and some of the variations that were observed.

Figure 10 shows the divergence functions obtained for two transcortical tracks in the same preparation, one at the maximal site and one 0.5 mm posterior to that site. Slightly over two hours elapsed between the completion of the maximal site track and the start of the posterior track. During the intervening time two more tracks, whose divergence functions represent novel variations, were explored. These data are reported later in this section. It can be noted that the crossover values of the two functions shown

Figure 10. Transcortical divergence of the recruiting response peak amplitude: example 2.

Tri-probe 19A was located at A +7.3 mm for the maximal site. The BC tip spacing was 92 μ while the CA spacing was 142 μ. The stimulating probe was located in the dorsal portion of the medial dorsal nucleus (A +7, L 2.5, V +5.5). Experiment 0187-22.


DIV (GRAD V_s), $\frac{\Delta}{\Delta s}\left(\frac{\Delta V}{\Delta s}\right)$ —— nv/μ/μ
−60
−40
−20
0
+20
+40
+60
200
600
1000
1200
DISTANCE FROM PIA —— MICRONS
max. site
0.5 mm post.

in Figure 10 are about 400 μ. However, the area-weighted mean depths of the positive and negative lobes are somewhat different. Figure 11 exemplifies those divergence functions obtained from tracks exhibiting deeper crossover levels and broader negative and positive lobes.

Figures 9 through 12 show 8 transcortical divergence functions which are representative of the 17 tracks that were used for this dissertation. Two of the 17 tracks were slightly atypical. In Figure 12 the tracks located 0.5 and 1.0 mm posterior to the maximal site have small negative lobes in the 950-1400 μ range. Also, the divergence for the 0.5 mm posterior site has two values at the same 530 μ level. The time required to change the recorder paper separated the taking of data for the widely spaced points at the 530 μ level. Cortical movement during the paper change interval greatly diminished the validity of the depth information for that track. Therefore, the source-sink separation value for this track was excluded from the tabular results.

Divergence function corssover values, and the locations of 16 transcortical tracks with respect to the maximal site, are shown in Table 2. The maximal site location for the 17th track (experiment 26) was undetermined. The thalamic sites of stimulation corresponding to the 17 tracks are also shown. These data indicate a mean crossover value of about 600 μ at the maximal site. With increasing distance from the maximal site in both anterior and posterior directions, the crossover depth increases to 700 μ

Figure 11. Transcortical divergence of the recruiting response peak amplitude: example 3.

Tri-probe 19A was located at A +5 mm for the maximal site. The BC tip spacing was 92 μ and the CA spacing was 142 μ. The stimulating probe was positioned in the medial dorsal nucleus (A +8, L 2.5, V +4.5). Experiment 6070-21.

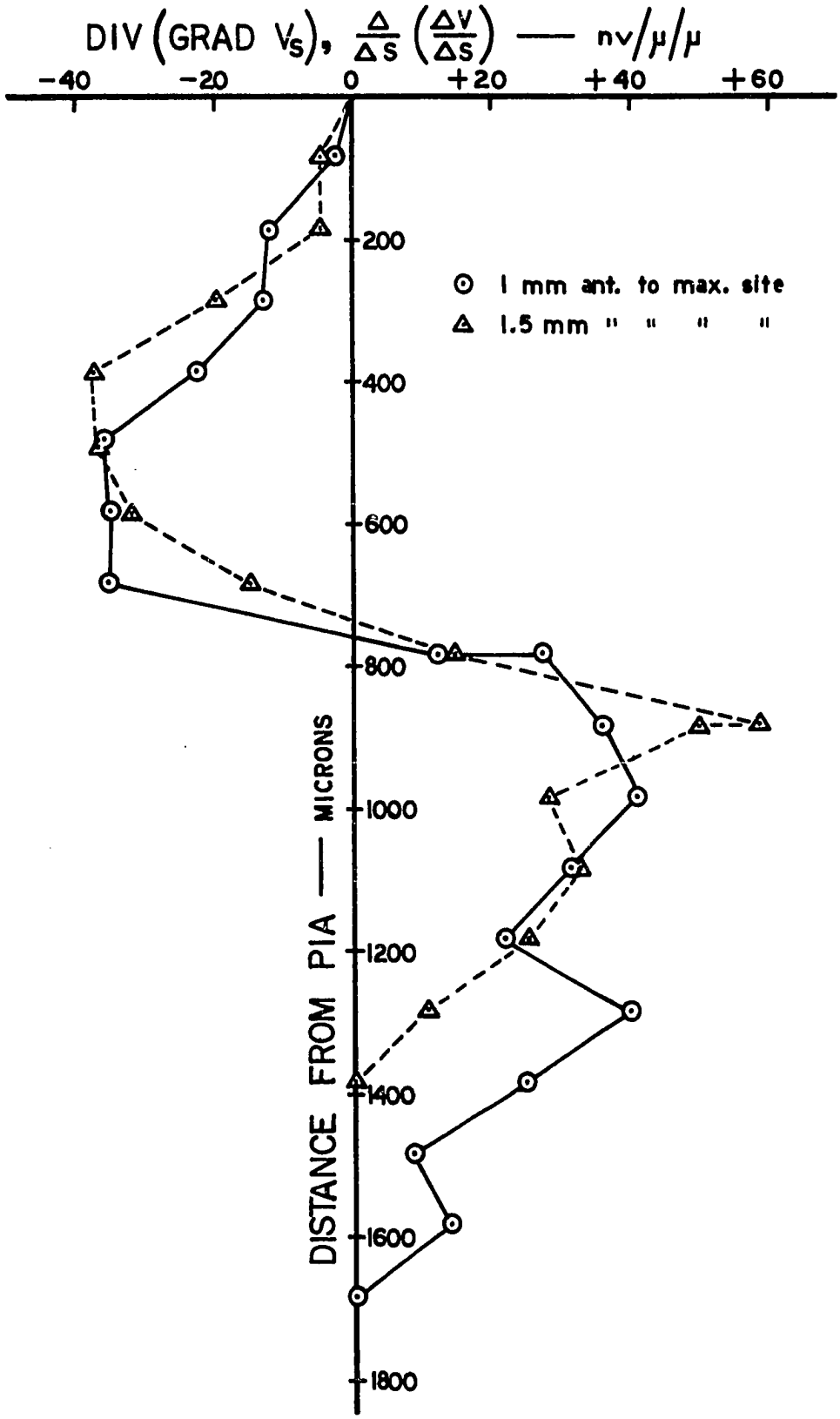

DIV (GRAD Vs), Δ/Δs (ΔV/Δs) — nv/μ/μ
-40
-20
0
+20
+40
+60
200
400
600
800
1000
1200
1400
1600
1800
DISTANCE FROM PIA — MICRONS
⊙ 1 mm ant. to max. site
△ 1.5 mm " " " "

Figure 12. Transcortical divergence of the recruiting response peak amplitude: example 4.

Of the 17 usable transcortical tracks, the two posterior tracks shown here were the only ones exhibiting negative values in the deep cortical levels. Triprobe 18A was located at A +5 for the maximal site. The BC spacing was 59 μ and the CA spacing was 70 μ. The stimulating probe was located in central lateral nucleus (A +8.7, L 3.5, V +3.0) for the maximal site and the 0.5 mm posterior tracks. For the 1 mm posterior track, the stimulating probe coordinates were A +8.5, L 3.5, V +2.5. Experiment 6071-24.

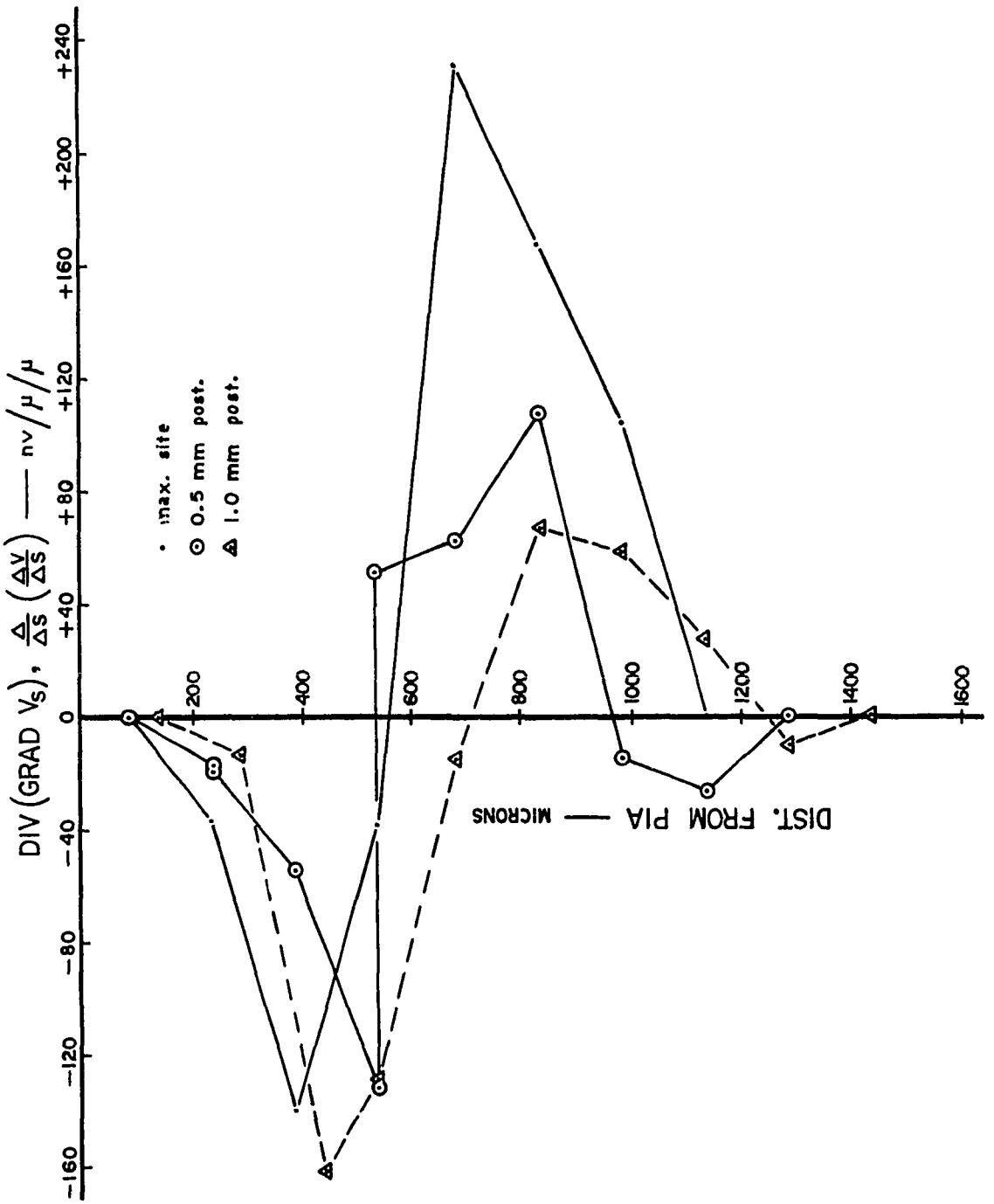

DIV (GRAD Vs), Δ/Δs (ΔV/Δs) — nv/μ/μ
+240
+200
+160
+120
+80
+40
0
-40
-80
-120
-160
200
400
600
800
1000
1200
1400
1600
DIST. FROM PIA — MICRONS
• max. site
⊙ 0.5 mm post.
△ 1.0 mm post.

