RETICULAR CONTROL OF CORTICAL UNIT ACTIVITY IN THE CAT

RETICULAR CONTROL OF CORTICAL UNIT ACTIVITY IN THE CAT

By

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SCOPE AND CONTENTS:

Extracellular action potentials were recorded from the suprasylvian gyrus of the cat and compared with slow potentials derived from the overlying cortical surface in experiments designed to investigate the influences of pontine reticular formation on these fast and slow voltage transients. When pontine afferents were blocked by mid-pontine lesion or reversible cooling, the number of recordable spontaneous and injury discharges was reduced as compared to that recorded from preparations with lower pontine, post-trigeminal, lesions. Bilateral gasserectomies in post-trigeminal preparations did not reduce unit activity. However, the mean and median average firing rates and the patterns of spontaneously-active cells were not demonstrably different for high and low pontine preparations.

Either EEG synchrony or desynchronization was found to co-exist with either high or low levels of unit activity.

ACKNOWLEDGMENTS

The ideas, technical knowledge, and general support of my advisor, Dr. G.K. Smith, is appreciated.

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Thanks are also due to Dr. W. Heron for critical reading of the manuscript.

To my wife, Margaret, goes my love for her hard work, love, and understanding.

TABLE OF CONTENTS

FIGURES

CHAPTER ONE

INTRODUCTION

In 1946, Magoun and Rhines (1946) reported that a ventromedial region of the bulbar reticular formation, when electrically stimulated, inhibited decerebrate reflex rigidity in the cat, as well as various movements induced by direct electrical stimulation of the motor cortex. The existence of such a powerful inhibitory mechanism, roughly localized in the region of the n. reticularis gigantocellularis of the medulla, reshaped the older notion of the bulbar level as being excitatory, a conclusion based on Sherrington's finding of rigidity of antigravity muscles following a decerebration which spared the bulb (Magoun and Rhines, 1946). The results of complete brainstem transections on postural tone in dogs (Keller, 1945) supported the concept of a bulbar inhibitory region as well as an antagonist pontine facilitatory region, which by electrical stimulation of a large region surrounding n. pontis oralis facilitated movements elicited by electrical stimulation of the motor cortex (Rhines and Magoun, 1946). Keller (1945) discovered chronic atonia and dysreflexia in cats maintained several weeks after complete transection at the level of the upper pons, after rostropontine medial lesions which spared

the lateral tissues, but not following bilateral rostro-pontine lesions which spared the medial reticular substance. These findings prompted Keller to conclude that the muscular atonia and dysreflexia both result from the "... release (of) a tonic tonus-inhibiting circuit from a superimposed antagonistic influence ..." (Keller, 1945, p. 285). Such a conception fit well with the localization of facilitatory (Rhines and Magoun, 1946) and inhibitory (Magoun and Rhines, 1946) reticular centres. And, as will be seen, the concept of antagonistic excitatory and inhibitory mechanisms has remained commonplace in evaluating research on the reticular control or modulation of other nervous structures.

Murphy and Gellhorn (1945a) reported consistent facilitation of cortically-induced movements during electrical stimulation of the hypothalamus. Unlike Rhines and Magoun (1946) who favored a descending reticular control as an explanation for motor facilitation following pontine reticular stimulation, Murphy and Gellhorn believed that hypothalamic stimulation directly activated the cortex, as for example when a sub-threshold cortical shock became effective in eliciting movement when paired with hypothalamic stimulation. Electrocorticograms taken before and after hypothalamic stimulation indicated a definite alteration in pattern which Murphy and Gellhorn described as an "... increase (in) rate and amplitude of discharge in the spontaneous electrocorticogram ..." (Murphy and Gellhorn, 1945a,

p. 362). Actually, the records clearly indicate an increase in waveform amplitude and slowing in wave period, a synchronized pattern (see Figure 1). In addition, strychnine spikes recorded at irregular intervals on the anterior sigmoid gyrus became "clustered" in groups of three closely-spaced spikes following hypothalamic stimulation. Such cortical alterations occurred in sensory and association cortex as well as the motor cortex, suggesting that the hypothalamic influence does not function specifically for motor system facilitation.

Rhines and Magoun (1946) performed control experiments to discount the role of the motor cortex as the site of facilitation due to hypothalamic or brainstem stimulation. They traced a pathway caudally from as far rostral as the basal ganglia through the basal diencephalon and brainstem to enter the spinal cord. Stimulation of this pathway facilitated movements elicited by electrical stimulation of either the motor cortex or the pyramidal tract after cortical extirpation, the latter result indicating direct facilitation of motoneurons or cord interneurons.

Meanwhile, Murphy and Gellhorn (1945b) performed strychnine neuronography experiments which elaborated hypothalamocortical pathways through the dorsomedial thalamus and cited experiments showing an effect on cortical rhythms following hypothalamic lesion (Obrador, 1943) or stimulation (Morison, Dempsey and Morison, 1941).

The parallelism between electrocortical "activation" and motor facilitation was, in Murphy and Gellhorn's experiments, probably not significant, i.e., the two effects following hypothalamic stimulation may be unrelated, as Rhines and Magoun (1946) suggested. However, in order to unequivocally demonstrate that such hypothalamic stimulation facilitates movements only by descending reticular influences, Rhines and Magoun would have to have demonstrated that interruption of the reticulo-spinal pathway, while sparing the motor cortico-spinal tract, resulted in no facilitation of cortically-induced movements during hypothalamic stimulation. Therefore, it remains possible that hypothalamic stimulation facilitates at both cortical and spinal levels.

These experiments illustrate clearly the difficulty of accounting for brainstem functions either on the basis of only one measured effect (e.g., muscular movements in Rhines and Magoun's experiments) or in terms of only one type of effect common to all responses (e.g., "activation" in Murphy and Gellhorn, 1945a). In the former, a strictly downstream measure (limb musculature response) led the experimenters to disregard corticipetal influences from brainstem stimulation, by now a common finding (Moruzzi and Magoun, 1949; French, Verzeano and Magoun, 1953), particularly in the motor cortex (Whitlock, Arduini and Moruzzi, 1953; Parma and Zanchetti, 1956; Steriade, 1969; Steriade, Iosif and Apostol, 1969). In the latter case, Murphy and

Gellhorn referred to cortical rhythms during hypothalamic stimulation as "activated" when in fact the tracings would now be termed deactivated or synchronized[1] as compared to pre-stimulation patterns (see Figure 1). Motor facilitation was present, suggesting either that descending hypothalamic influences function differently from ascending effects or that the electrocortical measure (synchrony) belies the underlying state of synaptic transmission.

Notwithstanding these complications, these important observations, especially the recognition of ascending reticular control of cortical potentials, marked the beginning of systematic study of the functional organization of the reticular core. By 1942, Dempsey and Morison (Morison and Dempsey, 1942; 1943; Dempsey and Morison, 1942a, 1942b, 1943) had described thalamocortical pathways, apparently independent of the classical afferent projections, which relayed a rhythmic cortical potential when certain "non-specific" thalamic nuclei were stimulated with low rate (8 - 12/sec.) electrical pulses. This rhythmic cortical response, called the recruiting response, appeared throughout the cortical surface and could be evoked from mid-line nuclei ranging in location from the posterior commissure to

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1. Such terminology does not imply an understanding of the synaptic activity giving rise to these potentials. It is sufficient to point out that such waveforms are characteristically associated with passive, drowsy, or sleep states.

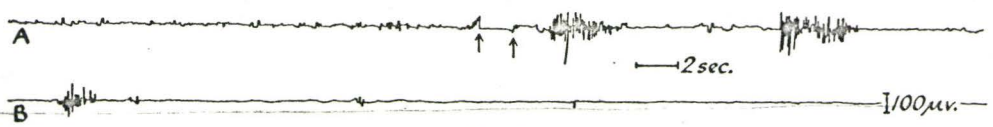

Fig. 5

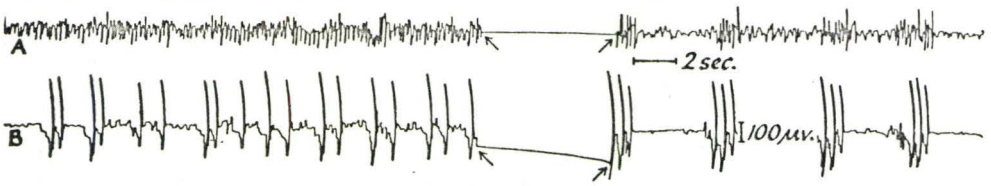

Fig. 6

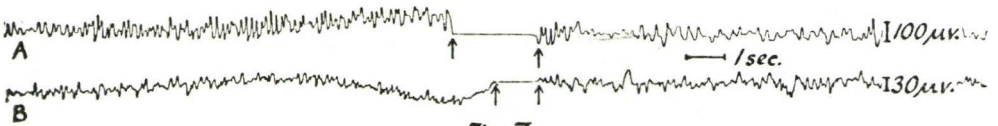

Fig. 7

FIG. 5. Effect of stimulation of the posterior hypothalamus* (between the arrows) on the e.c.g. of the ipsilateral posterior sigmoid gyrus. Dial potentials are suppressed for 4 minutes. Record B taken 3–4 minutes after record A. *All records from right to left.*

FIG. 6. Simultaneous recording of the e.c.g. from the posterior sigmoid gyrus (A) and the anterior sigmoid gyrus (B) after the latter had been strychninized locally. Upper record shows increased background activity, lower record shows increased frequency of strychnine spikes as the result of stimulation of the ipsilateral posterior hypothalamus (between arrows).

FIG. 7. Simultaneous recording of potentials from posterior sigmoid gyrus (A) and contralateral posterior hypothalamus (B) before and after stimulation (between arrows) of the latter. Note increase in amplitude and frequency in A but no significant change in B.

Figure 1. Taken from Murphy and Gellhorn, 1945a, p. 352.

the anterior limb of the internal capsule. Recruiting potentials were said to be equivalent to "spontaneous" barbiturate spindles, (i.e., traversing common fibers to share common cortical neurons), since evoked recruitment was aborted by an on-going barbiturate spindle and vice-versa (Dempsey and Morison, 1942b), and the frequency (8 - 12/sec.), polarity (usually surface negative), amplitude, and cortical distribution of the two waveforms were virtually identical (Dempsey and Morison, 1942a). That recruiting potentials involved an extralemniscal fiber system was indicated by the long latency (usually 20 - 35 msec.) for evoking the recruiting response (Dempsey and Morison, 1942a) and the fact that specific evoked responses to sciatic nerve stimulation could be superimposed on "spontaneous" barbiturate or recruiting potentials during "non-specific" dorsomedial stimulation, but not during "specific" VPL stimulation (Dempsey and Morison, 1942b). These facts supported the concept of a midline thalamic "pacemaker" whose fibers terminate on cortical neurons probably independent of the well-known, bushy, specific afferent terminals distributed in layers III and IV of sensory cortex. Accordingly, Li, Cullen, and Jasper (1956) found that the specific evoked response (surface positive) reversed phase (to deep negative) at this level (III and IV), whereas inversion (to deep positive) of surface-negative recruiting or "alpha" rhythm (decerebrate spindles) potentials occurred through-

out layers II to V with varying magnitude, suggesting a widespread and unique distribution of unspecific afferents on cortical neurons. Additionally, neurons discharged to nonspecific thalamic stimulation with latencies greater than 10 msec. and usually between 15 and 40 msec., (discharge latency for cortical neurons is about 1 msec. after specific thalamic stimulation), (Li et al., 1956).

Dempsey and Morison (1942a) had also noted that high frequency stimulation (20 - 120/sec.) of the same midline nuclei effective for evoking recruitment blocked synchronized potentials (recruitment) including "spontaneous" barbiturate spindles. Moruzzi and Magoun (1949) abolished synchrony in encéphale isolé, chloralosed, and barbiturized cats by high rate (50 - 300/sec.) stimulation of reticular regions from medulla through the pons and midbrain tegmentum to the dorsal hypothalamus and subthalamus. This reticular response, characterized by cortical desynchronization which outlasted the stimulus appeared simultaneously throughout the cortex after a long latency. That this response was conducted extralemniscally was shown by: (1) the absence of a somatosensory response at either thalamic (VPL) or cortical (sigmoid gyrus) levels following a single reticular shock, (2) the fact that primary sensory evoked potentials are unaltered by brainstem reticular stimulation, and finally, (3) the persistence of widespread electrocortical desynchronization (the reticular response) from reti-

cular stimulation after bilateral section of medial and lateral lemnisci (Moruzzi and Magoun, 1949). In demonstrating that the non-specific thalamic nuclei are correspondingly desynchronized during reticular stimulation, the authors suggested a direct brainstem regulation of the non-specific thalamic pacemaker, either inhibiting the rhythmic thalamocortical outflow or driving it at a higher frequency, each with the same result: cortical desynchronization.

The important distinction between an active inhibitory, as contrasted with an excitatory mode of action, suggested a functional organization along the reticular core, with brainstem structures (in medulla, pons, and midbrain) viewed as desynchronizing "centers" with either a direct desynchronizing influence on the cortex or an indirect influence on cortex by direct inhibition of the thalamic synchronizing "centers". The remainder of this discussion will detail the anatomy of such proposed reticular "centers" (in both hindbrain and diencephalon) and their ascending fiber systems in order to evaluate the significance of reticular control on cortical surface and membrane potentials.

The anatomical boundaries of the "reticular core", by general agreement, extend from the corpus striatum throughout the brainstem and spinal cord. This anatomical designation implies a similarity or continuity in reticular substance from the spinal gray throughout the mesencephalon

and diencephalic structures. In fact, reticular substance throughout the medial regions of the spinal cord and brainstem share a morphology which is best described as a network or reticulum of cells and fibers. The spinal gray, for example, is made up chiefly of cell bodies of different sizes, only some of them having medullated fibers but most of them projecting diffusely (Crosby, Humphrey and Lauer, 1962). Medullary and pontine gray substance have a similar macrostructure, heterogeneous cell aggregates interlaced with scattered fibers.

The term reticular formation has been retained by anatomists to describe this matrix of cell bodies and fibers ever since Allen (1932) noted that the "formatio reticularis" develops embryologically from those cells not concerned with the formation of motor nuclei or sensory relay nuclei. In this sense, the reticular core is defined more by what it is not than what it is. That is, nuclei along the longitudinal neuraxis which do not possess "specific" functional or histological characteristics are termed reticular nuclei. Such a classification may be too broad, since it is possible that certain nuclei, particularly association nuclei of thalamus (e.g., pulvinar) lack the properties of "specific" sensory or motor nuclei but do not function similar to other reticular nuclei, (e.g., multi-modal responsiveness and widespread "non-specific" electrocortical effects from stimulation). Attempts to

clarify this ambiguity of the structural organization of the reticular formation have led to experimental analyses of afferent components, efferent fibers, and the intrinsic organization or cytoarchitecture of reticular regions.

One of the chief properties of brainstem reticular formation is the overlapping, heterogeneous sensory convergence (Nauta and Kuypers, 1958; Scheibel, 1951) in the form of collateral branches or direct terminals from major ascending sensory tracts or spinal cells, e.g., anterolateral spinothalamic, spinoreticular and spinotectal tracts, spinocerebellar tracts, and descending trigeminal tracts (Scheibel and Scheibel, 1958). This reticular convergence has been confirmed by physiological studies showing activation or inhibition of the same single reticular unit by different sensory stimuli (Amassian and DeVito, 1954; Scheibel, Scheibel, Mollica and Moruzzi, 1955) as well as interaction (Hernandez-Peon and Hagbarth, 1954; Scheibel et al., 1955) and occlusion (Moruzzi, 1954). This is clearly illustrated in Figure 2, taken from Scheibel and Scheibel (1958).