TABLE 2

DIVERGENCE FUNCTION CROSSOVER VALUES
(MICRONS)

	TRI-PROBE SITE (MID-SSG)							SPRO SITE			
	ANTERIOR, mm			MAX SITE		POSTERIOR, mm					
EXPERIMENT	+1.5	+1.0	+0.5	0.0	STX	−0.5	−1.0	NUCL	A	L	V
6070-21	740	760			+5.0			MD	+8.0	2.5	+4.5
0187-22				400	+7.3	400		MD	+7.0	2.5	+5.5
0189-23			720	700	+8.0			Flds of Forel	+7.8	2.5	−2.0
6071-24				600	+9.0			CL	+11.0	3.0	+4.0
				550	+5.0	530	700	CL	+8.7	3.5	+3.0
6067-26			450	640	+8.0			CM	+7.3	2.5	+2.0
		600			+7.0			LD	+10.5	3.0	+6.5
	UNDETERMINED MAXIMAL SITE ; CROSSOVER AT 500 μ *							CL	+9.8	3.0	+3.8
6452-27		900		700	+6.0	900		LD	+10.0	3.0	+5.6
Σ	740	2260	1170	3590		1830	700	10290	10790 *		
No. of Tracks	1	3	2	6		3	1	16	17 *		
x̄	740	753	585	598		610	700	643	634		

or greater. This trend is supported by the data from two tracks which were excluded from Table 2. The 17 tracks represented in Table 2 were positioned along the midline of the suprasylvian gyrus while these latter two tracks (from experiment 6071-24) were 0.5 mm medial to that midline. With respect to the maximal site of A +9, one track (0.5 mm anterior and 0.5 mm medial) had a crossover depth of 660 μ. The other track (0.5 mm medial to the maximal site) had a crossover depth of 780 μ. It must be admitted that curvature of the gyrus and possible nonparallel alignment of the tri-probe with the apical dendrites may have contributed to an apparent increase in depth of the crossover values.

As mentioned in Analytical Procedures on p. 57 the negative lobes of the divergence functions represent sinks, or regions of diminished positive ion densities, while the positive lobes represent sources, or regions of increased positive ion densities. To evaluate the separation distance between the source-sink regions, an area-weighted mean depth value was estimated for each sink and source for each transcortical track. The area-weighted mean depth value for a lobe was defined as that depth which divided the area of the lobe into two equal parts. The difference between the two mean depth values defined the source-sink separation distance. These values, shown in Table 3, could be reliably determined for 14 of the 17 transcortical tracks shown in Table 2. For all transverse cortical sites the mean separation distances fell in the range from 400 μ to slightly over 500 μ.

TABLE 3

SOURCE-SINK SEPARATION DISTANCE (MICRONS)

	TRI-PROBE SITE (MID-SSG)							
	ANTERIOR, mm			MAX SITE	POSTERIOR, mm			
EXPERIMENT	+1.5	+1.0	+0.5	0.0	−0.5	−1.0		
6070-21	440	410						
0187-22				460	300			
0189-23			400	440				
6071-24				Indeterm.				
				340	Indeterm.	450		
6067-26			390	600				
		Indeterm.						
	UNDETERMINED MAXIMAL SITE ; 600*							
6452-27		500		200	730			
Σ	440	910	790	2040	1030	450	5660	6260*
No. of Tracks	1	2	2	5	2	1	13	14*
$\bar{s}$	440	455	395	408	515	450	435	447

The results shown in Table 2 and 3 (14 tracks) are schematically presented in Figure 13. The six Brodmann layers (Sholl, 1956, p. 48) are outlined with dotted lines for reference. The track at the far right of the figure was not referred to a maximal site due to faulty experimental technique. It can be noted that the positive ion sinks are located in layer II and the upper part of layer III. The positive ion sources are located in layers III, IV, and V. Though shown as discrete points in Figure 13, the recruiting response source-sink locations are more properly represented by laminar surfaces or regions as suggested by the lobular nature of the transcortical divergence functions.

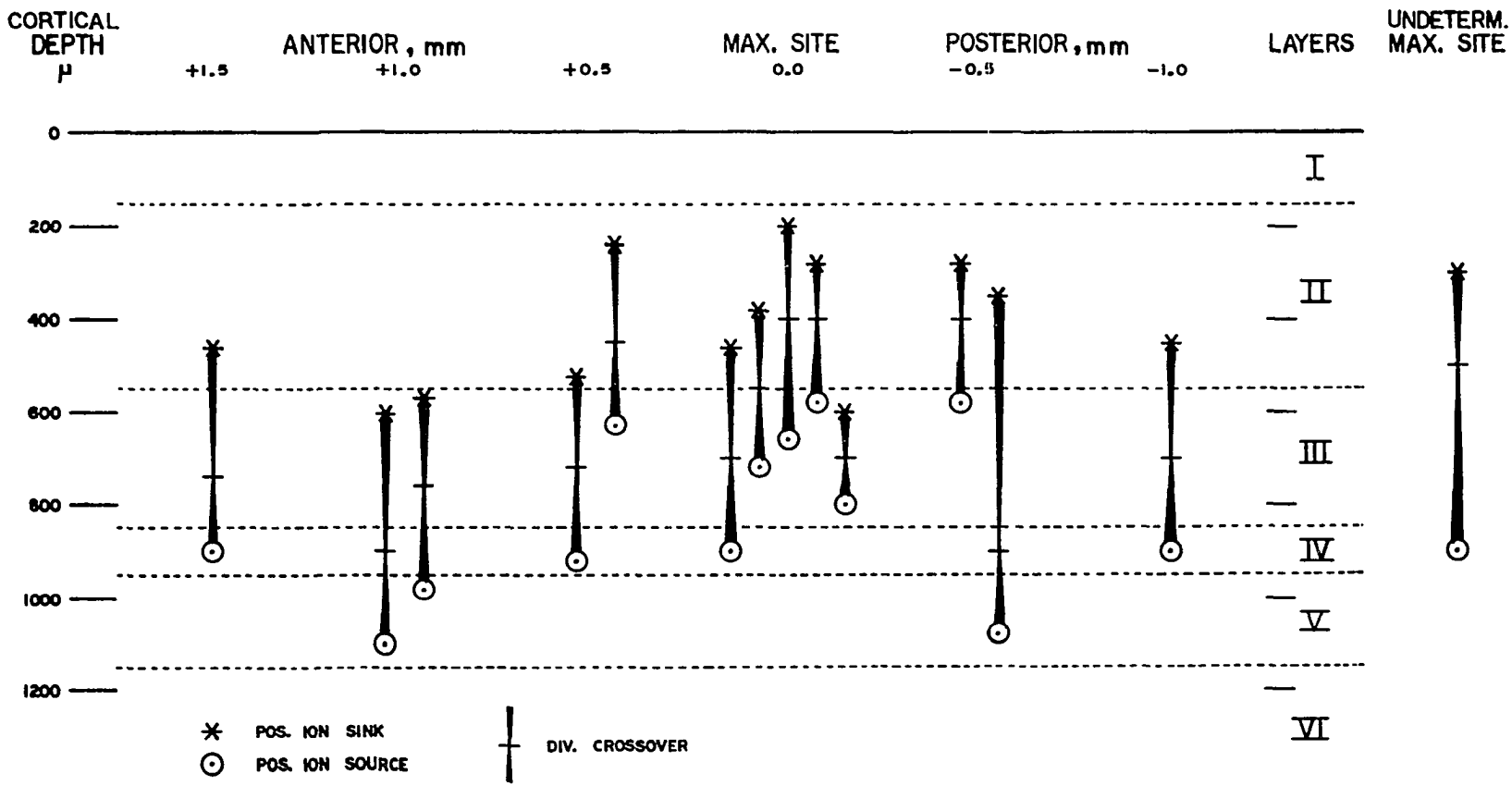
Variations from Normal Results

There are two variations from the "normal" results to be considered. These variations resulted from high frequency stimulation effects and from incremental changes in the stimulating probe site.

Effect of high frequency stimulation. The experimental protocol for two transcortical tracks at the same cortical site was revised in the following way. In addition to the standard 5/sec, 13-pulse stimulus train applied at each transcortical setting of the tri-probe, a 50/sec train of about 2 secs duration was also applied for reasons not developed in this dissertation. Specifically, the procedure (at a given cortical level) was to record the previously-defined 5/sec stimulus-response train.

Figure 13. Schematic diagram of positive ion sinks, sources and divergence cross-overs for 14 transcortical tracks.

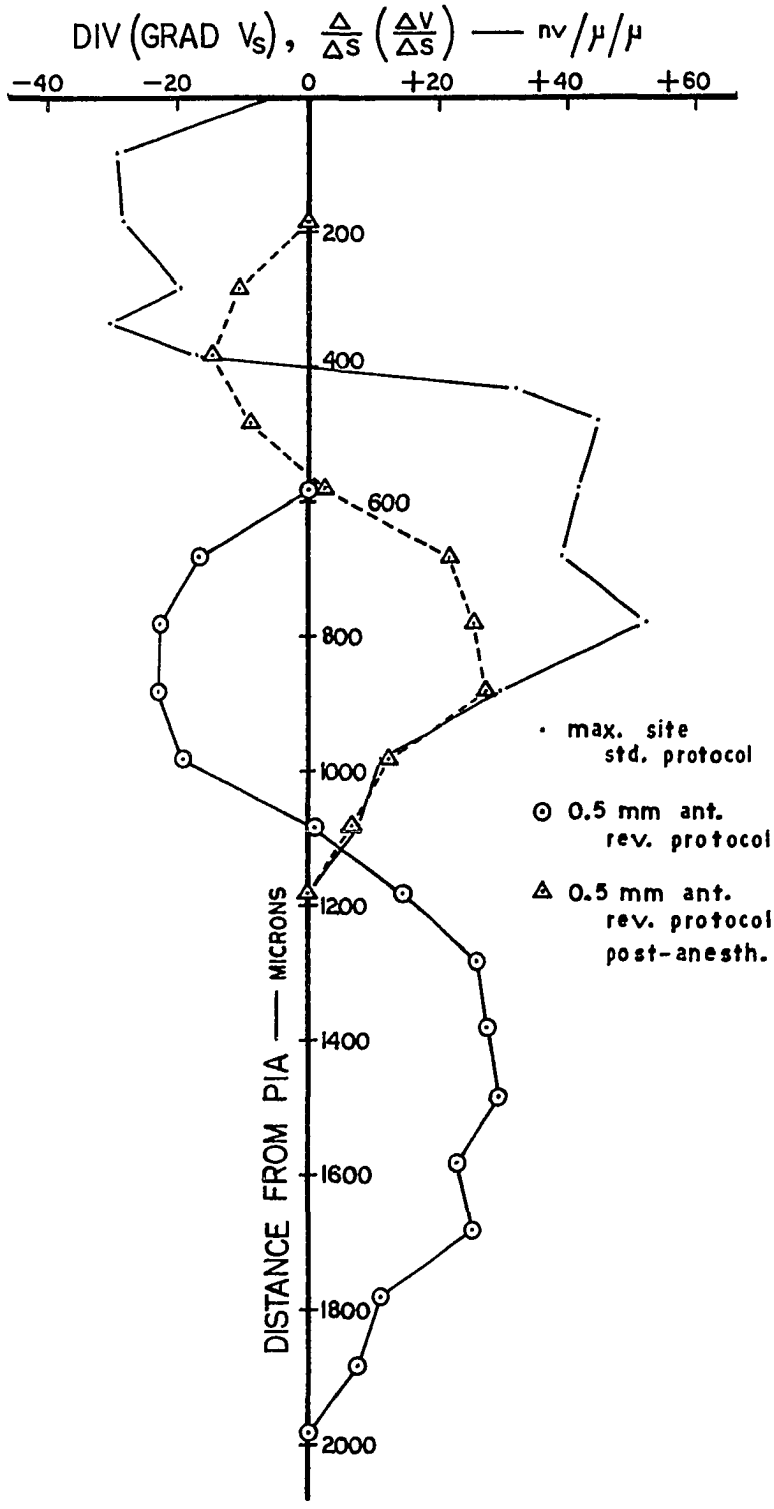
The ion sinks and sources are plotted as discrete points which, in fact, represent the area-weighted mean depths of the negative and positive lobes of the transcortical divergence functions. The Brodmann layers (Sholl, 1956, p. 48), indicated by the dashed lines and labeled to the right of the figure, are included for reference information.


CORTICAL DEPTH μ
ANTERIOR, mm
+1.5
+1.0
+0.5
MAX. SITE
0.0
POSTERIOR, mm
−0.5
−1.0
LAYERS
UNDETERM. MAX. SITE
0
200
400
600
800
1000
1200
I
II
III
IV
V
VI
POS. ION SINK
POS. ION SOURCE
DIV. CROSSOVER

After a 60 sec intertrial interval, a 2-sec train of 50/sec pulses was applied and the cortical responses recorded. Then the tri-probe was incrementally lowered into the cortex and, after the usual 60 sec intertrial interval, the two-step stimulus-response conditions were repeated. The 5/sec stimulus response data were analyzed as previously described and the transcortical divergence functions determined. These results are shown in Figure 14, which includes for reference purposes, the transcortical divergence at a maximal site which was obtained from the use of the standard protocol. Upon completion of the transcortical track, plotted with circled points, a supplementary dose of anesthesia (10% of initial) was administered. Twenty minutes later the second transcortical exploration at the same cortical site was initiated. The results of this second track are plotted with triangular symbols. In comparing these two tracks one must consider that the tissue trauma produced by the first transcortical passage of the tri-probe might affect the results of the second track. Also, careless technique in removing cerebrospinal fluid, prior to determining the pial surface location in stereotaxic coordinates for the first track, would have introduced a depth error corresponding to a deeper-than-usual response. It is difficult to conceive, however, that this error would have been as great as 400 μ.

Effect of varying the stimulating probe site. The transcortical track at anterior +1.0 mm, experiment 6067-26, was a partial track. Transcortical exploration was stopped with the tri-probe tip A at 700 μ, slightly below the inversion zone. The

Figure 14. Transcortical divergence of the recruiting response peak amplitude: variation from normal. The maximal site divergence is included for reference (tri-probe 19A, A +7.3mm). The revised protocol consisted of adding a 50/sec stimulus train following the completion of the 5/sec train by the 60 sec intertrial interval at each transcortical setting of the tri-probe. See the legend of Figure 10 for additional information. Experiment 0187-22.


DIV (GRAD Vs), Δ/Δs (ΔV/Δs) — nv/μ/μ
−40
−20
0
+20
+40
+60
200
400
600
800
1000
1200
1400
1600
1800
2000
DISTANCE FROM PIA — MICRONS
· max. site std. protocol
⊙ 0.5 mm ant. rev. protocol
△ 0.5 mm ant. rev. protocol post-anesth.

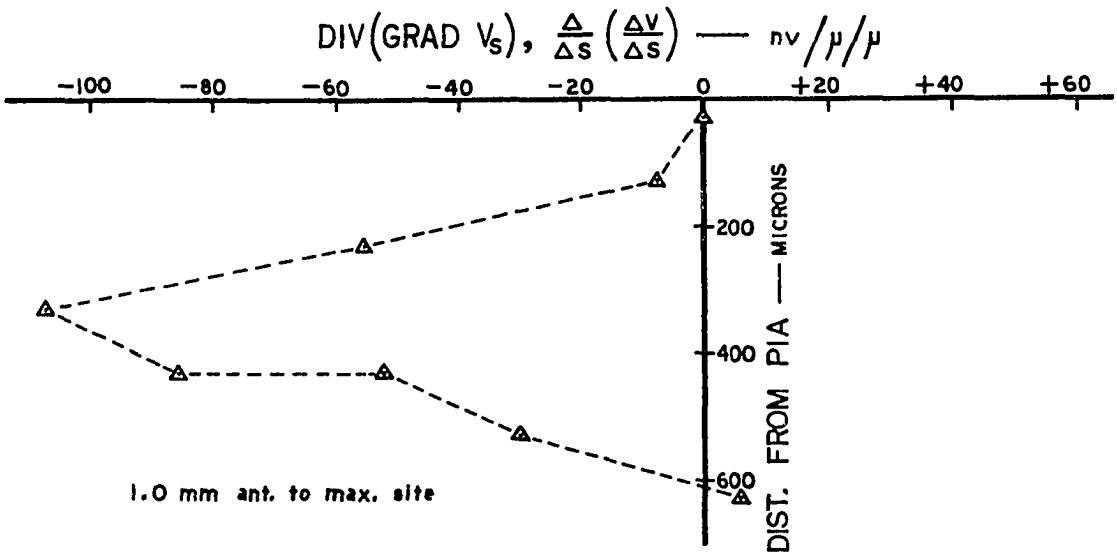
divergence for the superficial portion of the track is shown in the upper part of Figure 15. The standard protocol was altered in the following manner: with the tri-probe fixed in position (tip A at 700 μ), the stimulating probe was progressively advanced in 0.5 mm increments along the $+16^{o}$ sagittal angle through the lateral dorsal nucleus into the central lateral nucleus. At each position the recruiting response to the 5/sec stimulus train was recorded. The divergence function produced by analysis of these data is shown in the lower part of Figure 15. The data point at 0.0 in the lower part coincides with the deepest data point in the upper part. One interpretation of these results is that the divergence crossover, or inversion zone, moved upward in the cortex with the generally downward movement of the stimulating probe. Another possible explanation is that the cortical location of the maximal site is related to the thalamic site of stimulation and the apparent upward shift in inversion zone resulted from a transverse shift of the maximal site along the cortex. Of course, a third possible interpretation is a combination of the first two.

Post-stimulus Responses

Study of about 650 stimulus-response trains revealed occasional recruiting-like cortical responses following the cessation of the stimulus. This response was characterized by: (1) a waveform of the same polarity and the same general amplitude-duration shape as the last stimulus-driven cortical recruiting response; (2) the interval between the last driven response and the "spontaneous" event, or between the multiple response events, was

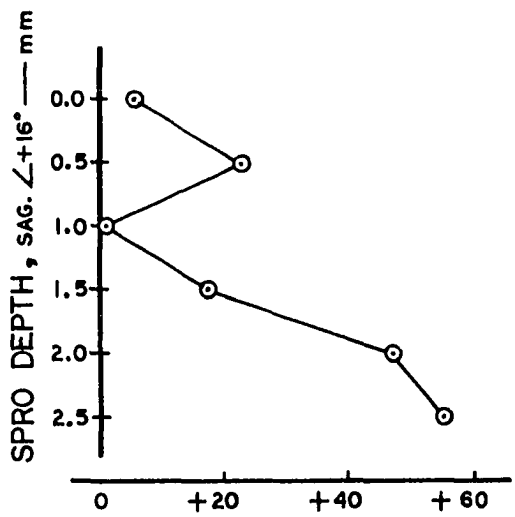
Figure 15. Effect of stimulating site on the transcortical divergence.

The upper part of the figure was a partial transcortical track with the tri-probe tip A fixed at 700 μ, just beneath the inversion zone. The value of the divergence at that point is identical to the value shown in the lower part of the figure for the stimulus site 0.0. The coaxial stimulating probe was advanced in 0.5 mm increments with cortical recruiting responses obtained at each position. Tri-probe 18A with a BC spacing of 59 μ and a CA spacing of 70 μ was used. The maximal site was at A +8 mm. Experiment 6067-26.



STIMULUS SITE

Nucl.	A	L	V
LD	+10.5	3	+6.5
LD	+10.4		+6.0
LD-CL	+10.3		+5.5
CL	+10.1		+4.8
CL	+10.0		+4.4
CL	+ 9.8		+3.8



approximately that of the stimulus period, 188 msec; and, (3) the duration was inversely related to the amplitude. This response was called the post-stimulus cortical response, or PSR.

Figure 16 illustrates an example of a single PSR occurrence. Cortical responses to the stimulating pulses 12 and 13 indicate that the tri-probe tips are below the inversion zone. There is a slight differential response in the B-C channel which exhibits a waning trend. The single PSR occurred 152 msec after the last stimulus-driven recruiting response.

Out of 635 possible opportunities for the PSR to occur, there were 161 PSR events consisting of one or more responses. Forty-seven had two or more responses while 21 showed three or more. There were 6 of the 161 events exhibiting 4 or more PSR's. An example of a four-PSR event is shown in Figure 17. The PSR's are most evident in the monopolar records from tips A and C. They are also distinguishable in the tip B record and in the differential record, B-C. There were only 2 out of the 161 PSR events which exhibited 5 sequential responses.