Spinal cells, coursing in the anterolateral funiculus, which distribute to various cell groups in brainstem reticular formation (spinoreticular path) also terminate in nuclei of the thalamic reticular system (intralaminar, n. centralis lateralis but not in parts of centrum medianum) and in the magnocellular portion of the medial geni-

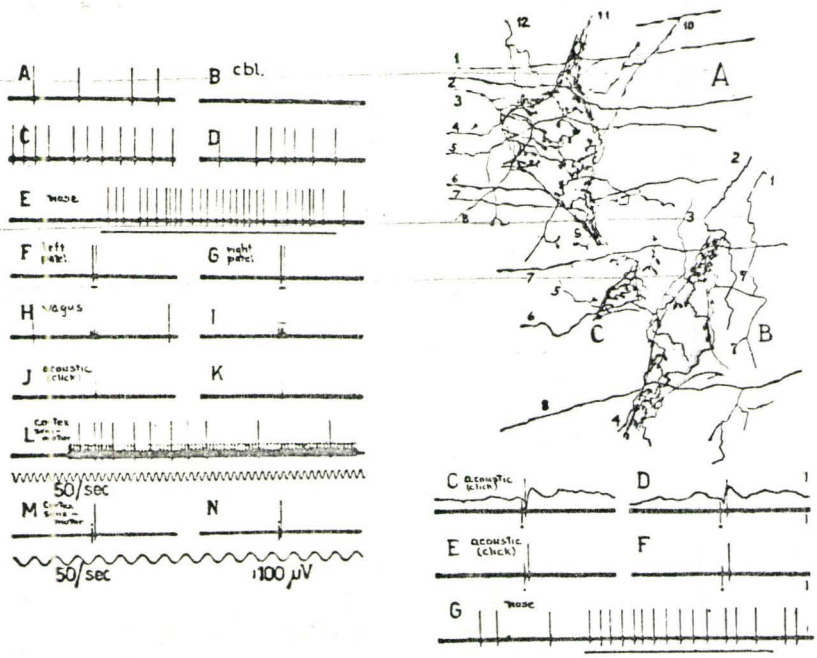

FIGURE 1. Convergence of heterogeneous afferents upon single elements of the brain stem reticular core, demonstrated physiologically and histologically. Strips A through N and C through G illustrate patterns of spike discharge of two elements of the bulboreticular formation. A is firing spontaneously; B is inhibited by cerebellar polarization; C rebounds following cessation of polarization; D returns to a more normal discharge pattern; E is stimulated by nose pressure; F and G are stimulated by patellar tendon taps bilaterally; H and I are unaffected by short trains of vagal stimulation, and J and K are unaffected by auditory clicks; L is driven by repetitive cortical stimulation; M and N show with the aid of an expanded time base that the latency of the corticifugal discharge to the bulboreticular unit is very short. Strips C, D, E, and F show that another bulboreticular unit which is sensitive to pressure to the nose (G) can also be driven by auditory clicks. This rather minimal effect is unmasked when the spontaneous activity of the unit is inhibited by cerebellar polarization.

A, B, and C are bulboreticular cells in a 10-day-old kitten, lying within several hundred microns of each other. Axons from a number of fiber systems were traced to these cells, although only the terminal portions are shown. Horizontally running fibers such as A, 1 through 7, and B, 6 through 8, appeared to belong to spinoreticular and long reticuloreticular components, while B, 1 through 4, approached from the dorsal and lateral aspects of the bulb representing sensory collaterals and cerebelloreticular collaterals. (Slightly modified from Scheibel *et al.*[28])

Figure 2. Taken from Scheibel and Scheibel, 1958.

culate (Mehler, Feferman and Nauta, 1960). Neurons in these regions may respond to tactile stimulation from widespread, often bilateral, receptive fields (Kruger and Albe-Fessard, 1961), and the responses are not usually modality-specific, as for example, when neurons are activated by auditory stimuli, (Poggio and Mountcastle, 1960). That these neurons are excited by a spinothalamic pathway, rather than through the "specific" dorsal white columns has been proved by Whitlock and Perl (1959, 1961; Perl and Whitlock, 1961), who observed that neurons from these "non-specific" thalamic regions were fired from scattered, bilateral receptive fields following cord transections which spared only one anterolateral funiculus. The polysensory nature of neurons in the centrum medianum has been verified, as these neurons respond to somatic, visual, and auditory stimuli (Bowsher, Mallart, Petit and Albe-Fessard, 1968). Generally, neuron activity in the thalamic reticular system is evoked from bilateral locations of the skin without somatotopic arrangement (Kruger and Albe-Fessard, 1961; Perl and Whitlock, 1961).

Notwithstanding this evidence of diffuse and multimodal input into the brainstem and thalamic reticular nuclei, it should be pointed out that neurons in the "specific" ventralis posterolateralis may be activated through an isolated anterolateral funiculus (spinothalamic tract) from widespread areas of the skin (Perl and Whitlock, 1961;

Whitlock and Perl, 1959) and Mehler et al. (1960) have traced fibers from the anterolateral tract into VPL to confirm a direct pathway. Furthermore, in most of the published results a degree of "graded" non-specificity exists, i.e., effective stimuli for different reticular neurons may vary from unimodal wide receptive fields, to bimodal, to multimodal effectiveness (e.g., touch, pressure, pain). Cutaneous, visual and auditory evocation of certain reticular units is rarely reported.

Finally, the reticular formation need not be regarded as a common sensory channel, since it apparently does not receive input from all known afferent pathways. Mehler and Nauta (Nauta and Kuypers, 1958) showed that the dorsal funiculus projects directly to ventral thalamic nuclei with only a minor collateral from the medial lemniscus to the paramedian region of the pons. Morillo and Baylor (1963) further showed that reticular responses to sciatic shocks survived dorsal column section and direct stimulation of dorsal column failed to evoke reticular responses. Whether or not some medial lemniscus input actually bypasses the reticular formation is still an open question, since Jacobson (1955) has traced terminal degeneration into reticular areas of medulla and pons following cuneate nucleus lesions.

Along with this multimodal, non-specific input, a second feature of reticular substance is its complicated

arrangement of heterogeneous ascending and descending fibers. It has been noted that the principal spinoreticular input terminates dorsal to the inferior olive (Morin, Schwartz and O'Leary, 1951; Morin, 1953) roughly equivalent to the nucleus gigantocellularis of Meesen and Olszewski (1949), (see Figure 3, taken from O'Leary, Kerr and Goldring, 1958, p. 190). Scheibel and Scheibel (1958), using Golgi-prepared normal material, confirmed that the maximum degree of overlap and synaptic convergence occurs in the region of n. gigantocellularis, where large somata give rise to long ascending and descending fibers with numerous collaterals in transit. Within the brainstem, these collaterals were seen to distribute diffusely to reticular formation, cranial nerve nuclei, cerebellum, periacqueductal gray, and rostrally to colliculi, extrapyramidal nuclei, and geniculates. Fiber pathways, through collateral branches, were totally dispersed (Scheibel and Scheibel, 1958). Besides collateral conduction, large fibers often bifurcated into craniad and caudad trajectories, with the latter sometimes synapsing on an ascending cell. Earlier, Ramon y Cajal (1909) had observed long and short axons, some of which crossed the midline. Scheibel and Scheibel (1958), observing frontal and sagittal sections, confirmed this midline-crossing but denied the prevalence of Golgi type II (short-axoned) cells. They attributed short-distance conduction to short collaterals and long-latency conduction

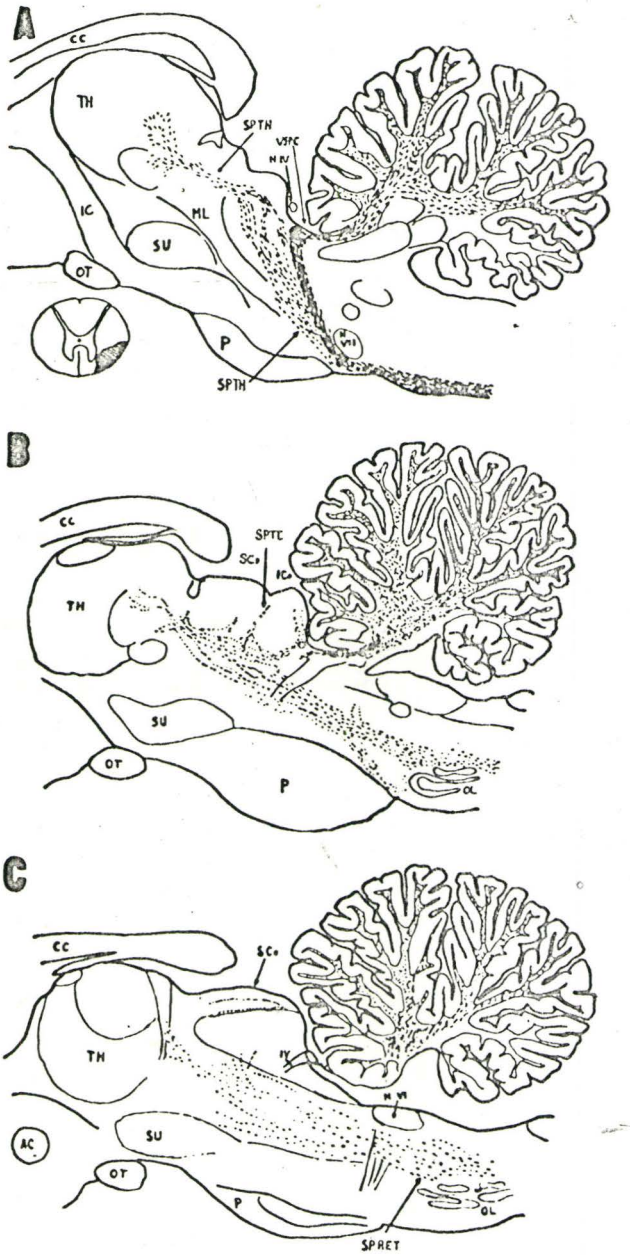

FIGURE 2. Lateral (A), intermediate (B), and medial (C) parasagittal Marchi sections through a macaque brain stem, to demonstrate the course of spinoreticular fibers. The corresponding ventrolateral column had been cut at C-1 two weeks previously. The combined ascending bundle is illustrated by the compact area of degeneration visible in A. Note the wealth of the degenerated reticular fibers in A, B, and C. (From Morin *et al.*[14])

Figure 3. Taken from O'Leary, Kerr and Goldring, 1958, p. 190.

to "collateral-loops" with synaptic delays, (see Figure 4, taken from Scheibel and Scheibel, 1958, p.44).

Attempts to study the functional organization of brainstem reticular nuclei have started from detailed analyses of reticular cytoarchitecture (Meesen and Olszewski, 1949; Olszewski and Baxter, 1954; Olszewski, 1954). Olszewski (1954), for example, has pointed out the inconsistency in anatomical nomenclature for nuclei within the brainstem reticular formation and has described 98 cell masses within the human lower brainstem. The point of his study was simply "... that the discovery of morphological differences points to the presence of functional differences. Accordingly, we may expect that future investigation will disclose functional differences between all nuclei of the lower brain stem." (Olszewski, 1954, p. 75). Although adjacent nuclear masses were often undifferentiated and certain nuclei contained a mixture of large and small cells, both aspects confusing the picture of Olszewski's proposed reticular structure, such an approach has stimulated and complemented more detailed studies of fiber connections within the central brainstem as well as of ascending and descending pathways.

For example, Brodal (1958) has differentiated nuclei on the basis of fiber projections and connections. He offers a rudimentary organization of the brainstem core into three parts, with cerebellar-projecting nuclei distinct

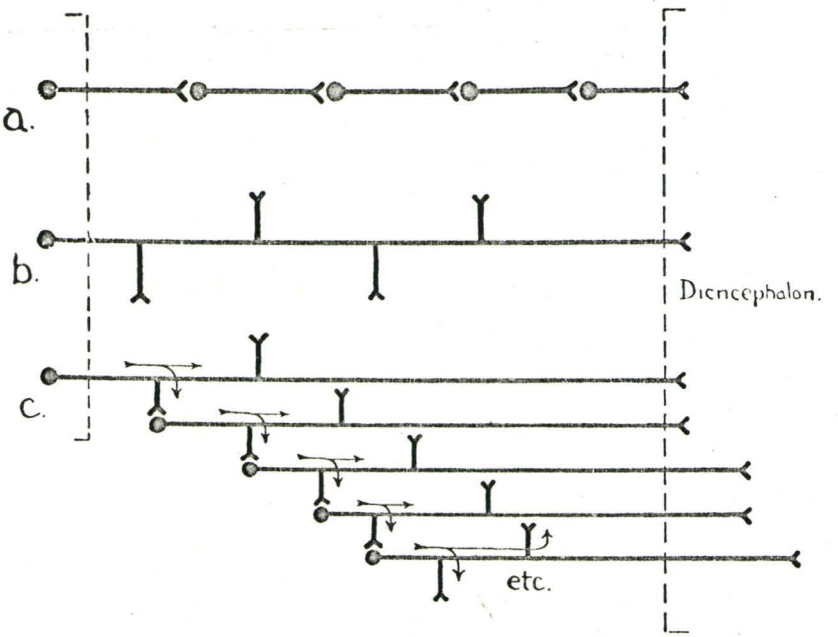

FIGURE 10. Diagram showing several possible conduction circuits through the reticular core of the brain stem. *a* shows the type of chaining of short-axoned cells which has been hypothesized by Moruzzi and Magoun and by a number of other workers to explain conduction characteristics marked by slow transmission, long latency, and recruiting. *b* shows a single long-axoned cell, reaching from bulb (dotted line at left) to diencephalon and illustrating the type of conductor which has been found in very large numbers in the reticular formation. *c* illustrates that the many collaterals of long conductors, as in *b*, may provide for more circuitous paths through the reticular core producing increasing lateral dispersion and increasingly long conduction times and longer latencies.

Figure 4. Taken from Scheibel and Scheibel, 1958, p. 44.

from medial nuclei, which receive primary afferent input and subserve effector functions, and each of these classes of nuclei are in turn further distinct from more laterally-situated nuclei, which receive collateral afferent input and "... may be more adapted to serve 'receptive' and 'associative' functions." (Brodal, 1958, p. 50). This tentative medio-lateral distinction was based primarily on the fact that the n. gigantocellularis in the medial medulla receives a copious afferent supply but its long axons project mainly caudad, hence the emphasis on effector function. It should be recalled, in addition, that Magoun and Rhines (1946) inhibited motor activity by stimulation of this region (i.e., n. gigantocellularis). More recently, however, Sprague and Chambers (1954) have shown that stimulation of various points within the medial brainstem (including n. gigantocellularis) yielded ipsilateral limb flexor tonus and extensor inhibition with contralateral extension and flexor inhibition. Stimulation of lateral reticular formation produced an opposite pattern from the medial one. These two reciprocal postural patterns could be duplicated by direct cerebellar stimulation (presumably indirect reticular activation) of the vermal cortex and fastigial nuclei.

It appears that lateral divisions of the reticular formation may have very specific motor functions and further that both medial and lateral nuclei may be closely related to cerebellar outflow. This does not agree with

Brodal's schema of an anatomically-separate reticular organization. Nevertheless, the observation that nearly adjacent stimulation will produce markedly different electrocortical responses (Jasper, 1960) suggests that the reticular formation nuclei may be functionally distinct.

Many studies have shown that low rate stimulation of medullary structures near the region of the solitary nucleus (Magnes, Moruzzi and Pompeiano, 1961) or n. reticularis gigantocellularis (Favale, Loeb and Sacco, 1961) in quiescent preparations precipitates synchronization of the EEG after a long latency but outlasting the stimulus (considered, therefore, a non-specific response). Pontine or midbrain reticular stimulation at high frequencies under similar conditions more often produces an activated EEG (Moruzzi and Magoun, 1949). When the medulla is separated by lesion from these pontine activating structures (mid-pontine pretrigeminal preparation), abnormally long periods of low voltage fast cortical activity usually occur, (mean 78 $\pm$ 10 per cent as compared to a mean 37 $\pm$ 12 per cent for normals), for the post-operative recovery time. On the other hand, a complete brainstem transection at the rostral border of the pons (rostropontine) will usually induce less desynchronized activity (Batini, Moruzzi, Palestini, Rossi, and Zanchetti, 1959a). One conclusion about the functional organization of these reticular areas that accounts for these results is that the pontine nuclei (n. reticularis

tegmenti pontis and n. reticularis pontis oralis) whose giant ascending fibers may regulate the thalamic reticular system are themselves regulated by the massive medullary fiber systems of n. reticularis ventralis and n. reticularis gigantocellularis. Evidence that medullary structures may truly inhibit the activating "centers" has been given by the prolongation, following bulbar lesions, of EEG arousal (Bonvallet and Bloch, 1961), and of inhibition of Edinger-Westphal parasympathetic discharge (Bonvallet and Allen, 1963), both being indices of reticular activation.

The notion of an antagonistic deactivating influence originating in the medulla is further supported by a differential role of afferent input, i.e., medullary deafferentation increases activation, supra-medullary deafferentation decreases it. For example, Bonvallet and Allen (1963) showed that removal of cardiovascular afferents (IX and X) released inhibitory control of pontine activation (i.e., slightly prolonged phasic EEG arousal and Edinger-Westphal inhibition). On the other hand, withdrawal of pontine or midbrain afferents precipitate (Roger, Rossi and Zirondoli, 1956; Batini, Magni, Palestini, Rossi and Zanchetti, 1959b) or heighten (Batini, Palestini, Rossi and Zanchetti, 1959c; Bizzi and Spencer, 1962), EEG synchrony. By clamping the basilar artery in cats, Magni, Moruzzi, Rossi and Zanchetti (1959) were able to selectively depress either the medulla with a small vertebral artery injection of Thiopental,

producing EEG arousal, or the rostral pons with intracarotid Thiopental, producing synchrony. These differential effects of pharmacological blockade complement the findings of differential effects of deafferentation just cited and suggest that the afferent input to medulla or pons serves to maintain or reinforce the regulatory (inhibitory) activity of the respective reticular regions (medulla or pons). Withdrawal of medullary afferents would (and does) decrease the efficacy of its inhibitory control on the pons, whereas pontine deafferentation releases forebrain spindling. It also follows that stimulation of the vagal afferents (Bonvallet and Sigg, 1958), the sensory nucleus of the vagus nerve (Magnes et al., 1961) or carotid distension (Bonvallet, Dell and Hiebel, 1954) induces electrocortical synchrony.