Determination of the validity of a PSR event was complicated by the effect of the cardiac systolic pulsations on cortical movement. In some preparations each systole caused a transient pulsation of cortical movement which appeared as a small amplitude variation in the record. This effect could be sorted out in almost all instances by the unique variation of the signal and by its periodicity in the record. For example, in Figure 17, immediately following the fourth PSR, the negative-positive after-

Figure 16. Post-stimulus cortical response: single occurrence. Tri-probe 18A was located at a maximal site, A+7mm. The BC spacing was 59 μ and the CA spacing was 70 μ. Tip A was 1800 μ beneath the pial surface. The stimulating probe was positioned in the lateral dorsal nucleus (A +10.5, L 3, V +6). The vertical calibration was 0.5 mv with an upward deflection representing a negative potential. Experiment 6067-26.

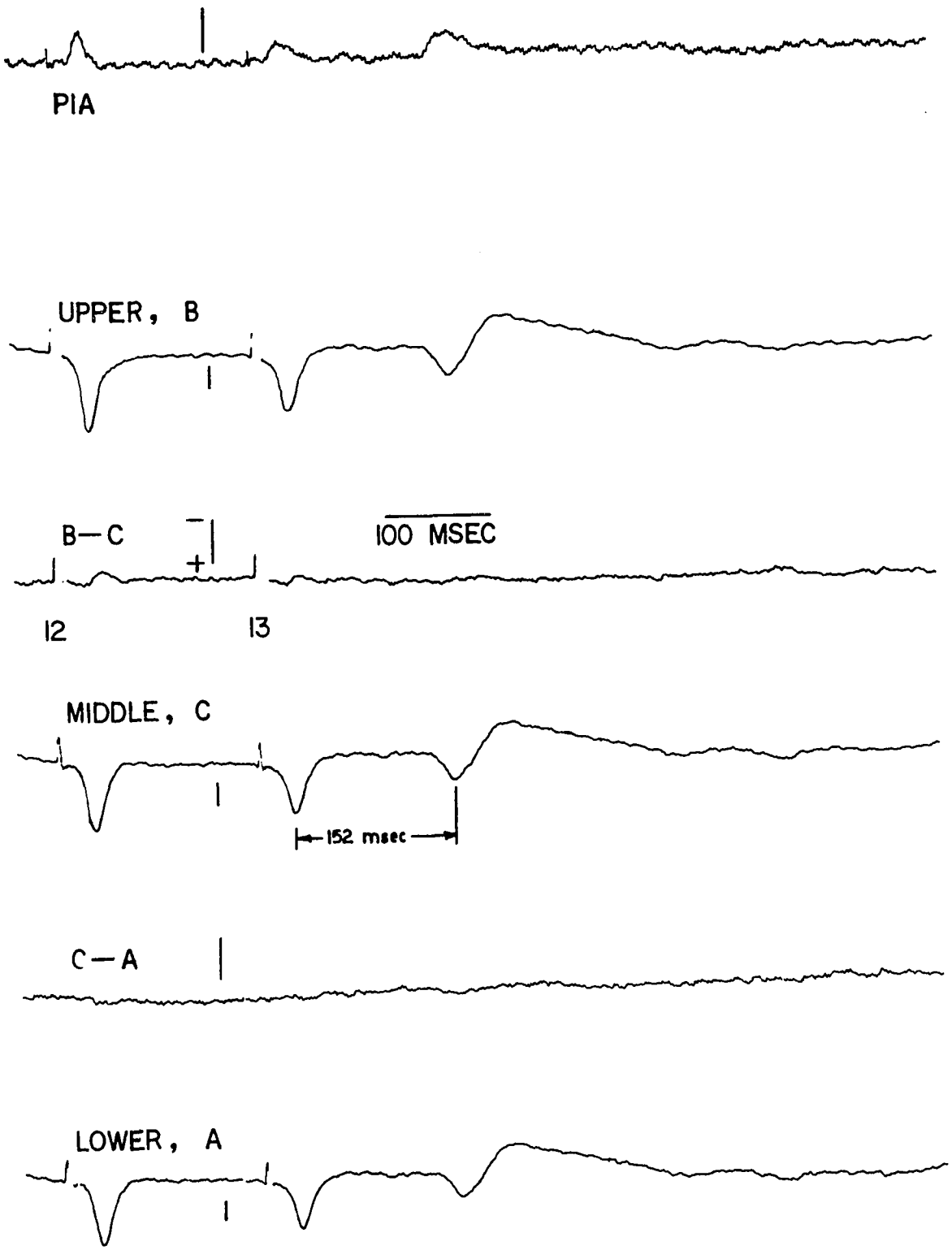
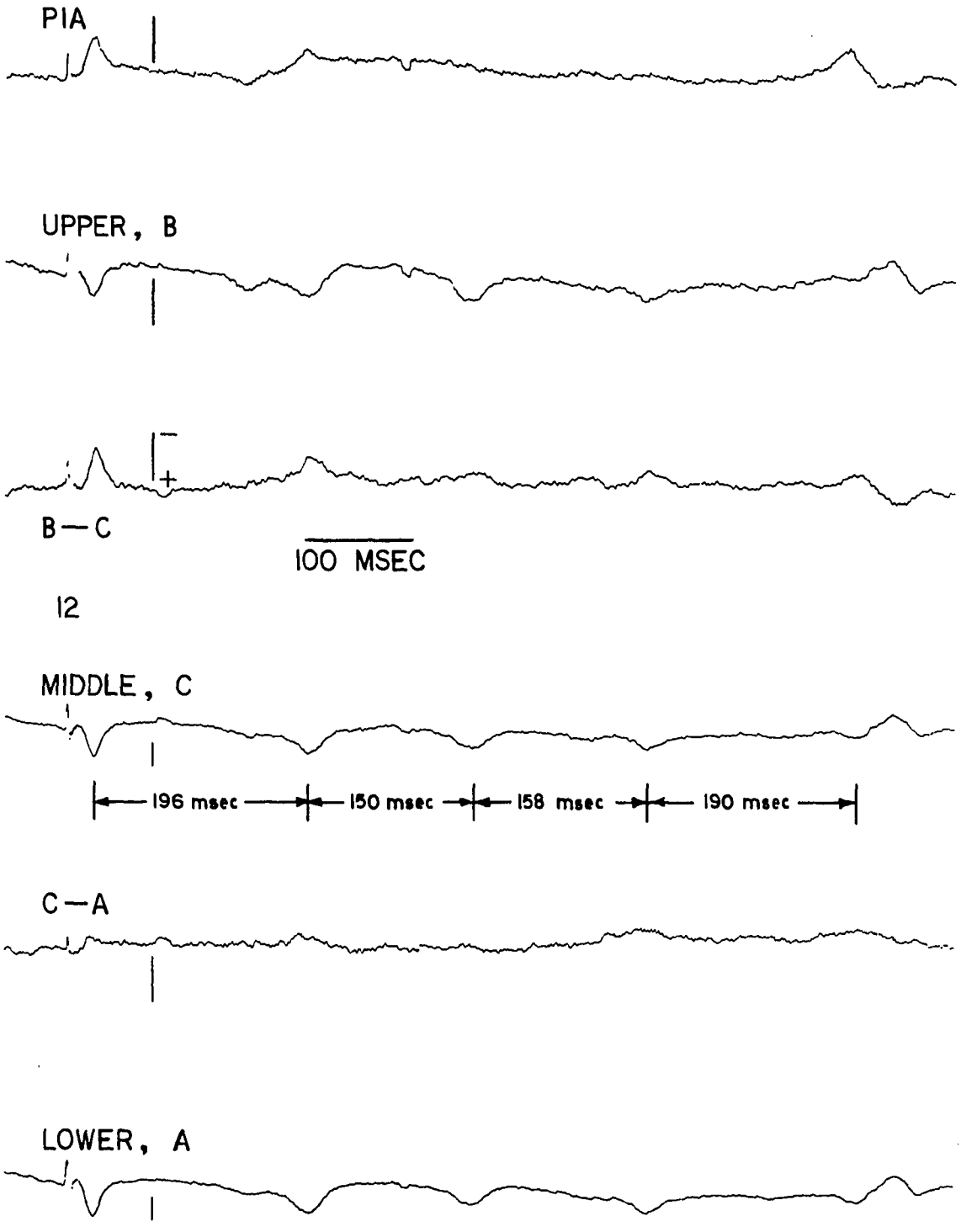

PIA
UPPER, B
1
B—C
100 MSEC
12
13
MIDDLE, C
1
152 msec
C—A
LOWER, A
1

Figure 17. Post-stimulus cortical response: four occurrences. Tri-probe 11B was 0.5 mm anterior to a maximal site at A +8 mm. Tip A was 1150 μ beneath the pial surface. The BC spacing was 79 μ and the CA spacing was 74 μ. The stimulating probe was located in the Fields of Forel (A +7.8, L 2.5, V -2). The vertical calibration was 0.5 mv and an upward deflection represented a negative potential. Experiment 0189-23.


PIA
UPPER, B
B—C
100 MSEC
12
MIDDLE, C
196 msec
150 msec
158 msec
190 msec
C—A
LOWER, A

potential prominent in B, C and A was caused by a cardiac systole. Where there was doubt as to whether an observation was due to valid cortical events or to the heart beat, the data were excluded from consideration.

The mean latency from the last stimulus-driven response peak to the first PSR peak for 161 events was 225 msec with a standard deviation of 63 msec. The mean latency between the first and second responses for 47 occurrences was 201 msec with a standard deviation of 70 msec. Between the second and third responses for 21 events, the mean latency was 179 msec with a standard deviation of 13 msec. Between the third and fourth responses for 6 events, the mean latency was 170 msec with a standard deviation of 13 msec. The two events showing five responses had latencies of 176 and 172 msec after the fourth PSR. Both of these events occurred in the same transcortical track in experiment 0189-23. This track also produced 7 PSR events having 1 or more responses; 6 PSR events having 2 or more responses; 4 events having 3 or more responses; and 3 events having 4 or more responses. These results from a single transcortical track suggest that the state of the preparation may be important to the incidence of the PSR.

The interpretation of these results is the basis of Chapter IV, DISCUSSION.

CHAPTER IV

DISCUSSION

This chapter presents an interpretation of the preceding results, including the variations from 'normal' effects and the additional findings.

Interpretation of Results

The results presented in Chapter III indicate that the cortical recruiting response phenomenon is generated by intracortical elements, substantiating the work of previous investigators. For all stimulus response trains from all experiments in this research, the peak amplitude of the recruiting response was time coincident at all cortical levels to within $\pm$ 4 msec, the time resolving capability of the Beckman Type R Dynograph. Thus, in contradiction to Li, et al. (1956b) but in support of Spencer and Brookhart (1961a) and Purpura, et al. (1964), these results show that the normal intracortical recruiting response is not a traveling wave like that which is associated with the augmenting response and the evoked potential, but is spatially fixed in the vertical direction.