However, an alternative conception of the role of afferent input on reticular structure is that these regions do not have intrinsic activity patterns but rather are a collection of "passive" sensory neurons which reflect the activity of the afferent input. In support of this conception are the findings of EEG desynchronization with high rate vagal stimulation (Bonvallet and Sigg, 1958) and EEG synchronization with low rate cutaneous stimulation which at higher rates produced desynchronization (Pompeiano and Swett, 1962a). These Group II cutaneous fibers ascend through the anterolateral funiculi (Pompeiano and Swett,

1962b) and distribute diffusely to medulla and pontine reticular regions (Scheibel and Scheibel, 1958). Direct electrical stimulation of the same point in the medulla at low rates producing synchrony and at high rates desynchronization is a common finding (Magnes et al., 1961; Favale et al., 1961; Abeles, 1968); although in some regions high frequencies have evoked brief periods of synchrony (Ingvar and Soderberg, 1958), sometimes obtainable only in anesthetized animals, (Kaada, Thomas, Alnaes and Wester, 1967).

A third hypothesis about brainstem reticular function which would account for these frequency-determined stimulation effects is that reticular neurons preferentially sensitive to either low or high rates of stimulation run intermingled throughout the reticular core. This notion, almost identical to the previous one, suggests that different neuron groups have specialized functions (synchronizing or desynchronizing) at low stimulation thresholds. Abeles (1968), in attempting to find threshold differences in so-called synchronizing or desynchronizing centers, found instead almost identical strength-duration curves for high frequencies producing desynchronization and low frequency pulses which elicited synchrony. This result may fit either of the last two hypotheses; however, neither of the last two hypotheses can accomodate the fact that reduction of stimulation (medullary deafferentation) may heighten activation.

The prevailing view of the organization of the non-specific brainstem is that nuclei at upper pontine levels (n. reticularis pontis caudalis, n. reticularis pontis oralis and others) emit fibers which exert a direct (usually inhibitory) control over autonomic (e.g., pupils), somatic (e.g., digastric reflex), and EEG activity (e.g., recruiting responses or spontaneous electrocortical synchrony). It should be recalled that midpontine pretrigeminal (Batini et al., 1959a) and pre-bulbar lesions (Bonvallet and Allen, 1963), both of which spare n. r. p. oralis, result in activated indications (e.g., EEG arousal, pupil tonus) while a rostropontine lesion (Batini et al., 1959a) a few millimeters rostral will interrupt ascending pontine fibers and induce electrocortical synchrony. Small electrolytic lesions which reliably produce EEG synchrony have been localized to n. reticularis pontis oralis, with surrounding regions having variable effects (Camacho-Evangelista and Reinoso-Suarez, 1964).

Bulbar inhibitory neurons are capable of regulating this pontine outflow, even suppressing these more rostral activating structures. The balance of control between these two non-specific levels is apparent in the encéphale isolé preparation, which exhibits alternating periods of EEG synchrony and activation. This balance can be shifted toward activation by deafferentation at the bulbar level (Bonvallet and Allen, 1963), which reduces bulbar inhibi-

tory efficacy, or shifted towards deactivation after pontine deafferentation with (Batini et al., 1959b) or without an intact medulla (Roger, Rossi and Zirondoli, 1956).

The finding that long axon projections may pass through the long axis of the brainstem from caudal medulla to rostral thalamus or striatum (Scheibel and Scheibel, 1958) has been reaffirmed by Nauta and Kuypers (1958) on the basis of axonal degeneration studies. Long ascending axons originate from the medial gigantocellular regions of the medulla (e.g., n. medullae oblongatae centralis, n. gigantocellularis) and pons (e.g., n. pontis oralis, n. reticularis tegmenti pontis) and ascend through certain longitudinal tegmental tracts[1] in common with "specific" sensory pathways, leaving a picture of intermingled, parallel "specific" and "non-specific" ascending sensory conduction. The more laterally situated reticular regions consist of small-celled groups whose axons trace a broad-banded lateral tegmental fasciculus which turns medially to join Forel's tractus fasciculorum at the level of the main trigeminal nucleus. These lateral parvocellular groups emit shorter axons, a considerable proportion going medially into gigantocellular groups.

Forel's tegmental tract is considered to be the main

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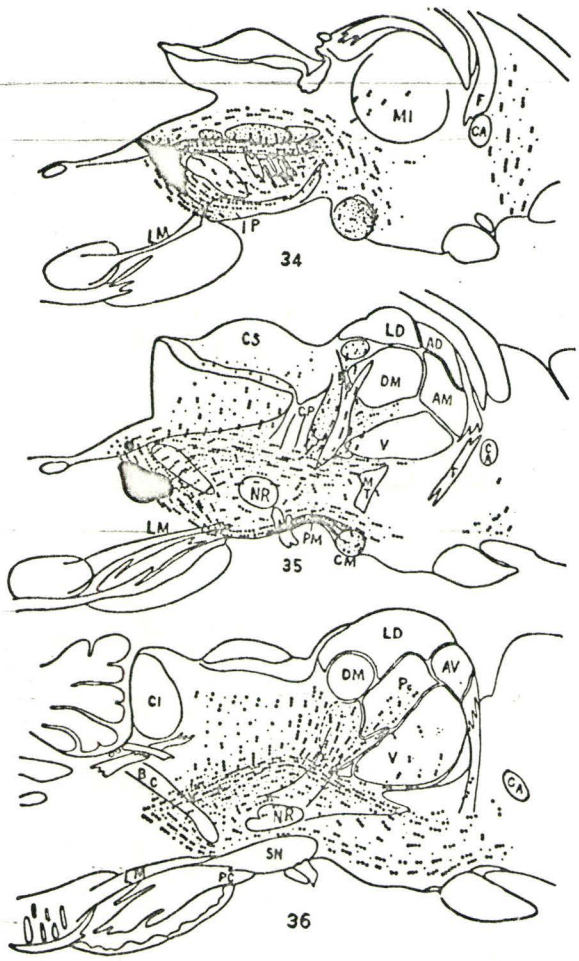
1. "... a group of compactly degenerated ascending fiber bundles, ventrolateral to the medial longitudinal fasciculus ..." (Nauta and Kuypers, 1958).

ascending pathway for reticular projections. The progressive thinning of axonal degeneration following discrete reticular lesions as well as the profuse terminal degeneration noted in the bundles of this tract during its rostral course suggests that it contains an abundance of short ascending fibers for widespread intrareticular conduction. However, it is difficult, on the basis of axon degenerations following a reticular lesion, to say whether the sequential terminations originate from reticular neurons or result from the interruption in transit of long fibers of the spinal lemniscus terminating in the medial core and sharing ascending pathways with reticular projections, as previously mentioned (Nauta and Kuypers, 1958).

Fibers ascending through the brainstem reticular formation may leave the midbrain via Forel's tract (Nauta and Kuypers, 1958), which at that level corresponds to the central tegmental tract (Morin, 1953), and project diffusely to the diencephalon with a distinct bifurcation dorsally to thalamus (parafascicular nucleus - centromedian complex) and ventrally to subthalamus (field H of Forel) (see Figure 5). Starzl, Taylor and Magoun (1951) traced these rostral pathways and found desynchronization to a repetitive brainstem stimulus and evoked potentials to single shocks in sub- and hypothalamus extending into the internal capsule, and the same results more dorsally into ventromedial thalamus, where the non-specific "synchronizing" nuclei may be



FIGURES 34–36. Case CT 29. Degeneration of ascending fiber systems charted from sagittal sections in a case of paramedian lesion of the caudal midbrain tegmentum. Symbols as in preceding figures.

Figure 5. Taken from Nauta and Kuypers, 1958.

directly inhibited. Other experiments have shown that interruption of these pathways unilaterally induces cortical synchrony unilaterally (Bach-y-Rita et al., 1969). This ascending reticular pathway has been further confirmed by Brodal and Rossi (1955), with an emphasis on projections to intralaminar thalamus, dorsal hypothalamus, septum, and pre-optic region. Fiber degeneration may extend from the central mesencephalon via the internal capsule to the head of the caudate nucleus or disperse in the globus pallidus and putamen (Nauta and Kuypers, 1958). Degeneration into "specific" thalamic nuclei is uncommon, and direct reticulocortical fibers have never been evidenced.

Since at the thalamic level only the "specific" nuclei have abundant direct projections to the neocortex, the non-specific thalamocortical pathways are not well-defined. For, although non-specific thalamic fibers are known to synapse in "specific" thalamic areas (Nauta and Whitlock, 1954), these latter nuclei project only to limited areas of the neocortex and the characteristically widespread cortical responses to non-specific thalamic stimulation survive total specific nuclear destruction (Hanbery and Jasper, 1953), remaining or even increasing in sensory cortex (Jasper, Naquet and King, 1955).

Retrograde degeneration of intralaminar thalamic nuclei occurs following total decortication (Murray, 1966) and less severe retrograde deformities of non-specific cell

nuclei have been reported as evidence of some direct thalamocortical projections (Nashold, Hanbery and Olszewski, 1955). However, the paucity of degenerative changes in these non-specific nuclei suggests that direct fibers to the neocortex are few in number, probably considerably outnumbered by multi-synaptic fiber pathways which would account for the long latency required for the appearance of recruiting potentials throughout neocortex following non-specific thalamic stimulation.

Rostrally-orientated fibers from the thalamic reticular system generally pass through the anterior limb of the internal capsule, as evidenced by severe intralaminar degeneration following capsular damage (Nashold et al., 1955; Nauta and Whitlock, 1954). For this reason, the striate bodies have been thought to relay non-specific activity to cortex, especially in view of the fact that caudate-cortical afferents, like recruiting potentials and unlike specific augmenting waves, do not activate corticospinal neurons (Purpura, 1959). However, caudate projections to neocortex are not well understood, and the latency for cortical recruitment may often be less with intralaminar than with caudate stimulation (Jasper, 1960). Recruiting potentials occur in neocortex after caudate removal (Ajmone-Marsan and Dilworth, 1953). Finally, Goldring, Anthony, Stohr and O'Leary (1963) have shown that the "caudate-cortical" evoked potentials in the cat neocortex resembling non-

specific responses (spindle and recruiting response) may be due to the proximity of internal capsule fibers to caudate stimulation; since in the monkey with non-contiguous caudate and capsule, these non-specific potentials were evoked only by capsular stimulation.

Lesions at different levels of the internal capsule resulted in differential degeneration within the non-specific nuclei, with the more ventral medial parts of the thalamus (midline nuclei, middle part of dorso-medialis) projecting to the ventro-medial capsule fibers and the more dorso-lateral nuclei (n. paracentralis, centralis lateralis and portions of centrum medianum) sending fibers through the dorsal portion of the capsule's anterior limb (Nashold et al., 1955). This topographical relation for thalamic outflow along the radiations of the internal capsule has been extended to projections onto various cortical regions, with the anterior pole of the thalamus (n. ventralis anterior, n. reticularis, and anterior limb of internal capsule) seen as the final filter for widespread distribution of cortical recruiting responses (Hanbery, Ajmone-Marsan and Dilworth, 1954). Small ventromedial lesions of the n. ventralis anterior, including the oral pole of n. reticularis and the medial portion of the anterior limb blocked frontal and motor cortex recruitment to centrum medianum stimulation which remained in posterior cortex. Conversely, contralateral frontal cortex recruitment was

unaffected by dorsolateral ventralis anterior and n. reticularis lesions which were large enough to abolish intrathalamic recruiting recorded from the spared region of n. ventralis anterior as well as recruiting in association and posterior cortex. This pattern of projection was essentially the same for stimulation of various non-specific thalamic nuclei caudad to n. ventralis anterior and n. reticularis. In 1949, Rose and Woolsey (1949) suggested such a distributory role for the reticular complex by noting that in rabbit and cat, "After various restricted cortical removals (degenerative) changes are present within different restricted sectors of the reticular complex." (Rose and Woolsey, 1949, p. 393).

The suggestion that the widespread occurrence of recruiting potentials may be conducted cross-cortically from a focalized projection point seems untenable since the latency differences for the appearance of recruitment from one cortical region to the next is usually smaller than the latency required for conduction through horizontal elements of the cortex. However, Velasco and Lindsley (1965) reported that total lesion of only the orbitofrontal cortex, where recruiting latencies are customarily shortest, was sufficient to block recruitment and decerebrate-induced spindling across the remaining cortex (Velasco and Lindsley, 1965), in subcortical white matter and in the thalamus (Velasco, Skinner, Asaro and Lindsley, 1968).

This puzzling result, obtained in unanesthetized, paralyzed cats, stands in direct contradiction to Dempsey and Morison's (1942a) report of recruitment remaining in posterior cortex after entire frontal cortex removal (or vice-versa) in nembutalized cats and Jasper's (Jasper et al., 1955) demonstration of true recruiting potentials in auditory cortex isolated except for thalamic radiations in cats paralyzed after withdrawal of surgical Pentothal anesthesia. For the latter two experiments, the effects of the barbiturates would have an enduring influence on cortical potentials, thereby enhancing the likelihood of synchronized or rhythmic potentials. The implication of Velasco's experiments are perhaps not to validate the idea of cross-cortical conduction but rather to suggest the existence of corticothalamic feedback as an important link in the production of rhythmic thalamocortical projections. The proximity of the orbital gyrus to the rostral thalamus (n. reticularis, n. ventralis anterior) and the prevalence of orbital gyral fibers to the thalamic nucleus reticularis (Nauta, 1964, p. 401-404) with its diffuse cortical projections would allow for a thalamo-cortico- thalamo-cortical circuit in time with the slightly longer latencies of non-frontal cortical recruitment. Orbital gyrus stimulation may enhance light barbiturate spindles across the neocortex and high frequency stimulation elicits the typical generalized EEG arousal (Kaada, 1960) also elicited in midline thalamus or midbrain tegmentum

(Moruzzi and Magoun, 1949) but not usually found in other cortical areas (Bremer and Terzuolo, 1954).

As previously mentioned, corticipetal afferents from non-specific thalamic nuclei are thought to be distinct from specific afferents due to evidence of a lack of mutual occlusion and a significant latency difference for cortical slow waves evoked by non-specific thalamic stimulation (recruitment) and specific thalamic stimulation (augmenting). In addition, phase reversals for these evoked waves throughout the cortical layers differ for recruiting and augmenting responses (or specific evoked potential), suggesting distinct synaptic terminations on cortical neurons (Li et al., 1956; Spencer and Brookhart, 1961). For example, Spencer and Brookhart (1961) recorded a short-latency (1 msec.) cluster of action potentials in layers III - V during the surface-positive phase of the augmenting response but irregular occurrences of long-latency extracellular spikes in these layers during the recruiting response. These authors concluded that specific afferents terminate on pyramidal cells in layers III - V to generate a deep "sink" (surface-positivity) which migrates to the superficial layers (surface-negativity) thereby inducing the positive-negative sequence of the augmenting response. Activation of non-specific afferents create only a small superficial "sink" (deep positivity) without evidence of synaptic location. Hence, although

the non-specific afferents which are thought to control the spontaneous surface potentials may be activated synchronously by non-specific thalamic stimulation, the resulting cortical waveform (recruitment) is apparently generated by cortical synapses very different from synaptic activation due to synchronous specific afferent volleys.

The importance of subcortical afferents for the maintenance of "spontaneous" cortical cell discharge has been emphasized by Burns' evidence of reduced or eliminated "spontaneous" cell activity in neurologically isolated cortical slabs as compared with cortex having intact subcortical connections (Burns, 1958). This might suggest that cell discharge patterns in the cerebral cortex are determined by non-specific afferents which also determine the patterns of the electrocorticogram. Li (1956), for example, has shown that stimulation of the non-specific nucleus centromedianus may increase the number of posterior sigmoid unit discharges evoked by VPL stimulation approximately 100 msec. later, in time with the latency for recruiting responses. Also, a close relationship between cortical spindle waves and unit discharges in motor cortex (Whitlock, Arduini and Moruzzi, 1953) is a common finding. On the other hand, high frequency stimulation of diencephalic or mesencephalic reticular formation, which produced electrocortical desynchrony, reduced or blocked unit activity in motor cortex (Calma and Arduini, 1954).

Such experiments reinforce the idea that the non-specific reticular system maintains or modulates the activity patterns of cortical neurons, (usually referred to as spontaneous activity due to the absence of intentional specific stimulation). Direct support for this view is shown in an experiment in which, subsequent to midbrain lesions, the spontaneous activity of Betz cells was changed from "rhythmic discharges" (i.e., intervals between spikes were usually 50 - 100 msec. but rarely less than 10 msec.) to "interrupted random discharges" (i.e., a clustered or burst pattern), (Martin and Branch, 1958). It is suggested that tonic brainstem reticular inhibition prevents successive discharges with short intervals and promotes regularity or rhythmicity of discharge pattern. This reticular control of discharge pattern may be abolished by a midbrain lesion.

Recent experiments evaluating the relationship between cortical neuron discharge and the electrocorticogram have demonstrated high correlations between spontaneous unit discharge and slow wave surface positivity (Fromm and Bond, 1964) or negativity in the region of the cell (Fox and Norman, 1968). Other experiments, however, have noted instances of dissociation either under natural conditions or experimentally induced. For example, Evarts (1961) and Murata and Kameta (1963) showed that spontaneous slow-wave EEG patterns during light sleep were often associated with an increase in the discharge rate of single cortical cells

over the rate that existed during the low-voltage fast (LVF) pattern of wakefulness. This rate increased even further in the transition to the activated (LVF pattern) phase of deep sleep or after arousal. Deepening anesthesia or hypoxia which did not significantly alter the production of high voltage slow (HVS) cortical waves did, however, abolish cell discharge (Li, McLennan and Jasper, 1952). Intracellular recordings from cortical neurons during analogs of spontaneous EEG activity (barbiturate synchrony or recruitment) have shown high cross-correlations between slow membrane transients (EPSPs and IPSPs) and surface potentials (Klee, Offenloch and Tigges, 1965), with or without neuron discharge (Creutzfeldt, Watanabe and Lux, 1966).

Why slow voltage transients (PSPs) of relatively small magnitude (10 - 20 mv) should be detected at the surface while larger (50 - 90 mv) fast transients (spikes) are not has been explained as due to temporal dispersion in potential gradients, i.e., potential fields generated by rapid membrane depolarization would be less likely to summate than fields due to long duration PSPs. While this explanation may hold for the "random" activity of the spontaneous EEG, even in non-random evoked cortical potentials, where many units fire in synchrony, the PSP contribution dominates. For example, Humphrey (1968b) recorded from inside Betz cells during antidromic activation and compared those membrane transients with a simultaneous extracellular

recording taken 0.4 mm. laterally. Synchronous action potentials did not summate to affect the extracellular record, which virtually duplicated the slow membrane fluctuations (PSPs).

This recent evidence has provided support for explanations of the electrogenesis of cortical surface potentials as due to summated membrane potentials. One version (Creutzfeldt et al., 1966) asserts that rapid membrane transients in the cortical depths are "seen" with an opposite polarity on the cortical surface, i.e., a quick EPSP induces a surface positivity, IPSP induces surface negativity. Due to electrotonic conduction which involves some delay along the soma-apical extent, the rapidly depolarizing soma is likely to have repolarized and may then become a "source" for the "sink" of the superficial apical dendrite, which depolarizes to produce surface negativity. It should be recalled that this account was used by Spencer and Brookhart (1961) to explain generation of the augmenting response. Soma transients with durations outlasting the soma-dendrite conduction delay would result in virtually uniform depolarization at soma and dendrite without a surface phase inversion. Hence, slow IPSPs would generate surface-positive surface potentials, long-lasting EPSPs producing surface negative waves (Creutzfeldt et al., 1966).

A preponderance of axo-dendritic synapses from nonspecific afferents has been suggested to explain the sur-

face-negative components of recruitment and barbiturate spindles. In support of this contention, Jasper and Stefanis (1965) report that surface spindles may occasionally precede in time the onset of an intracellular EPSP. Nevertheless, the importance placed upon dendritic depolarization as the generator of surface negativity either by electrotonic conduction (Creutzfeldt et al., 1966) or synaptic activation (Jasper and Stefanis, 1965) is questioned by the experiments of Jabbur and Towe (1961) and Humphrey (1968a). They showed that stimulation of the medullary pyramids, uncontaminated by co-stimulation of adjacent tissue (lemniscal afferents), resulted in activation of Betz cells and antidromic invasion of dendrites but only two surface positive waves could be recorded.

In addition to these findings that antidromic activation of dendrites may <u>not</u> give rise to surface-negative potentials, these explanations rely on an histological arrangement of pyramidal cells situated about 1 mm. below the surface and projecting their apical dendrites to the surface. Though such neurons exist in mammalian cerebral cortex, it would be an over-simplification to regard deep cortical potentials as somatic in origin and superficial potentials as reflecting dendritic "sinks" or "sources". Interpretations of the cortical elements providing electromotive force for cortical surface potentials would be improved by knowledge of (1) the size and depth distribution

as well as the dendritic lengths of neurons in the population from which surface potentials are recorded, and (2) laminar intracellular analysis of membrane potentials correlated with simultaneous surface potentials.

In consideration of the many experiments demonstrating reticular influences on the EEG and others of non-specific effects on single cortical neurons, the present research was designed to determine if these non-specific influences have a common basis. In the present experiments, in order to study the distribution of responding elements within the neuron population, extracellular action potentials were sampled in cat cortex exhibiting different spontaneous surface potentials, without experimental treatments such as electrical stimulation, differential anesthetics or hypoxia which affect synaptic transmission. By differential transection of the brainstem, ascending non-specific fiber systems of the reticular core induce characteristic EEG patterns. In the experiments to be reported, it will be shown that certain non-specific afferents also exert an influence on the activity of neurons in the suprasylvian gyrus; although it was found that this change in activity was sometimes unrelated to the pattern of the surface slow potentials. As a result, these experiments lead to the conclusion that the density of active units in a cortical neuron population bears no necessary relation to the form of the surface slow potentials recorded from the vicinity of that population.

CHAPTER TWO

METHOD

Subjects and Surgical Preparation

All experiments were acute and were performed on healthy cats weighing between four and eight pounds. Pregnant females were rejected.

Surgical anesthesia was induced by ethyl chloride and maintained by ether. Tracheotomy was then performed on all cats, the femoral vein being cannulated in some animals to administer drugs and the femoral artery in others so as to measure blood pressure. The animals were placed in a David Kopf stereotaxic instrument after Xylocaine jelly had been placed on the ear bars and nose clamp. A Xylocaine solution (lidocaine hydrochloride) was injected into the head and neck region for certain experiments in which the trigeminal (V) nerves, which transmit sensation from the head, were left intact.

A midsaggital incision and removal of the temporal muscles exposed the cranium from the naso-frontal suture to behind the lambdoidal ridge. In experiments in which the trigeminal nerves were bilaterally sectioned at their foramina, the entire temporal muscle was removed to expose the mandibular ramus, which was chipped away with bone

cutters. The origin of the masseter muscle along the contour of the skull was scraped away to expose the foramen rotundum (maxillary portion of the Vth nerve), orbital fissure (ophthalmic portion), and foramen ovale (mandibular portion). All the nerves passing through these foramina were severed, including the oculomotor (III), trochlear (IV) and abducens (VI) motor nerves to the eye that exit through the orbital fissure. In one cat (X26), bilateral gasserectomies by step-wise electrolytic lesions (3 mA passed for 20 seconds for each step) were performed five weeks before a PoT transection. During those weeks, trigeminal nerve damage was verified in part by the inability of the cat to masticate.

Before surgical anesthesia was discontinued, a complete transection of the brainstem was performed at one of three levels on each cat by the method of blunt traction using a U-shaped, wire knife stereotaxically guided through holes in the cranium (see Figure 6) down through the cerebellum to the base of the brainstem (see Figure 7). This procedure isolated the entire forebrain and some of the hindbrain from the remainder of the central nervous system. In preparations in which the section was rostral to the fifth nerve rootlets (pretrigeminal lesions), afferent input to the isolated forebrain was possible through cranial nerves I (N. olfactorius) and II (N. opticus). When there were complete lesions caudal to the fifth nerve (post-tri-

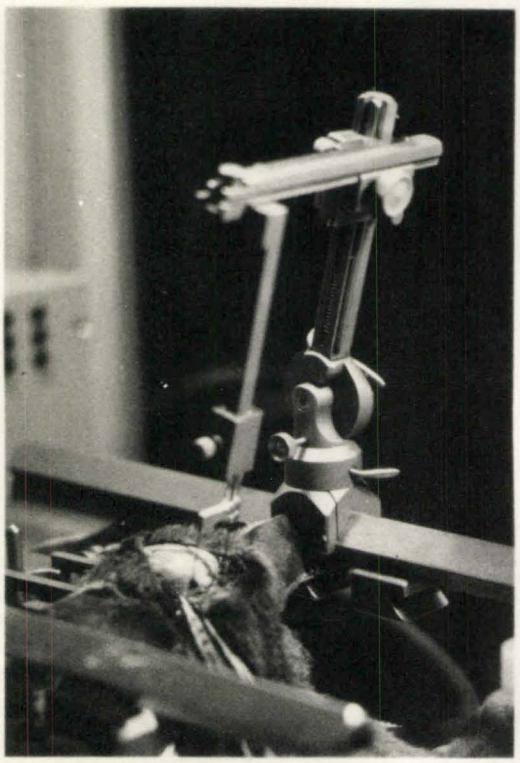


Figure 6

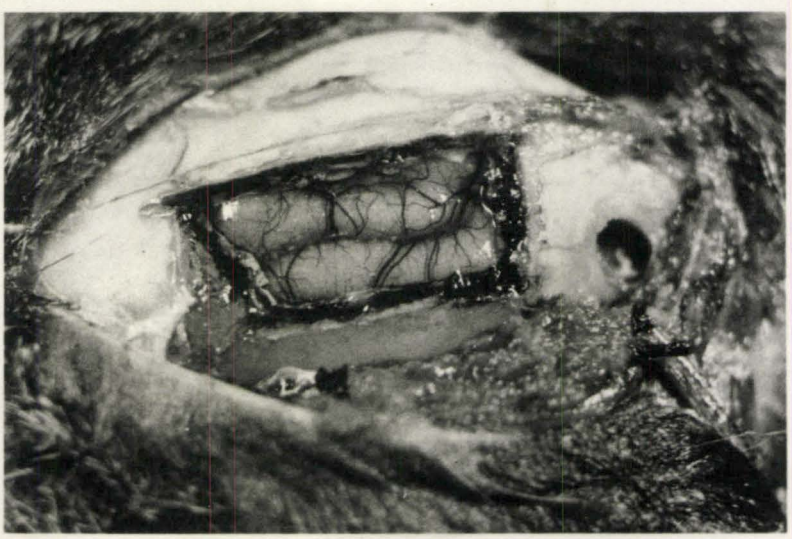


Figure 9

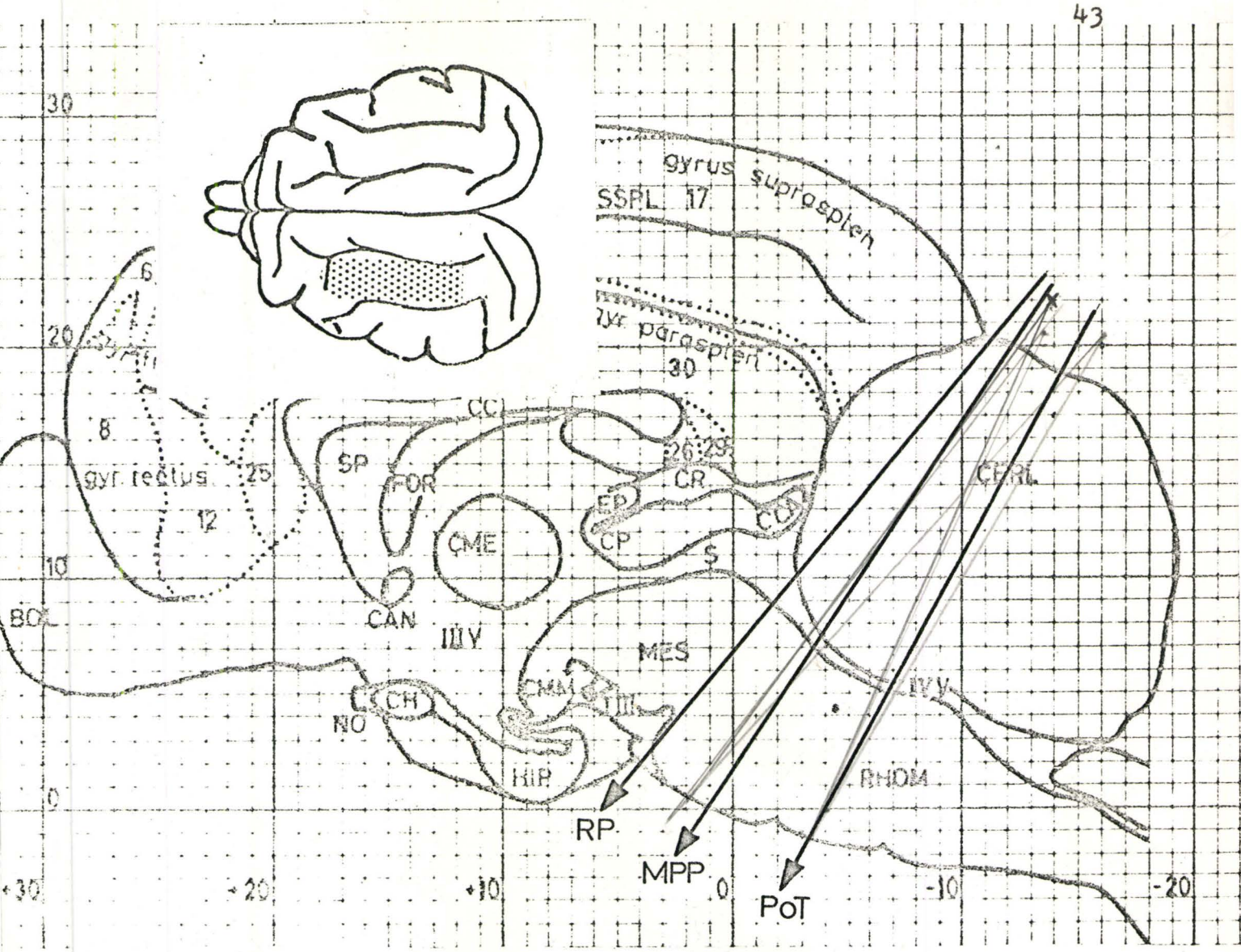


Figure 7. Lateral view of a sagittal section of the cat brain in a drawing. The arrows represent planes of transverse brainstem section:
RP - Rostropontine
MPP - Midpontine pretrigeminal
PoT - Post-trigeminal
Insert at upper left of page is a dorsal view of the major gyri of the cat cerebral cortex. The speckled area represents the anterior and median suprasylvian gyrus from which the recordings were made.

geminal lesions), the input from sensory fibers of N. trigeminus (V) which synapse in the mesencephalic nucleus was available; however, the descending tract to the spinal nucleus of N. trigeminus was interrupted. For the results reported in Experiment I, the position of the transverse section along the longitudinal neuraxis was manipulated as the independent variable.

In Experiment II, neuronal conduction through the pontine region of the brainstem was blocked by cooling that region, using two specially constructed cryogenic probes (for details see Appendix: Cryoprobe System) which could be stereotaxically placed on either side of the pontine reticular formation (see Figure 8). Blood pressure changes due to the temporary inactivation of a descending pontine influence on cardiovascular centers in the medulla were prevented by a lower pontine (post-trigeminal) transection. Therefore, in these cooling experiments the presence or absence of ascending pontine influences served as the independent variable.

Following decerebration and insertion of the cooling probe, ether was discontinued. If breathing stopped, artificial respiration (Large Animal Respirator, Model 607, Harvard Apparatus Co. Inc., Dover, Mass.) was supplied and CO_2 content in expired air was regulated to between 2 and 3 per cent CO_2 (CO_2 Analyzer Model 2000, Harvard Apparatus). A unilateral or bilateral craniotomy (approximately 4 X 3 cm.)