Whereas Spencer and Brookhart (1961a) were not able to define the intracortical site of origin for the recruiting complex, the results presented here show that the laminar sites of

action are simultaneous sources and sinks situated in the upper two-thirds of the cortex (Figures 9 through 13). The sink was always observed superficial to the deeper source with the exception of the one experiment shown in Figure 12 where additional low-amplitude sinks were observed in the 1000-1400 μ range. The results in Table 2 show that the intracortical recruiting response does indeed occupy an extensive part of the suprasylvian gyrus whose anterior-posterior limits are not defined by this work. Furthermore, these data suggest that the inversion zone is closest to the pial surface in the vicinity of the maximal site, tending toward slightly deeper levels with increasing anterior or posterior distance from the maximal site. In contrast, there is no apparent relationship between the source-sink separation distance and the track location with respect to the maximal site. For 14 tracks the mean source-sink separation distance was 447 μ with a standard deviation of 133 μ.

The results of Tables 2, 3 and Figure 13 imply that simple dipole concepts inadequately describe the intracortical recruiting response phenomenon. Instead, the proposal is made that this phenomenon is more accurately described by parallel laminar surfaces or sheets, separated by about 500 μ, and generally following the contours of the gyrus. In the vicinity of the maximal site, both surfaces bulge slightly upward. The superficial surface of this concomitant pair is characterized by a diminished density of positive ions, while the deeper surface is defined by an increased density of positive ions. These polarized surfaces are transitory

in time. The time dependent properties are characterized by the duration of each recruiting response. Whether these polarized laminar-spaced surfaces exhibit columnar properties (Scheibel and Scheibel, 1958; Globus and Scheibel, 1967; Chow and Leiman, 1970) remains for future work.

The divergence functions can be divided into two groups. One group is exemplified by the 0.5 mm posterior track of Figure 10. Note that the negative and positive lobes are rather sharply defined. The other group had broader lobes, some of which exhibited bimodal properties, e.g., the negative lobe of Figure 9 and both lobes of the maximal site track of Figure 10. The significance of these differences is not clear. The bimodal property (e.g., Figure 9) is suggestive of two closely spaced ionic sinks rather than one. Testing of this suggestion will require more refined potential measurement techniques or a different experimental design.

The 14 transcortical tracks in Figure 13 show a depolarization of neuronal elements in layer II and upper III concomitant with a hyperpolarization of neuronal elements in layers III, IV and V. Using the histological data reported by Sholl (1956, p. 48-49), the hypothesis is proposed that the intracortical recruiting response is a synergistic modulation of pyramidal cell activity in the different laminae of the cortex. Specifically, the pyramidal cells of layer II are facilitated during the response, presumably by synaptic effects on basilar dendrites or directly on the somas. At the same time, the pyramidal cells predominately located in

layers III, IV and V are inhibited, perhaps by synaptic effects on apical dendrites. This synergistic modulation is a kind of push-pull or gating control of pyramidal cell activity in the superficial cortex versus pyramidal cell activity in the deep cortex. The time coincidence of the source-sink modulation supports the idea that the neuronal triggering and dynamic response mechanisms are the same for the source and the sink, despite the physically separate transcortical locations. This modulation hypothesis is supported by: (1) behavioral observations of the 'arrest reaction' of skeletal muscle activity during the orienting response (Jasper, 1960); (2) facilitatory-inhibitory effects of nonspecific thalamic pulses when properly timed with specific thalamic pulses (Li, 1956a; Torii, et al. 1965; Morillo, 1961); (3) inhibition of pyramidal tract volleys with nonspecific thalamic stimulation (Brookhart and Zanchetti, 1956); and (4) quieting of unit activity in layer VI during the recruiting response (Spencer and Brookhart, 1961a).

The recruiting response phenomenon might be considered the extreme limit of the modulation concept, namely, a gating process which is switched on or off. In the normal intact animal, the nonspecific thalamic effect at the cortex is conceptualized as a smoothly graded process, differentially modulating neuronal activity in the two major pyramidal cell laminae. Interaction of the specific afferents via layer III neurons with the synergistic nonspecific effects on pyramidal cells in layers II and IV, V suggests that here may be a neuronal analog of the comparator

function, or error detector, so essential in servomechanism feedback loops.

Variations from Normal Results

Discussion of the variations resulting from high frequency stimulation and from the incremental changes in stimulating probe site is presented below.

Effect of high frequency stimulation. In considering the effect of the revised protocol mentioned in Chapter III, the reader should take note that the results shown in Figures 10 and 14 come from the same preparation with a common site of thalamic stimulation, the medial dorsal nucleus. The maximal site data of Figure 10 was taken before (and the 0.5 mm posterior track was taken after) the revised protocol procedures leading to the two novel divergence functions of Figure 14. The rounded nature of the divergence lobes of the novel tracks are in contrast with the more common angular nature exemplified by the divergence profiles of Figure 10. As mentioned in Chapter III, faulty experimental technique could have introduced an error in depth location of the Figure 14 divergence plotted with circled points. It is highly unlikely, however, that such an error would have amounted to as much as 400 μ, the depth difference between the initial start of the two negative lobes. The source-sink separation distance of 630 μ for the circled-symbol divergence, in contrast with the separation distance of 460 μ for the triangular-symbol divergence, supports this reasoning.

These limited results suggest that the revised protocol altered the cortical state so that the source-sink surfaces of the intracortical recruiting response appeared deeper in the cortex than was usual for the standard protocol. Level of anesthesia of the preparation is believed to have only a moderate influence on depth location of the recruiting response source-sink surfaces, since the depth variations for the 17 transcortical tracks are not extensive and were obtained from preparations at various levels of anesthesia. The influence of high frequency stimulation on the intracortical location of the recruiting response needs further study.

Effect of varying the stimulating probe site. The results of Figure 15 raise interesting questions about the relationship between the inversion zone depth from pial surface and the thalamic stimulation site. For the results shown, the inversion zone appeared to 'move upward' as the stimulus probe progressed from the lateral dorsal nucleus down into the central lateral nucleus. Support for this argument, over that for a transverse shift in the maximal site, is obtained from the transcortical track labeled "undetermined maximal site" in Tables 2 and 3 and shown to the right of Figure 13. Data for this particular track were obtained with the stimulating probe located in the final site of Figure 15 (A +9.8, L 3, V +3.8). The track was labeled "undetermined maximal site" because the experimental procedure for searching out a maximal site was not performed for the particular thalamic site of stimulation. This track was located 0.5 mm

anterior to the partial track whose results appear in the upper part of Figure 15. One can note that the divergence crossover for the "undetermined maximal site" track is 500 μ as compared with a crossover value of about 600 μ for the partial track of Figure 15. This comparative result supports the premise of an apparent upward shift in inversion zone, as implied by the results shown in Figure 15. Consequently, despite the limited data, there is evidence that the depth from the pial surface at which the inversion zone is encountered is related to the site of thalamic stimulation.

Ancillary Findings

A report of the post-stimulus response (PSR) has not been found in the literature. Chang (1950) advanced the concept of cortico-thalamic reverberating circuits in explaining his observations of repetitive waves. In response to single pulses applied to peripheral sensory organs, sensory nerves or afferent tracts, Chang reported repetitive waves from the sensory cortex whose periodicity was about 90 msec. This periodicity coincides with the alpha rhythm. The initial latency to the first wave in the set was about 100 msec.

As reported in EXPERIMENTAL RESULTS, the PSR periodicity was of the order of the thalamic stimulation period, 188 msec. The mean latency to the first PSR was 225 msec with mean periods of 201, 179, 170 and 174 msec for succeeding PSR's when multiple responses occurred. As with Chang's findings, the initial latency to the first event was longer than the succeeding periods.

If the PSR's are grouped by categories defined by the number of responses following a stimulus train (i.e., 0, 1, 2, 3, 4, and 5), the frequency of occurrence resembles a Poisson distribution. This resemblance suggests that the underlying neural process may be a random process. The 25% frequency of occurrence of one or more PSR's for all experiments underscores the lability of this unusual response.

Circulatory reverberatory circuits of thalamo-cortical interaction were also postulated by Verzeano, Lindsley and Magoun (1953). The PSR's observed in this work certainly exhibit some of the properties of a damped reverberation. Does the PSR represent some kind of conditioning or learning phenomenon, or is it merely the result of chloralose anesthesia partially releasing inhibitory controls on the thalamo-cortical interaction? The data are insufficient to answer these and other related questions.

CHAPTER V

SUMMARY

The involvement of the thalamus with behavioral characteristics has been reported by many investigators. That portion of the thalamus classified as nonspecific has been implicated by some investigators as playing an important role in the formation of attention, especially with regard to novel or relevant sensory stimuli. Effects at the cortex of nonspecific thalamic activity induced by electrical stimulation have been extensively studied, but the functional mechanisms by which this afferent activity influences cortical function and the precise location of these mechanisms in the cortical layers remain undefined. Thus, the objective of this dissertation was to determine the laminar site(s) of functional action at the cortical level of the nonspecific thalamic afferents evoked by low frequency electrical stimulation.

The cortical recruiting response was selected as the indicator of the nonspecific thalamic activity. The laminar site of functional action was determined by locating (with respect to the pial surface) the principle sources and sinks of ions which produced the recruiting response. The potential field, generated by the ion movement and/or the physical separation of ion densities having a net charge difference, was detected by a triple barreled

microelectrode probe (tri-probe). The three tips of the tri-probe were separated about 100 μ along the probe axis so that simultaneous measurement of the potential between the two pairs of tips would facilitate the determination of the spatial gradient of potential. With sequential transcortical steps a profile of the gradient of potential normal to the pial surface was obtained. The divergence, derived from this gradient of potential profile, was interpreted in terms of the ion source and sink locations.

Adult cats, anesthesized with 5% alpha chloralose, were placed in a stereotaxic instrument and the suprasylvian gyrus was surgically exposed. A coaxial stimulating probe was positioned in a nonspecific thalamic site. The electrical stimulus consisted of 0.5 msec rectangular pulses presented at a rate of 5/sec for a fixed time interval such that 12-13 pulses were applied to the stimulating probe. All recording electrodes (silver silver-chloride) were directly coupled to the amplifying/recording system. A cotton wick electrode recorded pial surface activity with respect to a reference site on the frontal bone. A transverse exploration of the surface of the suprasylvian gyrus was conducted with the wick electrode to locate a maximal response site for recruiting responses (peak amplitude criterion). Transcortical explorations were conducted at or near this site with the tri-probe. Recordings of the difference in potential between the two pairs of tips and between each tip and the remote reference site were made in addition to the pial surface activity. The recordings from 6 cats provided 21 transcortical tracks for analysis.