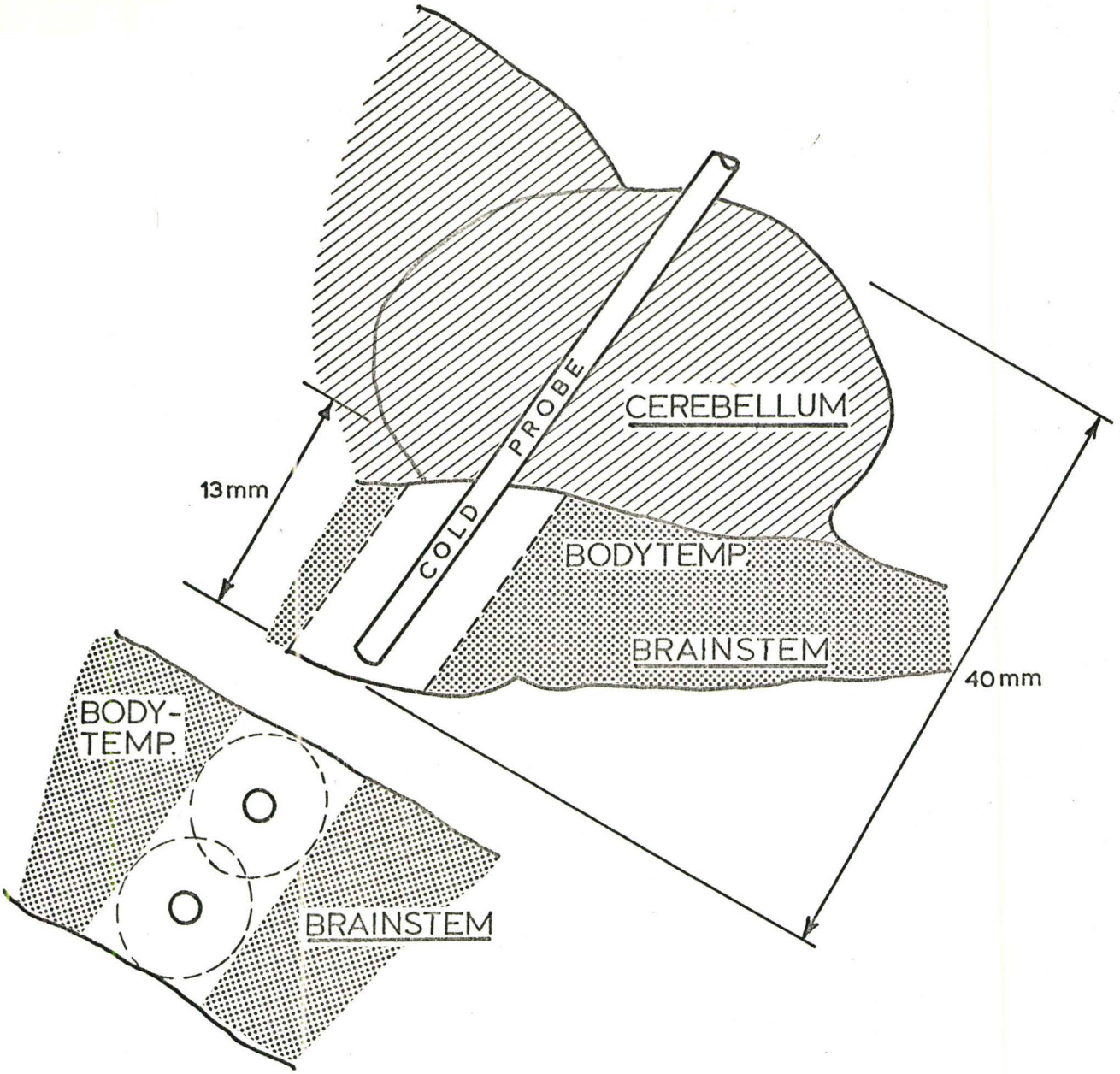


Figure 8. Top drawing is a side view of a sagittal section of the cat brain with cryogenic probe inserted. Bottom drawing is the dorsal aspect of the brainstem with two circles depicting approximate location of each cryogenic probe and approximate region of cooling (white area).

followed by a removal of the dura exposed the middle suprasylvian gyrus (see Figure 9, page 42) and portions of the marginal (lateral) and ectosylvian gyri. The right lateral ventricle beneath the posterior suprasylvian gyrus, the side from which no recordings were made, was drained to relieve brain swelling, when necessary.

The skin around the cranium was sutured to a metal ring to form a well which was filled with mineral oil heated to 37° C to keep the cerebral cortex moist and warm. The abdomen of the cat was placed on a heating pan (Thermistemp Temperature Regulator Model 71, Yellow Springs, Ohio) which automatically regulated rectal temperature to 37° C. The thoracic spinal column was suspended by stretching one hind limb and tying it to the rear of the Faraday cage; this procedure suspended the rib cage in space and reduced movements of the spinal cord and brain during lung expansion.

Following certain neurological tests of peripheral responsiveness (see below) gallamine triethiodide (Flaxedil, Poulenc Ltd., Montreal, approximately 15 mg/kg i.p. or 10 mg/kg i.v.) was administered at approximately 100 minute intervals as a paralyzant to prevent reflex movements and restoration of normal or apneuistic breathing which would compete with the artificially controlled adequate ventilation.

Procedure and Data Collection

A brief neurological examination was given following surgery, preceding paralysis and electrophysiological recording. Indications of peripheral responsiveness were used to determine if the cat's behavior was consistent with the intended level of brainstem damage and to confirm the integrity of certain brainstem nuclei, (see Results). Tests included indications of the presence or absence of: (1) horizontal and/or vertical eye tracking movements, (2) normal pupil diameter, (3) pupillary light reflex, (4) limb resistance to passive movement, (5) hindlimb movements to abdominal scratch and (6) spontaneous breathing. Pupil diameter was monitored periodically throughout the recording sessions.

Potentials of the EEG at the cortical surface were derived from saline-soaked wick, or platinum ball-tip, electrodes placed on the surface of the anterior or median suprasylvian gyrus near the point of microelectrode penetration. These potentials were referred to a chlorided silver-wire ground electrode located in the killed right temporal muscle and led through a push-pull A.C. preamplifier (Grass Model P-5, Grass Instruments Inc., Quincy, Mass.) with 3 db attenuation at 0.1 Hz and 100 Hz. Transcortical potentials (electrocorticogram or ECoG) and extracellular neuronal discharges (action potentials or spikes) were recorded from micropipettes (1 - 3 microns tip diameter) filled with nearly-saturated NaCl solution. These potentials

in the cortical depth were referred to a 0.9% NaCl-soaked wick on the cortical surface above the micropipette. Non-polarizable Ag-AgCl electrodes were led from the wick and micropipette to a D.C. preamplifier specially constructed from two operational amplifiers (Differential Operational Amplifier Models P25 and P65, Philbrick Researches Inc., Dedham, Mass.) and designed for a differential gain of 1000, drift less than 5 uv/min, and 25 uv or less noise for action potential (300 Hz - 16 KHz) and slow potential (0 - 80 Hz) bandwidths, respectively. The input capacitance of approximately 15 pf was insignificant for the relatively low resistance (approximately 0.5 megohms) of the microelectrodes used. This D.C. preamplifier allowed measurement of slow potentials which would have been filtered with RC-coupled amplification. The broad-banded amplified signal was found to roll-off to half-amplitude at 16 KHz and further filtering separated the spikes (high pass filter, half-amplitude at 300 Hz) and the ECoG (low pass filter, half-amplitude at 80 Hz) for storage, along with the surface EEG and voice monitor, on four-channel magnetic tape (Model SP300, Ampex Corp., Redwood City, California).

Recording Procedures

Glass micropipettes of 1 to 3 microns internal tip diameter were suspended from a "weightless" microelectrode

holder (see Figure 10 taken from Smith and Smith, 1965) fixed to a rigid micromanipulator. The microelectrode tip

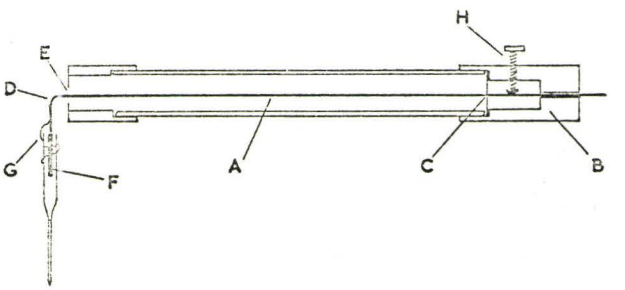


FIGURE 1 Modified "weightless" microelectrode carriage. The electrode is suspended from the stainless steel wire, *A*, which is anchored in a perspex cylinder, *B*, shown in cross-section. The wire is beaten out flat and bent to a right angle at *D* to allow the third dimension of free movement. A silver wire treated with chloride, *F*, makes contact with the electrolyte in the micropipette. The latter is clamped in the looped end of the spring wire, *G*. The wire passes through a small hole in the diaphragm *C* which acts as a fulcrum for the thumbscrew *H* bearing against the wire. The thumbscrew is adjusted so that spring just supports the weight of the pipette and so that its end hangs freely in the center of the slit *E*. The spring is protected within a glass barrel.

Figure 10. Taken from Smith and Smith, 1965, p. 49.

was lowered through the pia membrane and then withdrawn until it was judged to be as near to the pia as possible without encountering electrical noise, probably due to loss of contact with the cortex, when recording from the microelectrode. Nevertheless, since the position of the microelectrode tip directly beneath the pia was not marked electrolytically and verified histologically, no laminar analysis of cell activity was attempted. The presence and distribution of neuronal discharges in suprasylvian gyrus were

evaluated in different preparations by two procedures each of which involved measurement of extracellular action potentials during successive microelectrode penetrations:

(1) Cell count: cortical cells which discharged spontaneously or exhibited characteristic injury discharges (for explanation see Results) were counted as the microelectrode was lowered radially from under the pia for a distance of approximately 1.5 mm at a variable rate (median average rate 75 microns per minute), controlled manually and measured on a micrometer.

(2) Constant penetration: spontaneous and injury discharges were recorded continuously as the microelectrode was lowered radially from directly beneath the pia at a fixed rate of 5 microns per second for a pre-set distance (usually 1.5 mm) controlled by an electric motor.

Extracellular discharges from a single neuron (for criterion see Results) were recorded for long periods (5 to 10 minutes) in order to evaluate the effect of different brainstem lesions on the discharge patterns of spontaneously-active suprasylvian gyrus units and also to compare the firing pattern of these units with the coincident surface EEG and transcortical ECoG. The EEG and ECoG were also continuously monitored on an oscilloscope.

Data Analysis

Potentials recorded from the cortical surface (EEG

or ECoG) were analyzed by two methods: (1) visual inspection of waveforms displayed on EEG paper, or (2) estimates of the relative power of frequency components (power spectral estimates) computed on a digital computer (PDP-8I). One minute samples of the slow potentials were digitized at 20 msec intervals, a Fourier analysis of the autocovariance was performed for each sample, and the squared voltage estimates were plotted every one-quarter Hz. In subsequent figures, these voltages were not calibrated nor were the histogram plots corrected for total power. The ordinate will always be arbitrary and referred to as relative power.

Correlations between bursts of spike discharges and the phase of the transcortical potential (ECoG) were evaluated by another computer program called BOE (burst onset and end). The ECoG was digitized at 20 msec intervals and summated before (400 to 0 msec) and after (0 to 1000 msec) burst onset and end. Burst onset was statistically-defined as the first spike occurring after an interspike interval t_1 seconds or more (t_1 = 30 or 300 msec). Burst end was defined as the last spike before an interspike interval t_1 seconds or more (see Smith and Smith, 1965, for details). The average slow potential (ECoG) histogram prior to and following the burst onset spike and the burst end spike was displayed on an oscilloscope and photographed.

Statistical analysis of discharge patterns of individual neurons was performed by a computer program which

sampled at 10 msec intervals, timed the intervals between successive spikes, and accumulated a frequency density of these intervals, which was transformed to a distribution of intervals equal to or greater than t. This interval distribution was divided by the total sampling time in seconds and plotted on a semilogarithmic scale against t. Hence, the first data point on each interval distribution equals the average frequency of firing, in spikes per second.

Histology

At the end of the day's recording session, which usually lasted 6 - 8 hours post-operatively, the animal was killed by discontinuing artificial respiration. One common carotid artery was cannulated and the cephalic arterial system was perfused with physiological saline (0.9% NaCl), then 10% formalin. In the cat with electrolytic lesion of the trigeminal rootlets, potassium ferrocyanide was added to the formalin to produce a Prussian blue reaction. The fixed brains were blocked, frozen and parasagittal or horizontal sections (60, 80 or 100 microns thick) were cut, mounted, and nissl substance was stained with cresyl-violet echt or thionin.

CHAPTER THREE

EXPERIMENT ONE: RESULTS

Anatomy of Brainstem Lesions

Pretrigeminal preparations, i.e., complete section of the brainstem rostral to the fifth nerve rootlets (N. V in Figure 11), include midpontine or rostropontine damage. In the midpontine pretrigeminal (MPP) preparation (N = 10), where the cut is immediately rostral to the fifth nerve, ascending fibers from the medulla (e.g., R. gc. and R. pc. in Figure 11) and lower pons (R. p. c. in Figure 11) were interrupted along with N. VI motor nerves to eyes and all sensory input to the forebrain except that via N. I and N. II. Other motor nerves to the eye (N. III and N. IV) were spared and consequently vertical eye movements, normal or slightly closed pupils, and the pupillary light reflex could be detected in the MPP. Preparations (N = 5) with a rostropontine section, which interrupted connections between the superior colliculus and N. III, had completely closed pupils and no reflex changes with changes in illumination. No eye movements could be detected. These rostropontine lesions also interrupted ascending fibers from pontine reticular regions (e.g., R. p. o. and N. r. t. in Figure 11).

Figure 11. Horizontal sections (100 u) of the brainstem from a MPP preparation (X37) shown in the top row. Levels of RP and PoT transections depicted in the middle row. Drawings for comparison in the bottom row were taken from Brodal (1958, p. 35).

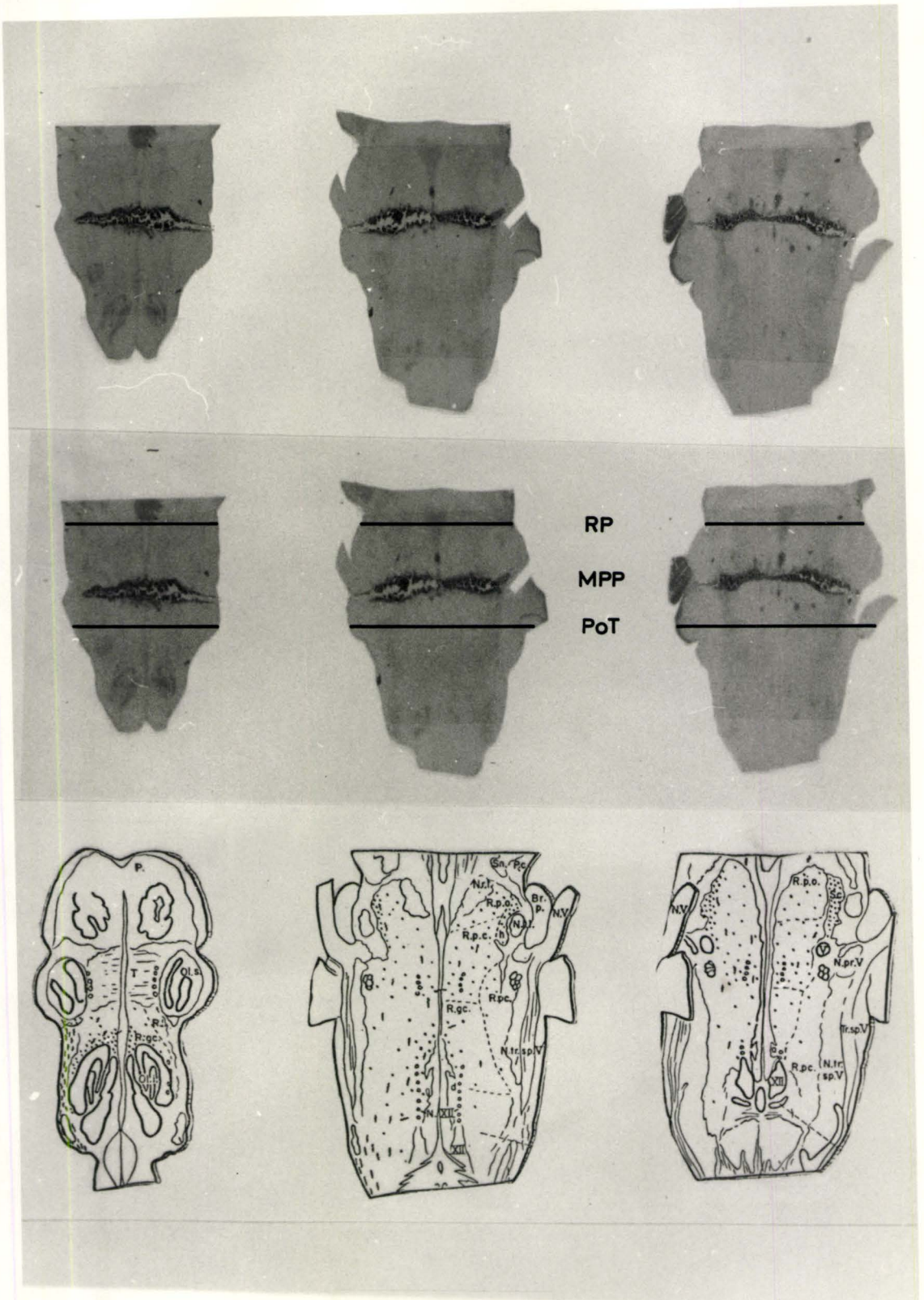

RP
MPP
PoT
P.
T.
Ol.s.
R.l.
R.gc.
Gn./Pc.
N.l.
R.p.o.
Br.
P.
N.V.
N.l.
R.p.c.
R.pc.
R.gc.
N.tr.sp.V
N. XII
XII
R.p.o.
N.V.
N.pr.V
tr.sp.V
R.pc.
N.tr.
sp.V
XII

Figure 11.

Post-trigeminal (PoT) transections (N = 6) through the caudal pons occasionally spared N. VI as indicated by the occurrence of horizontal tracking eye movements. Post-trigeminal lesions spared reticularis pontis caudalis (R. p. c. in Figure 11).

In some of the midpontine preparations, strands of the brachia pontis (Br. p.) or the pyramidal tract were occasionally spared. The locus and extent of damage for different preparations is shown in parasagittal and horizontal tissue sections chosen to reveal intact tissue (see Appendix, Figures 70 and 71). It can be seen that the sections are all made close to the transverse plane of the brainstem (i.e., perpendicular to its long axis). What, if any, influence cerebellar projections may have on MPP preparations with partially intact brachia pontis is unknown.[1] However, in all such preparations the brachia conjunctiva were severed, eliminating the principal cerebellar projections.

Effects of Different Brainstem Transections on Cortical Potentials

In most of the experiments, potentials from the suprasylvian surface were recorded with reference to killed muscle (this particular derivation termed the EEG)

---

1. Only pontocerebellar fibers are known to traverse the brachia pontis (Crosby et al., 1962, p. 206).

and simultaneously another adjacent surface electrode was referred to the micropipette in the cortex immediately below, i.e., a transcortical derivation (termed ECoG). These two methods of recording from nearly identical regions of the cortical surface detected strikingly different potential waveforms. In general, the transcortical derivation (ECoG) detected more slow waves, and the high frequency components (15 - 25 Hz) apparent in the EEG (surface electrode referred to killed muscle) were usually absent in the ECoG. This contrast between EEG and ECoG potential derivations was most evident in one PoT preparation in which cortical potentials were recorded during wakefulness and sleeping, as evidenced by the onset of slow cortical waves and simultaneous pupillary myosis. In the frequency analyses (power spectral estimates) and waveform tracings of Figure 12, during the LVF pattern of wakefulness, high frequency components are less prevalent and low frequency components more prevalent in the ECoG than in the EEG. During sleep, synchrony is more pronounced in the transcortical derivation (ECoG) than the relatively slight synchrony which develops in the EEG. This observed difference between recording configurations was evidently due to the different potential fields and cannot be due to the RC-coupling used to record the EEG, since this influenced only frequencies below 0.5 Hz. Furthermore, the power spectral estimates in which both EEG and ECoG were RC-

Figure 12. In these and subsequent analyses of cortical EEG (Figures 12 through 16), EEG tracings were recorded with 3 db attenuation at 0.1 and 100 Hz, ECoG tracings were D.C. to 80 Hz. For all power spectral estimates, both EEG and ECoG signals were further filtered for 3 db attenuation at 1.6 and 35 Hz. The ordinate is arbitrary and in each histogram the amplitude of individual points is proportional to the square of the voltage at that frequency. The calibration refers to the waveform tracings, surface negative up.

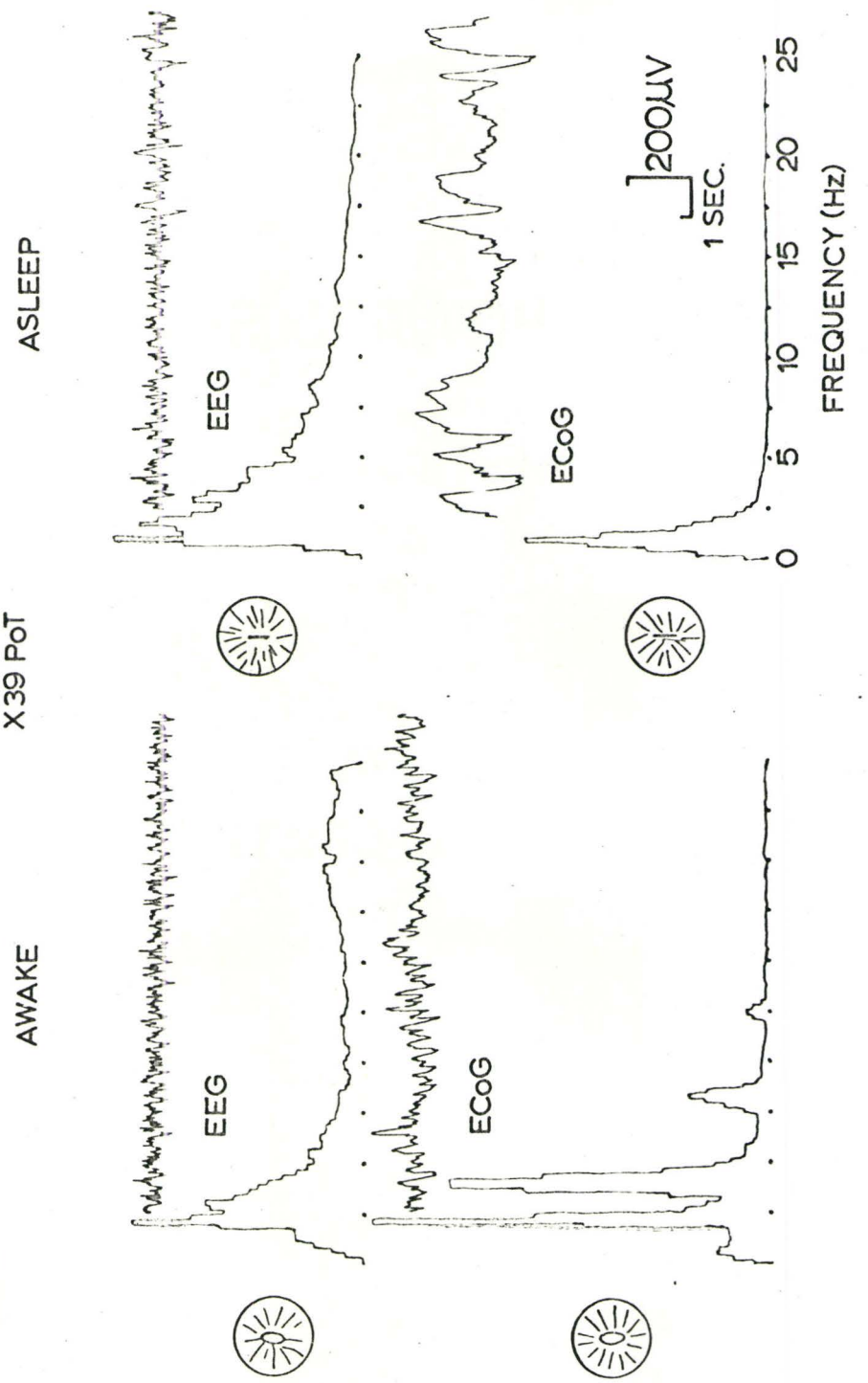

AWAKE
X 39 PoT
ASLEEP
EEG
ECoG
EEG
ECoG
200μV
1 SEC.
0
5
10
15
20
25
FREQUENCY (Hz)

Figure 12.

coupled, are strikingly different. This was a consistent observation and can be seen in Figures 13 through 15.

As mentioned in the introduction, numerous studies have concluded that activity of the nucleus reticularis pontis oralis (see R. p. o. in Figure 11) produces electrocortical desynchronization. From this it would be predicted that MPP and PoT preparations which spared n. r. p. o. would both exhibit desynchronized surface potentials while a RP preparation in which the ascending influence from n. r. p. o. was interrupted would be highly synchronized. This was generally true of all PoT, MPP (except one which was synchronized from the beginning of the recording session), and RP preparations for a variable time (usually more than two hours) following the transection. Later in the session there was a tendency for synchronized waveforms to appear. Either discrete spindles (phasic synchrony) or higher voltage slow waves were recorded from MPP and PoT preparations. Nevertheless, synchrony was more prevalent in preparations with more rostral sections than in those with more caudal ones.

This last point can be seen in the analyses of the frequency components (power spectral estimates) shown in Figures 13, 14, and 15. Despite the considerable variability evident in these examples, it is clear that the dominant slow components in PoT preparations were faster (3 - 4 Hz) than those of the MPP or RP (1 - 2 Hz) preparations.

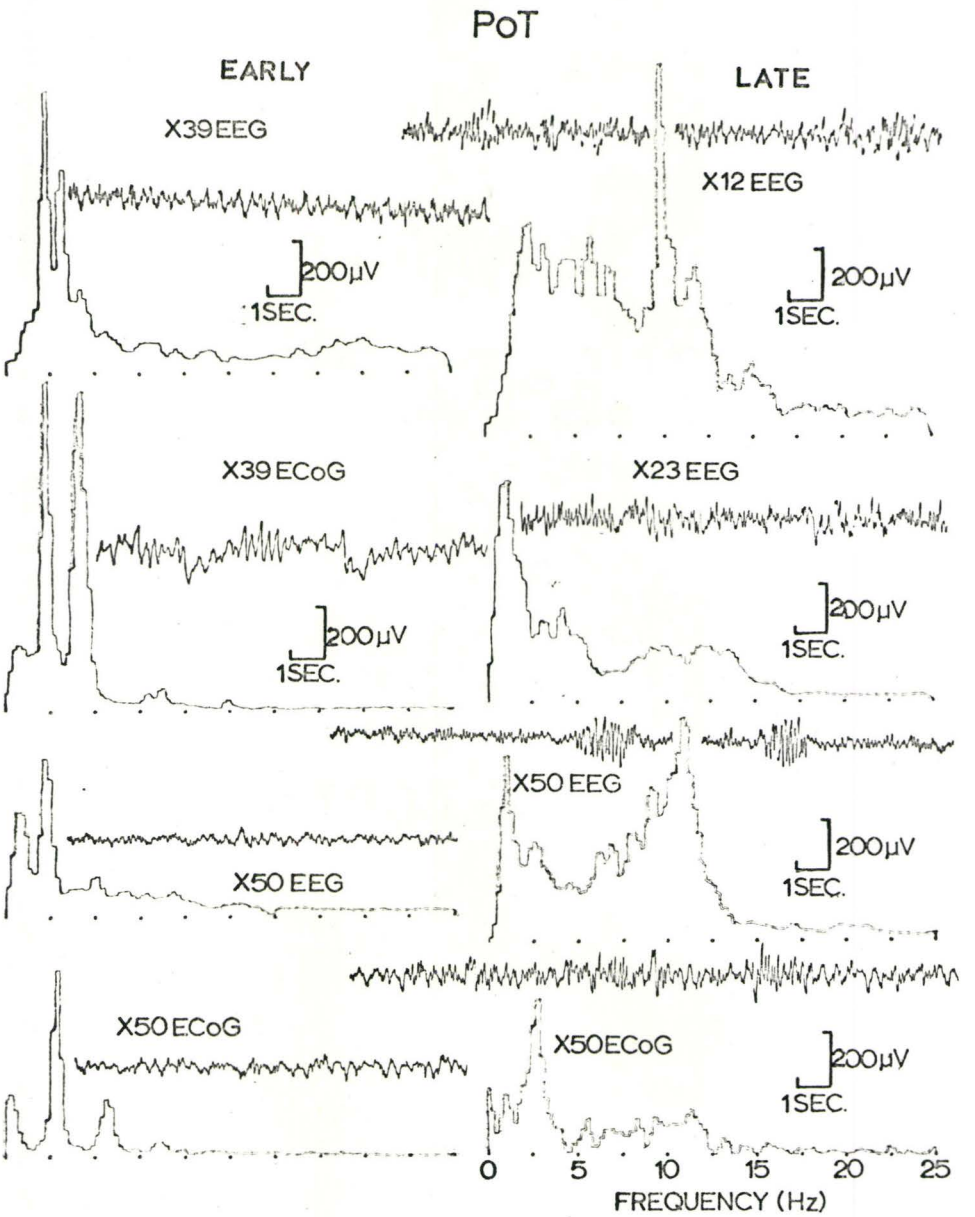

PoT
EARLY
LATE
X39 EEG
X12 EEG
200μV
1SEC.
200μV
1SEC.
X39 ECoG
X23 EEG
200μV
1SEC.
200μV
1SEC.
X50 EEG
X50 EEG
200μV
1SEC.
X50 ECoG
X50 ECoG
200μV
1SEC.
0
5
10
15
20
25
FREQUENCY (Hz)

Figure 13.

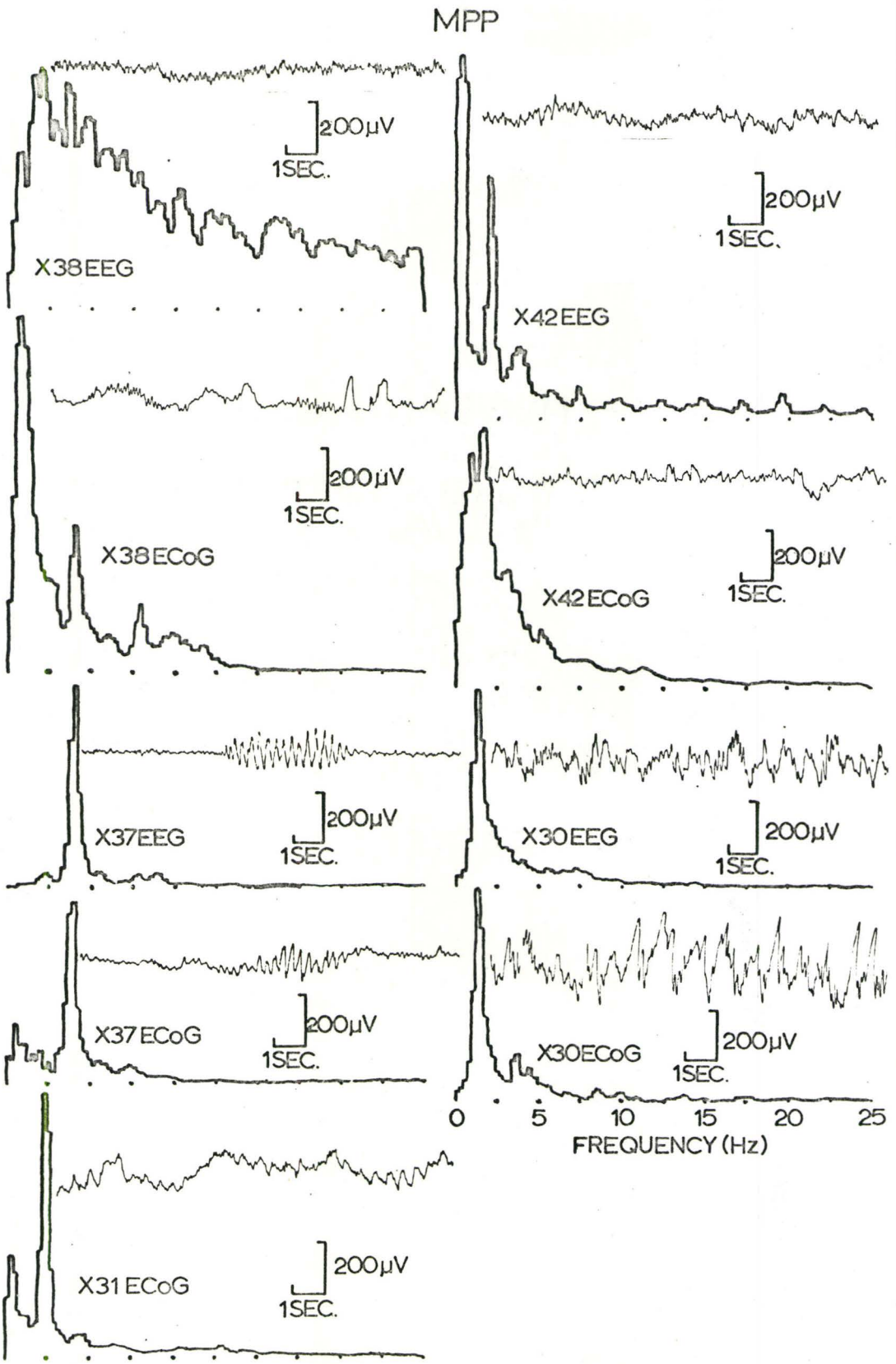

MPP
200μV
1SEC.
X38EEG
X42EEG
X38ECoG
X42ECoG
X37EEG
X30EEG
X37ECoG
X30ECoG
X31ECoG
0
5
10
15
20
25
FREQUENCY (Hz)

Figure 14.