The data show that the recruiting response was an intracortical phenomenon and, for a given response, was spatially fixed in the direction normal to the pial surface. The data suggest that the intracortical location of the phenomenon is related to the site of thalamic stimulation and to an antecedent stimulus having a 50/sec repetition rate. Interpretation of the divergence functions indicated the laminar sites of action of the nonspecific thalamic afferents (simultaneous ionic source-sink locations) were situated in the upper two-thirds of the cortex. The sink for positive ions was observed superficial to the deeper source. The mean source-sink separation distance was 447 μ with a standard deviation of 133 μ. The divergence function crossover value (equipotential point between the source and the sink) was about 600 μ beneath the pial surface at the maximal response site, increasing to 700 μ or greater with increasing anterior or posterior distance from the maximal site.

The proposal was made that, at the peak of the recruiting response, the recruiting response phenomenon was represented by parallel laminar regions or sheets, separated by about 500 μ and generally following the contours of the gyrus, whose superficial sheet was a region of decreasing positive ion density (or increasing negative ion density) while the deeper sheet was a region of increasing positive ion density (decreasing negative ion density). In the vicinity of the maximal site, both ion regions bulged slightly upward toward the pia. These polarized regions were transitory in time. The time dependent properties were determined

by the duration of each recruiting response. These results were further interpreted in terms of a depolarization of the neuronal elements in layer II and the upper part of layer III concomitant with a hyperpolarization of the neuronal elements in layer III, IV and V. It was hypothesized that the intracortical recruiting response represented a synergistic modulation of the pyramidal cell activity in different laminar regions of the cortex. This differential regulation of neuronal activity was thought to be a facilitation of the pyramidal cell activity in layer II while the activity of the pyramidal cells in layers IV and V was believed to be inhibited. This synergistic modulation was also likened to a push-pull or gating control of pyramidal cell activity in the two different regions. The recruiting response was thought to be the extreme limit of the modulation concept, i.e., an on-off gating process. For the normal intact animal the differential modulation of the regional pyramidal cell activity by non-specific thalamic afferents was thought to be a smoothly graded process. The suggestion was made that the differential modulation process may be a neuronal analog of the comparator function, or error detector, so essential to servomechanism feedback loops. Observations of occasional post-stimulus recruiting-like responses (PSR) were also reported. The PSR exhibited some of the properties of a damped reverberation, probably between thalamus and cortex.

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APPENDICES

APPENDIX I

FACILITIES AND INSTRUMENTATION

General Description

The facilities were located in the Whiteman House for Mental Health Research at the University of Oklahoma Medical Center. A special cage-like experiment "room" was constructed to provide structural support for an electrical shield. Bronze screen wire completely enclosed a space about 4½ ft by 4½ ft by 6¼ ft to shield the preparation and recording electrodes from electrical noise. The cage shield was electrically connected to a ground bus which in turn was connected by a heavy copper wire (2 gauge) to an earth ground outside of the building. Vinyl linoleum insulated the surgical table and pedestal from the cage shield. The surgical table, slightly larger than the Narishige Type SN-2 heavy duty stereotaxic frame, was mounted on a surplus dental chair pedestal which provided hydraulic control of vertical movement and manual rotation about the vertical axis. Accessory bars, clamps, and ring stands were attached to the stereotaxic frame or to the surgical table to provide for mounting of the reference electrode holder, the four preamplifiers and selector switch, a small 6 v DC light source, and other devices and instruments for use in the surgery or during the experiment. Surgical supplies, surgical

instruments, the secondary amplifiers, the differential amplifiers, and the rectal thermometer indicator were located on shelving around two walls of the cage. Cable passage between the cage and external instrumentation was by way of small ports located at the top and bottom of the cage. All cables to and from the electronic instrumentation had shields which were connected to the earth ground bus but which were insulated from the internal leads carrying the signals and signal reference. The signal reference leads were connected to the ground bus at the recorder. Ground loops were studiously avoided throughout all interconnections between the instrumentation components. The graphical recorder, oscilloscope, stimulator and isolation unit, the stimulus duration timing circuit, and the DC power supplies were located outside of but adjacent to the cage.

Electronic Instrumentation System

Electrical signals were led from the cortex by the tri-probe and the pial surface wick electrode (Figure 18), all with respect to the frontal bone reference site. The selector switch facilitated the experimental steps of recording zero potential (shorted) input or signals from the cortex. Transidyne General Corporation Type MPA-2 preamplifiers provided amplification of the electrical signals of 9.7 to 9.9. These preamplifiers utilized field effect transistors (FET) input stages with an input impedance adjustable from 200 meg up to near infinity. The input impedances of the preamplifiers for tri-probe signals B, C, and A were set

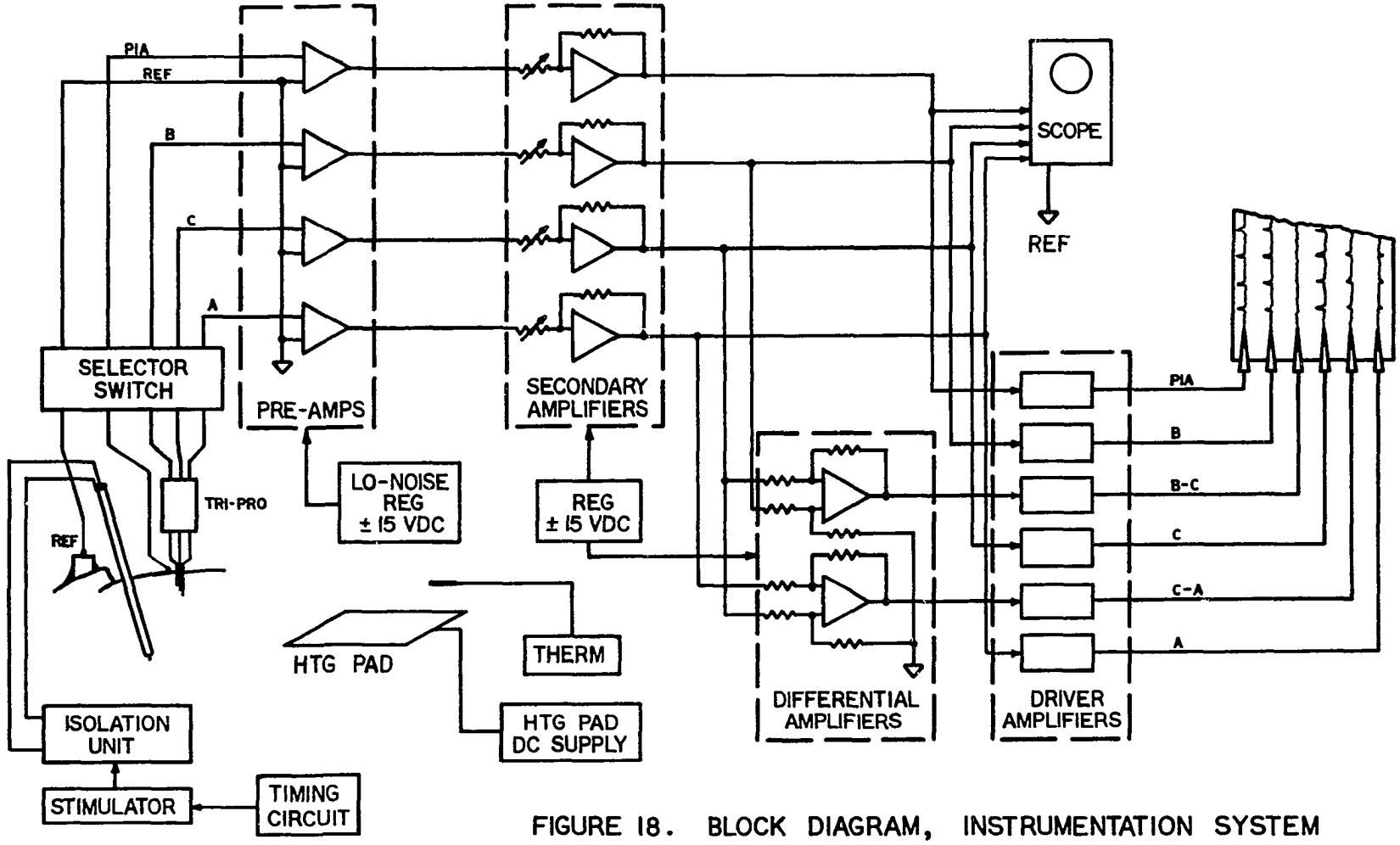


FIGURE 18. BLOCK DIAGRAM, INSTRUMENTATION SYSTEM

for the maximum value. The input impedance for the pial surface wick electrode was set at the lower value. The adjustments, compensating the input capacity of the probes, were set in accordance with the manufacturer's instructions. The low-noise regulated power supply (Transidyne General Type MPS-15) provided ± 15 v DC on a continuous basis to minimize thermal drift. The preamplifier outputs were directly coupled to the secondary operational amplifiers (Nexus Type PF85AU), each having a gain of about 10. Combined amplification of the preamplifiers and the secondary amplifiers was trimmed to 100 ±1% for each of the four channels. The low impedance, high-level outputs of the secondary amplifiers were directly coupled to three functional elements: the Beckman Type 482 pen-driver amplifiers; the differential amplifiers on the Tektronix Type 502 oscilloscope; and the two differential summing amplifiers. The differential summing amplifiers utilized Nexus Type QFT2 operational amplifiers in a conventional feedback circuit with an overall gain of 1. The single-ended outputs of the summing amplifiers, representing B-C and C-A, were directly coupled to the Beckman Type 482 pen-driver amplifiers. For simplicity, shorting switches at the inputs of the secondary and the differential summing amplifiers were omitted from the block diagram. The feedback circuits for both the secondary and differential summing amplifiers incorporated capacitance-limiting of the amplifier bandwidth to prevent high-frequency oscillation. Trimming of adjustments in the Beckman Type R Dynograph Recorder was performed to maximize the pen-recording system bandwidth, the major bandwidth-limiting element

in the total instrumentation system. Pen overshoot in response to a 20/sec squarewave input was limited to 10%. Regulated DC power for the secondary and differential amplifiers was provided by a Technipower Model DB 15 power supply.

The rectal thermometer and indicator unit was the Yellow Springs Instrument Model 46 TUC. The heating pad DC power was controlled by a Variac (General Instrument Co.) supplying alternating current to a full-wave bridge rectifier and a resistance-capacitor pi-section filter.

Unipolar rectangular voltage pulses were supplied to the coaxial stimulating probe via a Grass Instruments stimulation isolation unit, Model SIU4B. Generation of the stimulus pulses and control of the pulse width, pulse amplitude, and repetition rate were provided by the Grass Instruments Model S4B stimulator. Duration of the stimulus train was controlled by an external timing circuit. This timer utilized a unijunction transistor in a resistance-capacitance circuit to energize a relay via a silicon controlled rectifier. Time constants in the circuit were adjusted to provide about 2 secs of relay contact closure (12-13 pulses for a pulse repetition rate of 5/sec). This concludes the description of the instrumentation system.