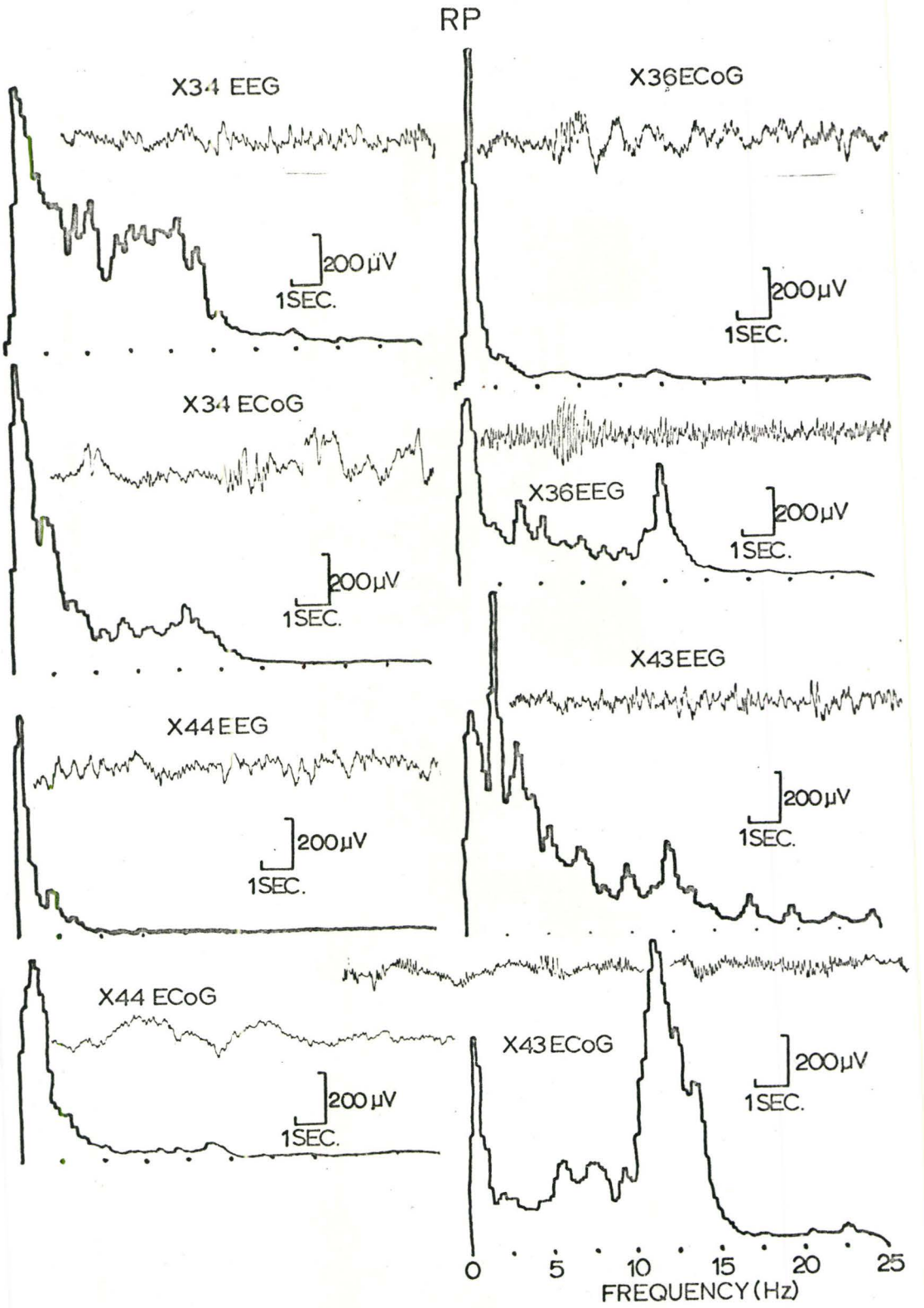

RP
X34 EEG
200µV
1SEC.
X36ECoG
200µV
1SEC.
X34 ECoG
200µV
1SEC.
X36EEG
200µV
1SEC.
X44EEG
200µV
1SEC.
X43EEG
200µV
1SEC.
X44 ECoG
200µV
1SEC.
X43ECoG
200µV
1SEC.
0
5
10
15
20
25
FREQUENCY (Hz)

Figure 15.

Furthermore, PoT preparations had greater relative power in the high frequency range (12 - 25 Hz) than RPs. Spindles (11 - 12 Hz) in PoT and MPP preparations developed late in the day but were usually immediately evident in RP preparations (compare Figure 13 right column with Figure 15). These findings differ from Batini's (Batini et al., 1959) experiments which employed electrolytic lesions in that she did not report the development of any significant degree of synchrony in MPP preparations. Zernicki (1964), however, who transected the pons (MPP) with a spatula, reports that a majority of such preparations were "... more or less synchronized and spindle bursts could be often observed." (Zernicki, 1964, p. 258).

Bilateral "gasserectomies" by electrolytic lesions were performed in one animal (X26) five weeks before a PoT transection. In three other cats (X14, X19, X22) the trigeminal nerves were severed at their foramina at the time of PoT transection. In all such "gasserectomized" PoT (PoT + V) preparations, the cortical surface potentials were highly synchronized and occasionally exhibited spindles (see Figure 16). The occurrence of synchrony and spindles after trigeminal deafferentation has been reported in the encéphale isolé (Batini, Magni et al., 1959) as well as the post-trigeminal preparation (Roger, Rossi and Zirondoli, 1956).

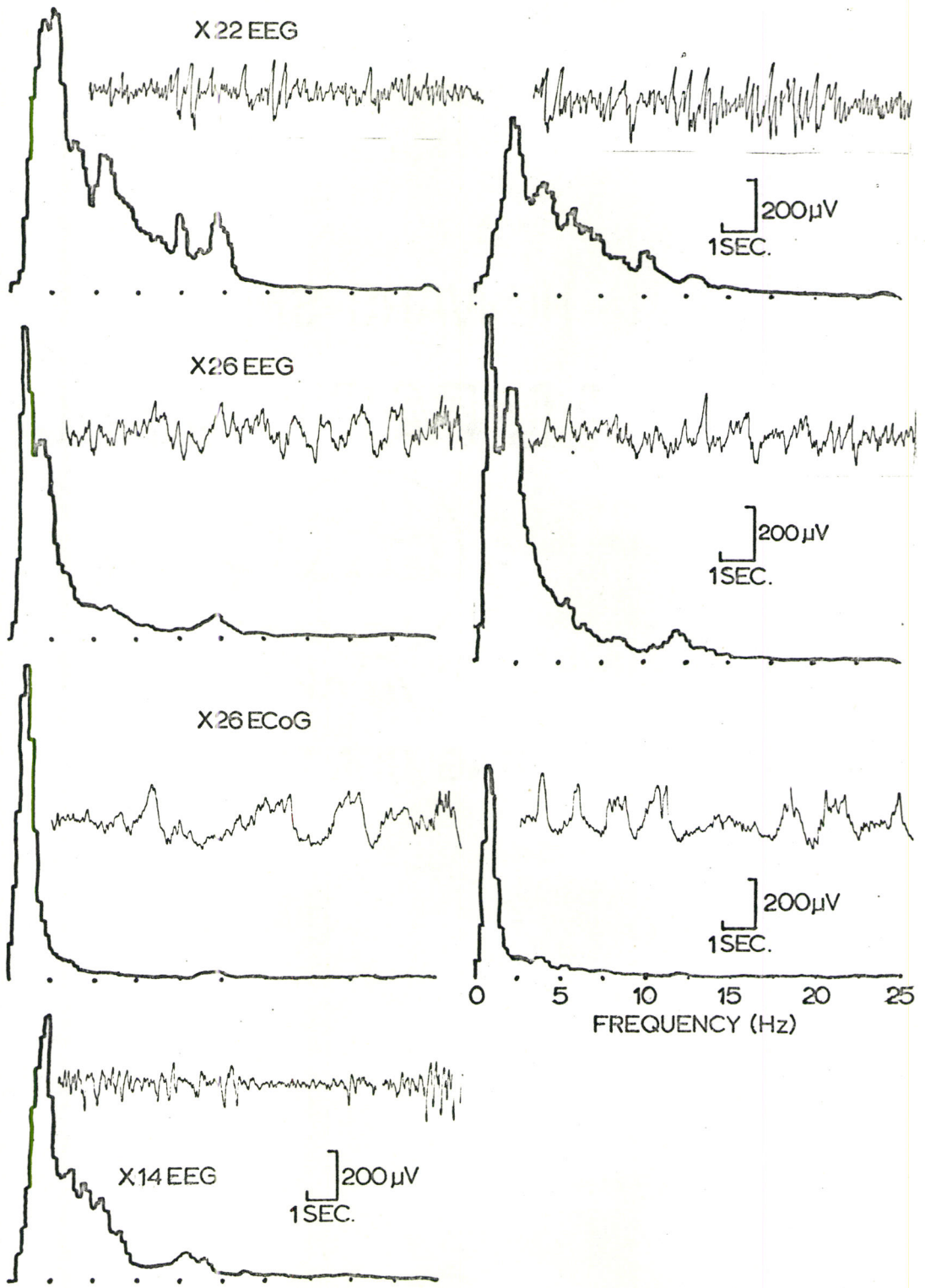

PoT+V
X22 EEG
200µV
1SEC.
X26 EEG
200µV
1SEC.
X26 ECoG
200µV
1SEC.
0
5
10
15
20
25
FREQUENCY (Hz)
X14 EEG
200µV
1SEC.

Figure 16.

Effects of Different Brainstem Transections on Neuron Discharge

In order to measure the relative density of spontaneously-active units within a neuron population beneath the slow wave recording electrode, a method of counting the number of spontaneously-active units encountered during several penetrations of the microelectrode was used. The experimenter simply lowered the microelectrode tip from a point beneath the pia until he detected extracellular action potentials of at least one cell and observed that these action potentials were either irregular and relatively infrequent (characteristic of spontaneous activity) or were characteristic of injury discharges (i.e., high rate, fixed interspike intervals, and eventually decreasing in amplitude). To avoid counting the same cell twice, injury discharges or spontaneous firing within approximately 100 microns of a previously counted cell were rejected.

This counting procedure led to the startling result that when spontaneous activity was high, injury discharges were also more prevalent. Conversely, when little spontaneous activity occurred, injury discharges were less frequent. In PoT preparations, both spontaneous activity and discharges due to microelectrode contact were more common than they were in either MPP or RP preparations. Due to the low occurrence of either form of discharge in these pretrigeminal preparations, the cells counted and listed

in Table 2 and summarized in Table 1 are a combination of those cells identified as spontaneously active and as discharging due to injury. A t-test comparison shows the number of PoT cells to be significantly larger than the number of MPP ($t = 10.66$; $p < .001$) or RP cells ($t = 10.43$; $p < .001$), which do not differ from each other ($t = 0.15$), (see Table 1).

Table 1

	PoT (N=5)	MPP (N=9)	RP (N=5)
Total cells	361	110	64
Cells/penetration	11.7	2.1	2.1
s^2	22.5	3.1	3.1
S.D.	4.8	1.8	1.8

t-tests: PoT vs. MPP: $t = 10.66$; $p < .001$

PoT vs. RP: $t = 10.43$; $p < .001$

MPP vs. RP: $t = 0.15$; n.s.

N = number of animals

Since it was found that spontaneous activity and injury discharge occurred more often after post-trigeminal than after pretrigeminal lesions, extracellular action potentials were sampled by an objective method (to avoid experimenter bias) in which the microelectrode was lowered through the cortex at a fixed rate (5 microns per second) from beneath the pia to a depth of 1.5 to 2.0 mm. Using

Table 2. Numbers of suprasylvian gyrus cells identified as spontaneously-active or discharging due to injury during consecutive penetrations. Each penetration was 1.5 mm. deep, radially from the pia. Microelectrode exploration rates varied about an average of 75 microns per minute.

Post-trigeminal (N=5)	Gasserectomized Post-trigeminal (N=4)	Midpontine Pretrigeminal (N=9)	Rostropontine (N=5)
Cat X39: 16, 10, 10, 1, 7, 3 = 47; average = 7.8	Cat X26: 8, 12, 6, 8, 11, 9, 10 = 64; average = 9.1	Cat X42: 3, 1, 1, 0, 0 = 5; average = 1.0	Cat X44: 3, 3, 3, 2, 2, 1 = 14; average = 2.3
Cat X29: 15, 6, 11, 7, 9, 12, 8 = 68; average = 9.7	Cat X22: 5, 9, 15 = 29; average = 9.7	Cat X38: 0, 0, 3, 5, 6, 3 = 17; average = 2.8	Cat X43: 3, 1, 0, 0, 2, 0 = 6; average = 1.0
Cat X23: 10, 15, 11, 7, 9, 7, 8 = 67; average = 9.6	Cat X19: 10, 7, 11, 13, 11 = 52; average = 12.4	Cat X37: 2, 3, 0, 3, 7, 7, 3 = 25; average = 3.6	Cat X36: 0, 2, 4, 5, 6, 5 = 22; average = 3.6
Cat X12: 18, 15, 16, 13 = 62; average = 15.5	Cat X14: 8, 9, 8 = 25; average = 8.3	Cat X33: 1, 3, 0, 4, 2 = 10; average = 2.0	Cat X34: 3, 2, 2, 2, 2, 0, 0, 0 = 11; average = 1.4
Cat X11: 17, 15, 20, 18, 17, 15, 15, = 117; average = 16.7		Cat X32: 3, 1, 1, 0 = 5; average = 0.8	Cat X8: 5, 4, 1, 1 = 11; average = 2.8
		Cat X31: 1, 0, 3, 0, 2, 2 = 8; average = 1.3	
		Cat X30: 1, 3, 3 = 7; average = 2.3	
		Cat X10: 3, 4, 3, 2, 1, 3, 4, 2 = 22; average = 2.8	
		Cat X9: 3, 0, 2, 2, 3, 1, 0, 0, 0 = 11; average = 1.2	
Total cells=361 Cells per penetration=11.7	Total cells=171 Cells per penetration=9.5	Total cells=114 Cells per penetration=2.1	Total cells=64 Cells per penetration=2.1

this method, it was possible to detect both spontaneous discharge from neurons near the moving microelectrode tip as well as the characteristic injury discharge "envelopes". Figure 17 schematizes the recording arrangement in this sampling technique. The cortical histology was modified from drawings of human cortex (Ranson and Clark, 1953, p. 323) and is not intended to represent cell lamination in the cat suprasylvian cortex. The scale given in microns refers to the depth of the microelectrode tip from an arbitrary zero point directly beneath the pia at the end of each recording strip. The figures show continuous recordings with elapsed time and distance from the pia going from left to right and following on without interruption to the next lower strip. Figures 17, 18 and 19 are examples representative of the range of activity which was found with various penetrations in post-trigeminal preparations. There is virtually no overlap in amount of recordable spike activity, however, in similar penetrations in MPP (Figures 20, 21, 22 and 23) or RP (Figures 24 and 25) preparations.

As can be seen in these figures, spike activity was always low in pretrigeminal preparations, regardless of the pattern of the EEG. At the beginning of each recording session, when all but one MPP exhibited desynchronized EEG patterns, samples of spike discharge indicated little activity. Later, when synchrony developed in some of these MPP preparations, the spike activity was not noticeably re-

Figures 17 through 28. Neuron activity (second channel) sampled by continuous penetration of the microelectrode at a fixed rate of 5 microns per second. The coincident surface EEG is recorded in channel one. Numbers on the scale refer to the depth, in microns, of the microelectrode tip from an arbitrary zero point beneath the pia at the end of each recording strip. The calibration refers to the EEG, surface negative up. Spike amplitudes are always arbitrary.

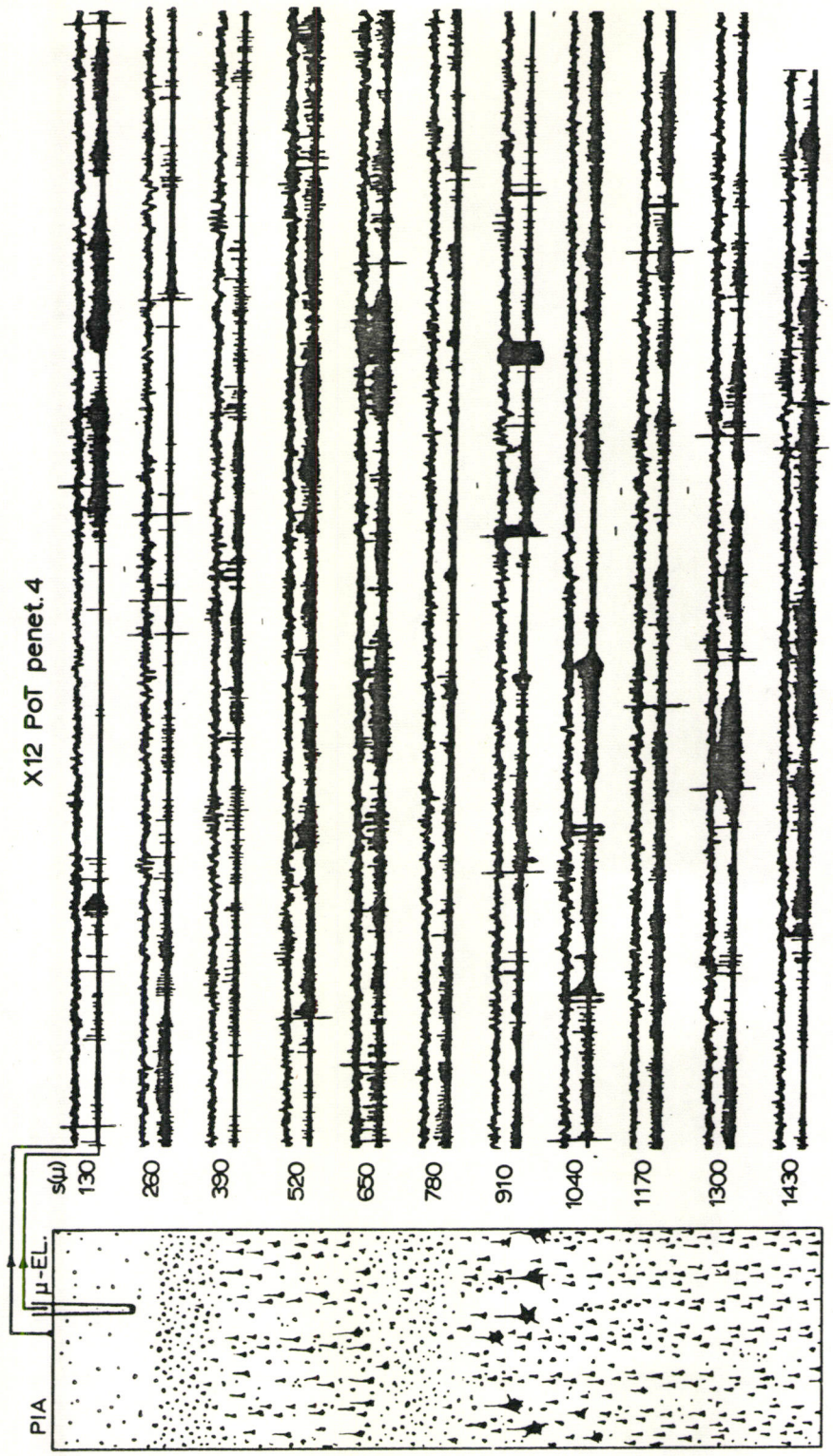

X12 PoT penet.4
PIA
μ-EL.
s(μ)
130
260
390
520
650
780
910
1040
1170
1300
1430
200μV
1SEC.