APPENDIX II

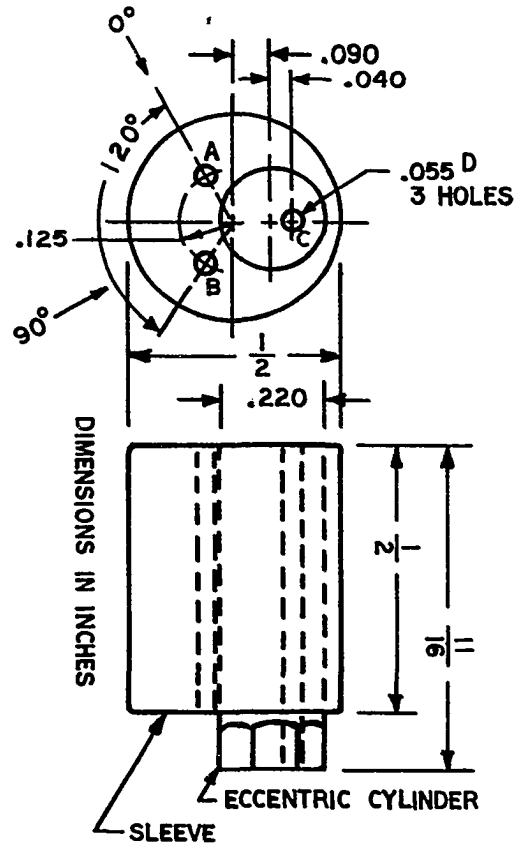
PROBES

A description of the fabrication and assembly procedures employed in producing the tri-probes used in this dissertation is presented. A brief description of the wick pipette preparation is followed by a discussion of the silver silver-chloride electrode fabrication.

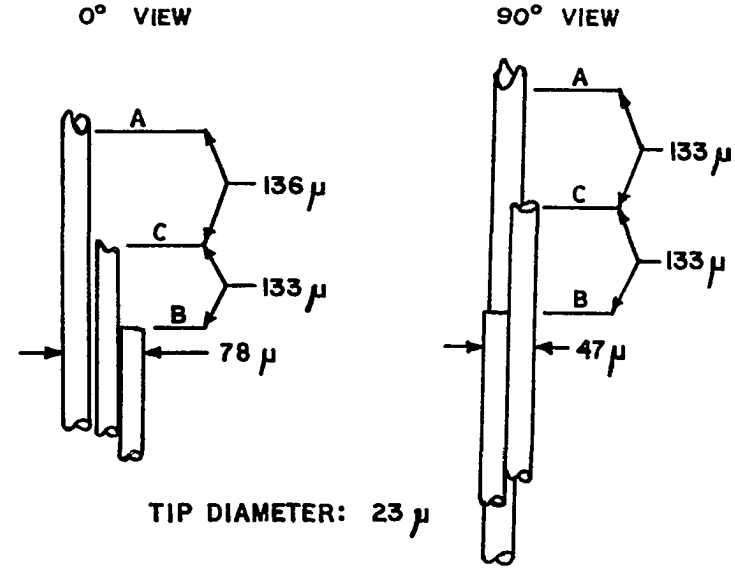
Tri-Probe

Production of the tri-probes, used in the experiments to detect potentials from closely spaced laminar regions of cortex, involved fabrication of the tri-probe holder, preparation of the three pipettes, and assembly of these parts.

Fabrication of the tri-probe holder was based on the design shown in Figure 19A. Plexiglas was used for the holder because of its easy machinability, acceptance of conventional cements, and its acceptable electrical and mechanical properties. The holder was fabricated in two parts, the sleeve and the eccentric cylinder. The sleeve was cut to length from ½ inch rod and mounted on a rotary table having x,y offset adjustments. Three holes were drilled, two of 0.055 inch diameter for the glass pipettes and



A. PIPETTE HOLDER



B. TIP CONFIGURATION: 12B

FIGURE 19. TRI-PROBE DESIGN

one of 0.220 inch diameter for the eccentric cylinder. The eccentric cylinder was turned down from a ¼ inch rod until it made a light slip fit with the 0.220 inch hole in the sleeve. The cylinder length was reduced to 11/16 inch and hexagonal flats were machined for about 3/16 inch along one end to facilitate eccentric cylinder rotation after assembly in the sleeve. A 0.055 inch hole was drilled 0.040 inch off-center but parallel to the cylinder's axis. The offset hole in the eccentric cylinder provided the two degrees of freedom necessary for assembly of three pipette elements along a common axis.

Pyrex glass capillary tubing (Industrial Science Associates) of 1.2-1.4 mm outside diameter by 7.5 cm long was cleaned with acetone and pulled into equal length pipettes using a David Kopf Model 700C vertical pipette puller. Heater current and the solenoid pull strength were adjusted to produce a short axial shoulder length combined with a small diameter but long tapered shank. Tip diameters were usually less than 5 μ at this stage. Using an optical magnification of 10 or 15 the tips were trimmed with surgical stitch-cutting scissors to about 20 μ outside tip diameter. The tip diameters were checked at a magnification of 120 with a microscope having a calibrated reticle. Careful inspection of the complete tapered shank was made to insure that glass defects (e.g. air inclusions, hairline cracks) were absent. Acceptable pipettes were cleaned with acetone drawn through the pipette tips by 20 inches of mercury vacuum applied to the barrel end. Drying of the pipette was accomplished by drawing air through

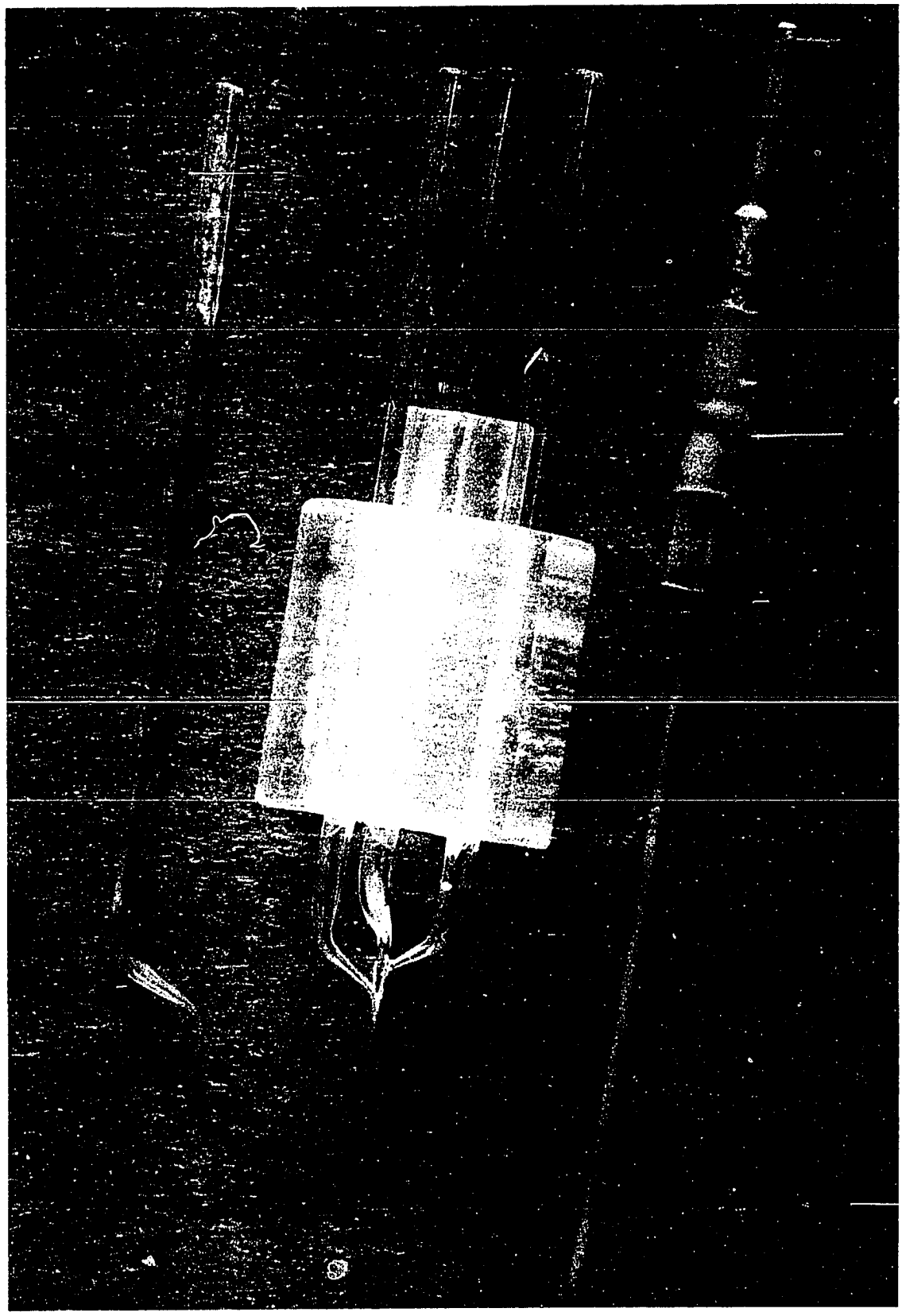
the pipette. The barrel end was lightly fired to fuse sharp edges which tended to scrape the silver-chloride coating during insertion of the electrode wires.

The next step was to bend the pipette tip about 60^{o} off-axis. A Unimat lathe, arranged for drill press operation, held the pipette. A circular one-turn coil of Nichrome wire, supplied with current from a filament transformer, provided the heat for softening the tapered shank near the shoulder of the pipette. A Variac (variable transformer, General Instrument Co.) provided adjustment of voltage to the filament transformer to facilitate temperature control. The circular microforge loop was mounted on a three-dimensional micromanipulator (Brinkman Instruments Model 3050) to facilitate positional control. A wedge-shaped push rod mounted on a second micromanipulator was used to force the tip and tapered shank to the desired angle.

The second bend, Figure 20, was accomplished with the aid of a half-loop of Nichrome wire whose temperature was controlled as described for the previous microforge. This half-loop was positioned between the first bend and the tip so that after completion of the bend the pipette tip would be 3.2 ± 0.4 mm offset from but parallel to the axis of the pipette barrel. The previously mentioned push rod was used to provide the slight force required to produce the bend. Care had to be exercised in these two bending operations to use the correct amount of heat (neither too much nor too little) and to prevent close proximity or contact of the microforge element with the glass. The resulting damage

Figure 20. Elements of a tri-probe

A pipette, ready for assembly into a tri-probe holder, is shown in the left side of the figure. A completely assembled tri-probe is shown in the middle of the figure. A silver silver-chloride electrode, ready for use in a pipette, is shown in the right side of the figure. Note the slightly larger diameter of the chlorided section as compared with the non-chlorided portion near the collar. Enlarged 4X scale.



to the glass invariably caused discarding of the pipette or consigning it to wick pipette use. After completion of the second bend, the lateral tip-offset distance was measured and the pipette classified according to its tip diameter and offset distance.

Assembly of the pipettes into the tri-probe holder (Figure 19A) was performed in two steps. The first step was to position pipettes A and B and cement them to the holder. The second step was to locate pipette C in relation to A and B. The first step was implemented by mounting the tri-probe holder vertically with the fluted end of the eccentric cylinder down. Two micromanipulators were positioned underneath the holder to provide independent vertical position control of pipettes A and B. Three pipettes were selected on the basis of common tip diameters and tip-offset distances to permit tri-probe assembly. For example, three pipettes of 3.6 mm offset each could not be assembled whereas offsets of 3.2 mm, 3.6 mm and 2.8 mm would permit assembly. Using an optical magnification of 10 or 15, pipettes A and B were vertically positioned to equally align their tips. Then the calibrated vertical micrometer control of one manipulator was adjusted to raise tip A until the desired overall spacing from A to B (usually about 200 or 250 μ) was established. The pipettes were gently rotated to minimize the separation of the tapered shanks. Then a small amount of cement (Duco Plastic cement diluted 1:1 with toluol) was placed on the junction of the pipettes with the holder. Capillary action would draw the cement into the hole around the pipettes. After the cement was set (about 10 mins) the contiguous alignment of the

tapered shanks and tips was carefully checked. If the tips were diverging outward from the confluence of the second bend, then the circular microforge loop was placed over the tip pair, the shanks were raised to softening temperature, and the shanks manually manipulated into parallel vertical alignment visually aided by an optical magnification of 15 or 20. This very delicate maneuver was facilitated by use of a fine wire (28 or 32 gauge) attached to the end of a cotton applicator stick.

The second step of assembly was to slip pipette C through the hole in the eccentric cylinder until it rested on the arm of a micromanipulator. The eccentric cylinder and the pipette were rotated to provide best overall alignment with A and B, the tips being viewed with an optical magnification of 20. The variance in pipette fabrication invariably required shank softening and alignment of the three tips at this stage. This alignment included the vertical positioning of the tip C midway between A and B. A great deal of patience and a steady hand were required to manually accomplish this vertical positioning and the parallel alignment of the three tips A, C, and B. Then a small amount of cement was applied to the junction of pipette C with the eccentric cylinder and also to the junction of the eccentric cylinder with the sleeve. After allowing time for the cement to set, a small amount of cement was applied to the confluence of pipettes A, B, and C. The end result was a sturdy structure capable of withstanding the mechanical forces associated with filling the pipettes, inserting and withdrawing the electrode wires, and "punching-through" the pia.

The tri-probe tips were inspected from two different angles (Figure 19B) at a magnification of 120 and a sketch made of the tip configuration. Dimensions were determined with reference to a calibrated reticle. Figure 19B also indicates typical variations in the fracture zones of the tips caused by the scissor-method of sizing the tip diameters. The tip spacing measurement was referred to the middle of the fracture zone of tip C. The BC and CA spacings were determined from this reference to the nearest edge of the fracture zones of tips A and B.

The tri-probe was prepared for use by filling each pipette with 0.9% saline. Twenty inches of mercury vacuum was connected to the barrel-end of each pipette with the tips immersed in the saline. After filling, a quantity of saline was withdrawn from each barrel by a syringe and needle. The length of the needle had been carefully established to just remove enough saline to equal that displaced by insertion of the silver silver-chloride electrode into the barrel. This step prevented overflow of saline as well as deleterious contact of the saline with the non-chloridized silver at the upper end of the electrode wire. After insertion of the wire electrodes into the barrels the tri-probe was ready for use (potentials stabilized) after about 15 minutes.

Table 4 lists the physical dimensions of the tri-probes used in the experiments providing the data for this dissertation.

Preparation of Wick Pipettes

Wick pipettes usually made use of pipettes rejected from

TABLE 4
TRI-PROBE DATA

TRI-PROBE NO.	TIP SPACING, μ		MEAN TIP DIA., μ	EXPERIMENTS
	BC	CA		
11 B	79	74	23	0189-23
12 B	123	129	23	6452-27
18 A	59	70	15	6071-24 6067-26
19 A	92	142	16	6070-21 0187-22

the tri-probe production. The tapered shanks were broken off nearly to the shoulder, or until a cotton thread, moistened and twisted, could be worked down through the pipette and out the "tip" end. The thread was drawn through the pipette until its upper cut-end reached the shoulder of the pipette. The "tip" end of the thread was cut to about 1 cm length and the pipette filled with 0.9% saline, flushing the wick in the process. The wick-pipette was stored completely immersed in 0.9% saline until used. At the time of use, the wick pipette was flushed with fresh saline solution. The wick was trimmed to provide about a 3 mm length beyond the pipette end. As described for the tri-probe pipettes, a quantity of saline was removed from the barrel to equal that displaced by insertion of the silver silver-chloride electrode into the barrel. Then, insertion of the wire electrode produced no overflow of saline from the pipette and no deleterious contact of saline with the non-chloridized silver at the upper end of the electrode wire. The wick electrode was ready for use about 15 minutes after insertion of the electrode wire into the barrel.

Preparation of Silver Silver-Chloride Electrodes

Only "fine" silver wire (99.9% pure silver) was used to make the wire electrodes (Janz & Ives, 1968). A 60 cm length of 30 gauge wire was cleaned of sulfide scale in a liquid cleaner (Speedip) and rinsed in running tap water. The wire was stretched to remove minor bends and kinks and cut into 5 cm lengths.

Connector pins (Amphenol 314980-20P) were machined to

reduce their overall length and to provide a uniform diameter. A 5 cm length of silver wire was soft-soldered to the pin in axial alignment with the connector pin. The wire was scrubbed with a household scouring pad (Rescue) and an abrasive cleaner (Ajax). After rinsing in tap water the wire was polished using jewelers gold-rouge and a buffing wheel driven by a motor-driven hand tool (Dremel Moto-Tool). The polishing was continued until visual inspection with a magnification of 1½ revealed no scratches. Polishing rouge was removed from the wire with cotton applicators soaked with acetone.

A 10 mm length of 1/16 inch Alphlex shrinkable tubing was slipped over the connector pin until about 3 mm extended out over the wire. A 6 mm length of PE 200 tubing was threaded over the wire and positioned under the shrinkable tubing until about 3 mm remained exposed. Heat was applied to the Alphlex tubing until it was tightly shrunk around the connector and the PE tubing. This process formed the PE tubing into a collar which fitted snugly on the barrel-end of the pipettes. A 3.3 cm length of glass capillary tubing was threaded over the wire and inserted into the PE collar. The silver wire was cut flush to the end of the glass tubing. The glass capillary tubing was removed and the wire carefully cleaned with acetone.

Chloridizing of the silver wires was performed in a 0.1 N HCl acid solution, saturated with silver chloride. Chloridizing operations were performed under normal laboratory light. Current density on the wire surfaces during the chloridizing operation was

limited to the range of 0.6 - 2.5 ma/cm^2 (Ives and Janz, 1961). The length of the wire converted to silver chloride was about 3 cm. For 14 electrodes a typical current was 4 ma. Best results were obtained with a periodic reversal of the chloridizing current. A timing apparatus was employed to provide for a current reversal period of 20% of the total cycle time. Thus, in a 75 sec cycle, there was current reversal for about 15 seconds. The duration of the chloridizing process varied from around 4 to 8 hours, dependent in part on the selected current density. The criterion for cessation of chloridizing was: (1) a uniformly smooth, even matrix of chloridized silver; and (2) a forward chloridizing current 5-10% less than the value of the reversal current. For example, the current might be 3.8 ma just prior to reversal and 4.1 ma just after reversal. (This variation in forward and reverse current was related to the thickness of the coating). The chloridizing process was stopped in that part of the cycle when the forward current just commenced to decline. The electrodes were rinsed thoroughly under running tap water and placed in distilled water overnight to remove traces of acid. The electrodes were air-dried and inspected for uniformity and quality of the coating. Electrodes were stored dry in a dust-proof container until ready for installation in the tri-probe and wick pipettes. This process produced a high yield of electrodes satisfying the requirement for less than 1 mv potential (DC) between any pair of electrodes installed in the tri-probe or wick pipettes with tips immersed in 0.9% saline.

Stimulating Probe

The stimulating probe was supplied by the laboratory of Dr. C. G. Gunn, Department of Medicine at the University of Oklahoma Medical Center, Oklahoma City, Oklahoma. The construction was based on a coaxial arrangement of a stainless steel wire inserted in and insulated from a section of stainless steel hypodermic tubing. The whole assembly was dipped in insulating material, cured in an oven and a conical point carefully ground to expose the inner and outer conductors. The outside diameter of the probe was 0.9 mm and the conical-shaped tip provided for 0.7 mm axial separation of the inner and outer conductors.

APPENDIX III

ABBREVIATIONS AND SYMBOLS

db	decibel; a measure of sound pressure
msec	millisecond; 10^{-3} seconds
sec	second
mm	millimeter; 10^{-3} meters
μ	micron; 10^{-6} meters
kg	kilogram
mg	milligram
gm	gram
cc	cubic centimeters
C	centigrade
β	beta
cm	centimeter
DC	direct current or direct coupled
μv	microvolt; 10^{-6} volts
AC	alternating current
mv	millivolt; 10^{-3} volts
S_A	distance of the tri-probe tip A beneath the pial surface
ΔV_{PIA}	amplitude of the recruiting response at the pial surface

ΔV_{B-C}	amplitude of the potential difference between the tri-probe tips B and C at the peak of the pial surface recruiting response
ΔV_{C-A}	amplitude of the potential difference between the tri-probe tips C and A at the peak of the pial surface recruiting response
$\overline{\Delta V}$	mean amplitude of the pial surface recruiting response
$\frac{\overline{\Delta V}}{\Delta V}$	equalization ratio
G_{BC}	spatial gradient of potential between the tri-probe tips B and C
G_{CA}	spatial gradient of potential between the tri-probe tips C and A
D, DIV	divergence of the gradient of potential
nv	nanovolt; 10^{-9} volts
S_A'	depth from the pial surface of the mean tip spacing
MID-SSG	mid-suprasylvian gyrus
SPRO	stimulating probe
Stx	stereotaxic location of the maximal response site in the anterior plane
MAX	maximal response
NUCL	nucleus
MD	medial dorsal
Flds	fields
CL	central lateral
CM	central median
LD	lateral dorsal
A	anterior
L	lateral

V	vertical
Indeterm	indeterminate
std	standard
rev	revised
SAG	sagittal