Figure 17.

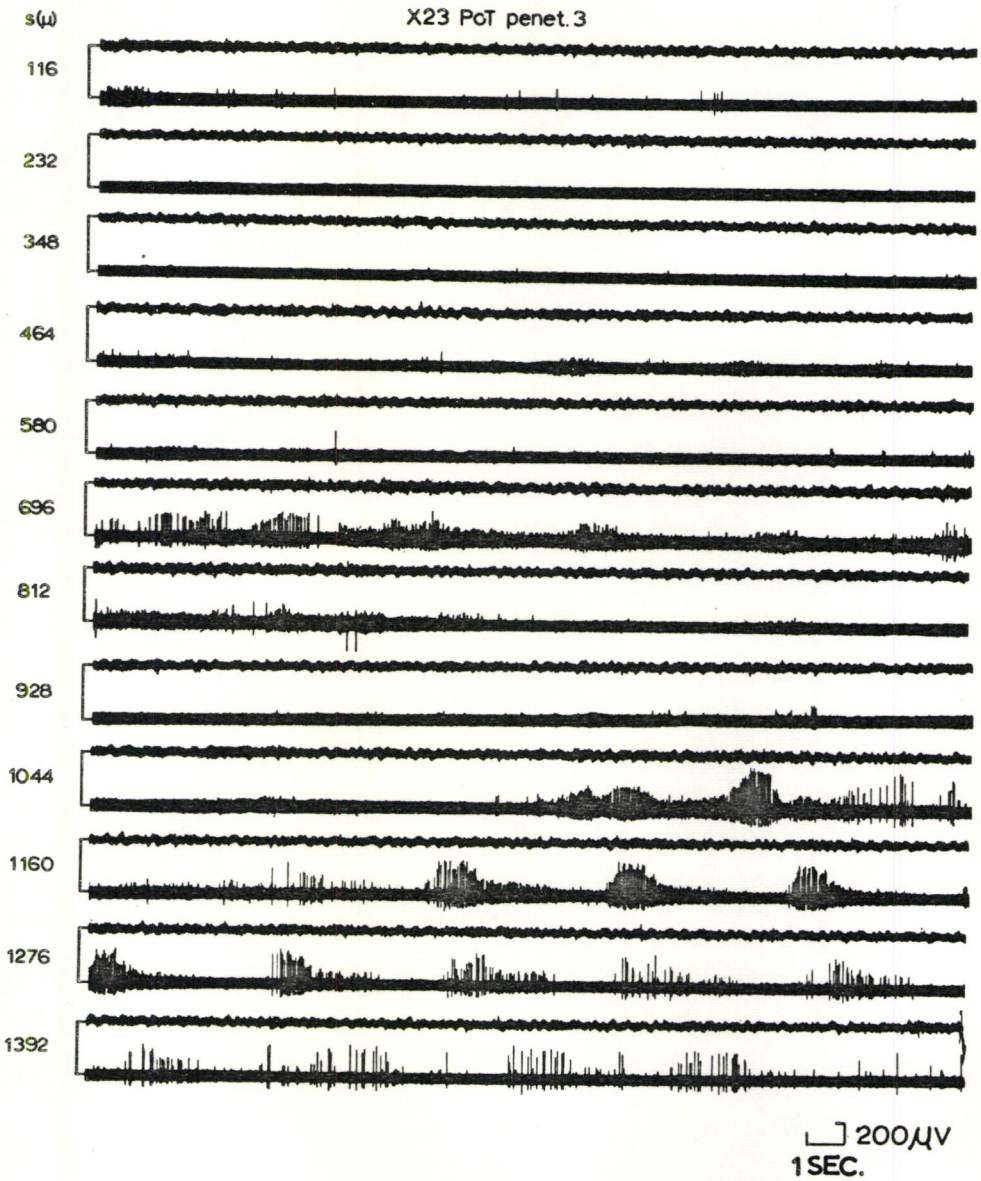

s(μ)
X23 PoT penet. 3
116
232
348
464
580
696
812
928
1044
1160
1276
1392
200μV
1 SEC.

Figure 18.

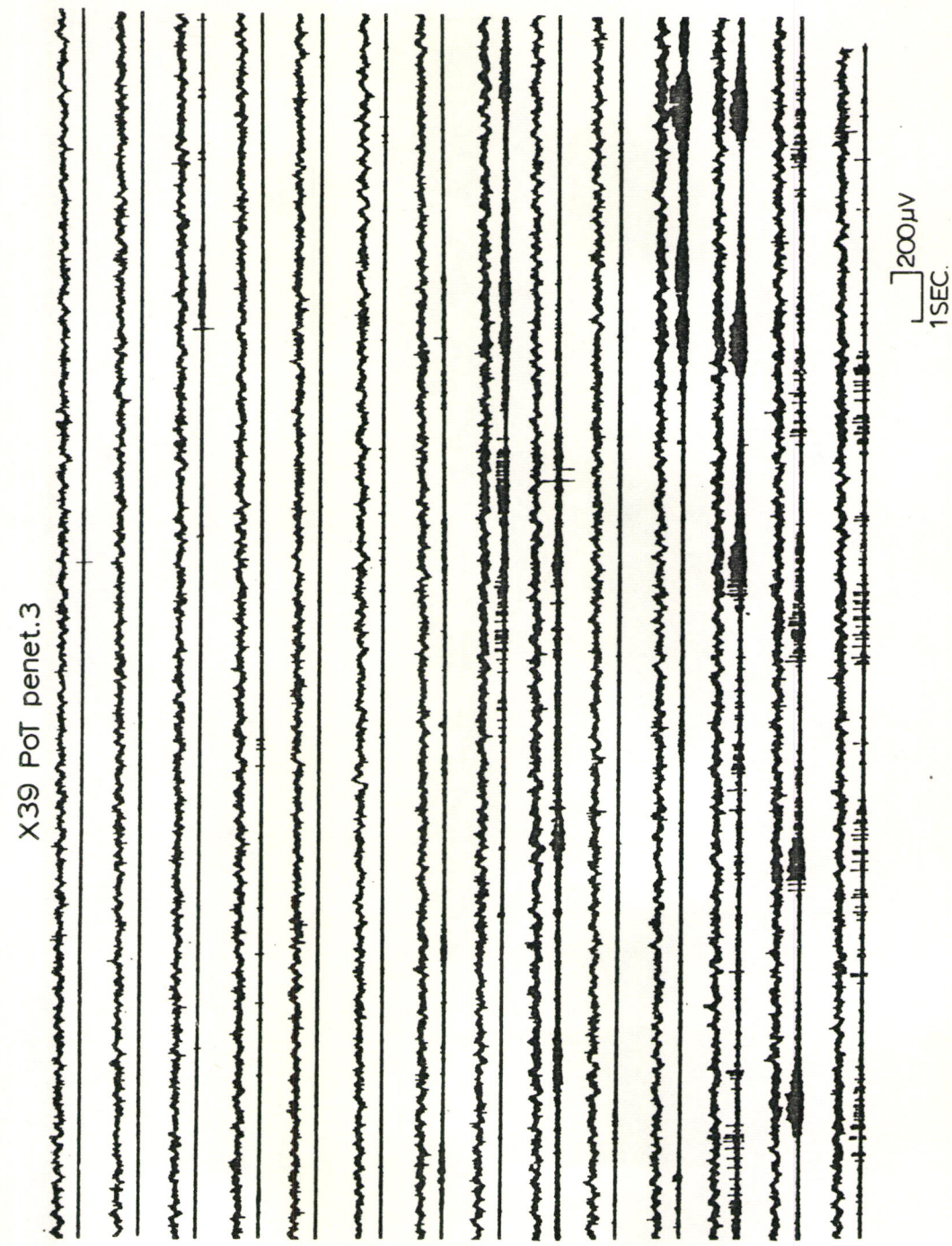

X39 PoT penet.3
s(μ)
127
251
376
500
625
749
874
998
1123
1247
1372
1496
1621
1745
200μV
1SEC.

Figure 19.

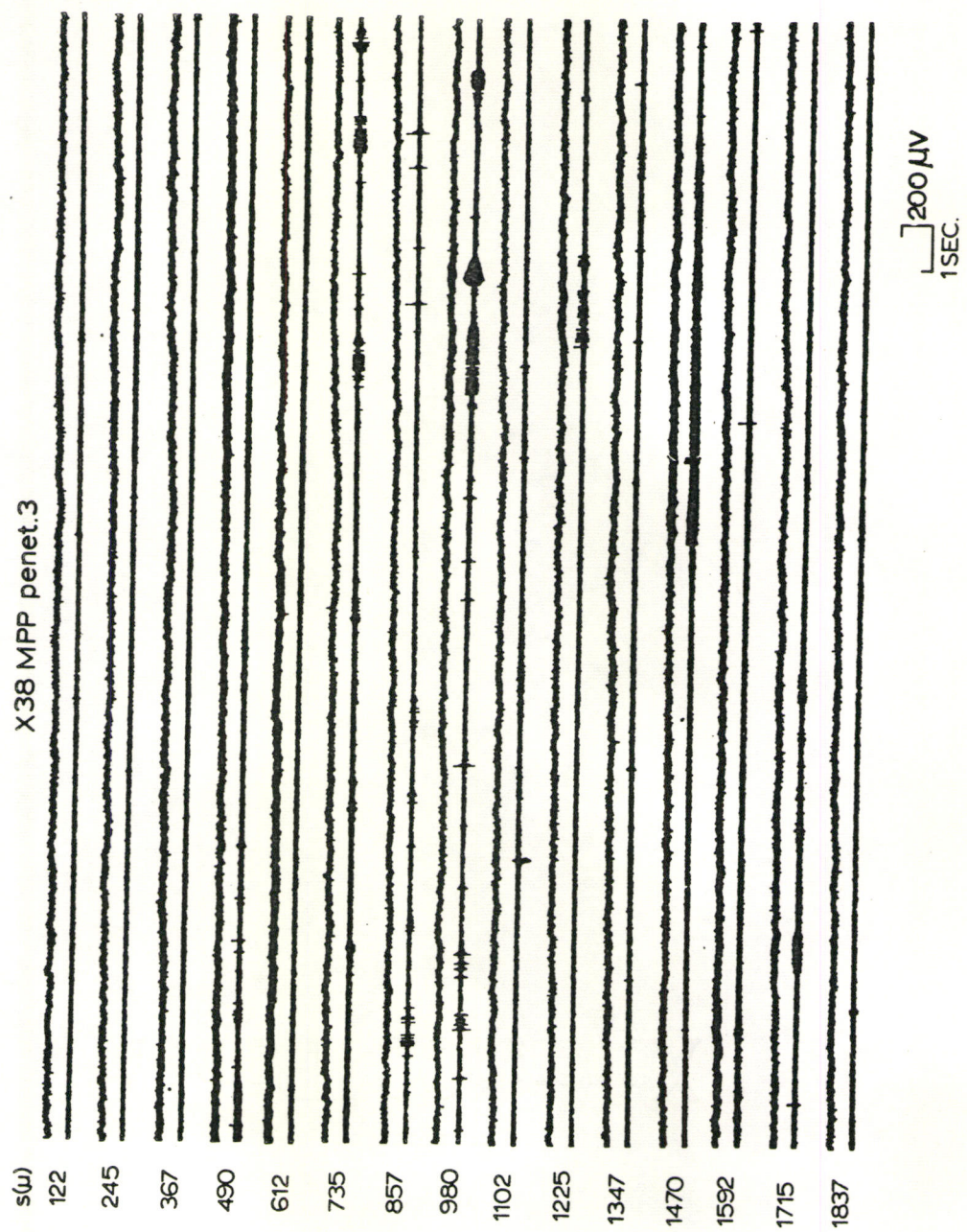

X38 MPP penet.3
s(ω)
122
245
367
490
612
735
857
980
1102
1225
1347
1470
1592
1715
1837
200 μV
1SEC.

Figure 20.

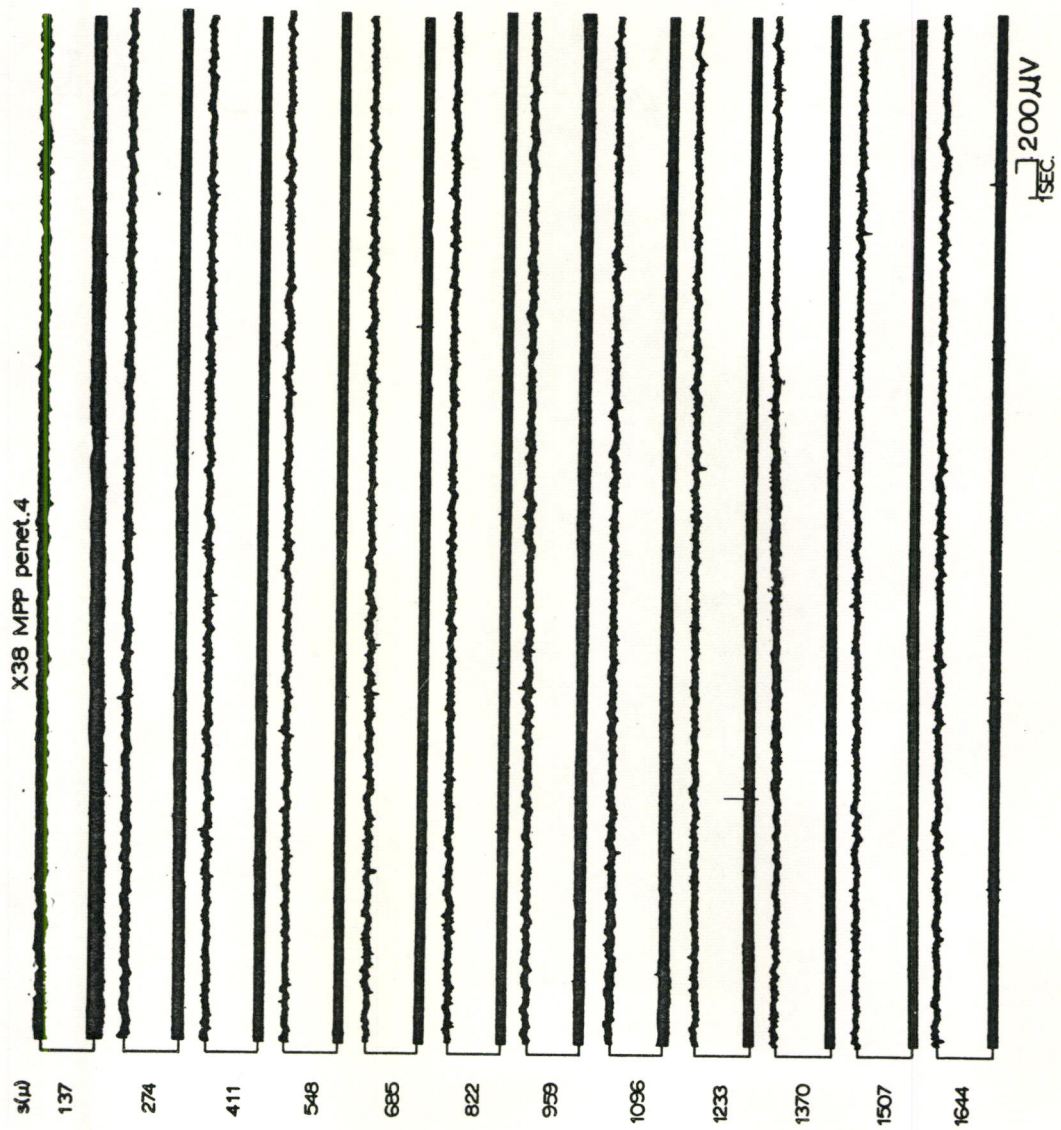

X38 MPP penet.4
s(μ)
137
274
411
548
685
822
959
1096
1233
1370
1507
1644
1SEC
200μV

Figure 21.

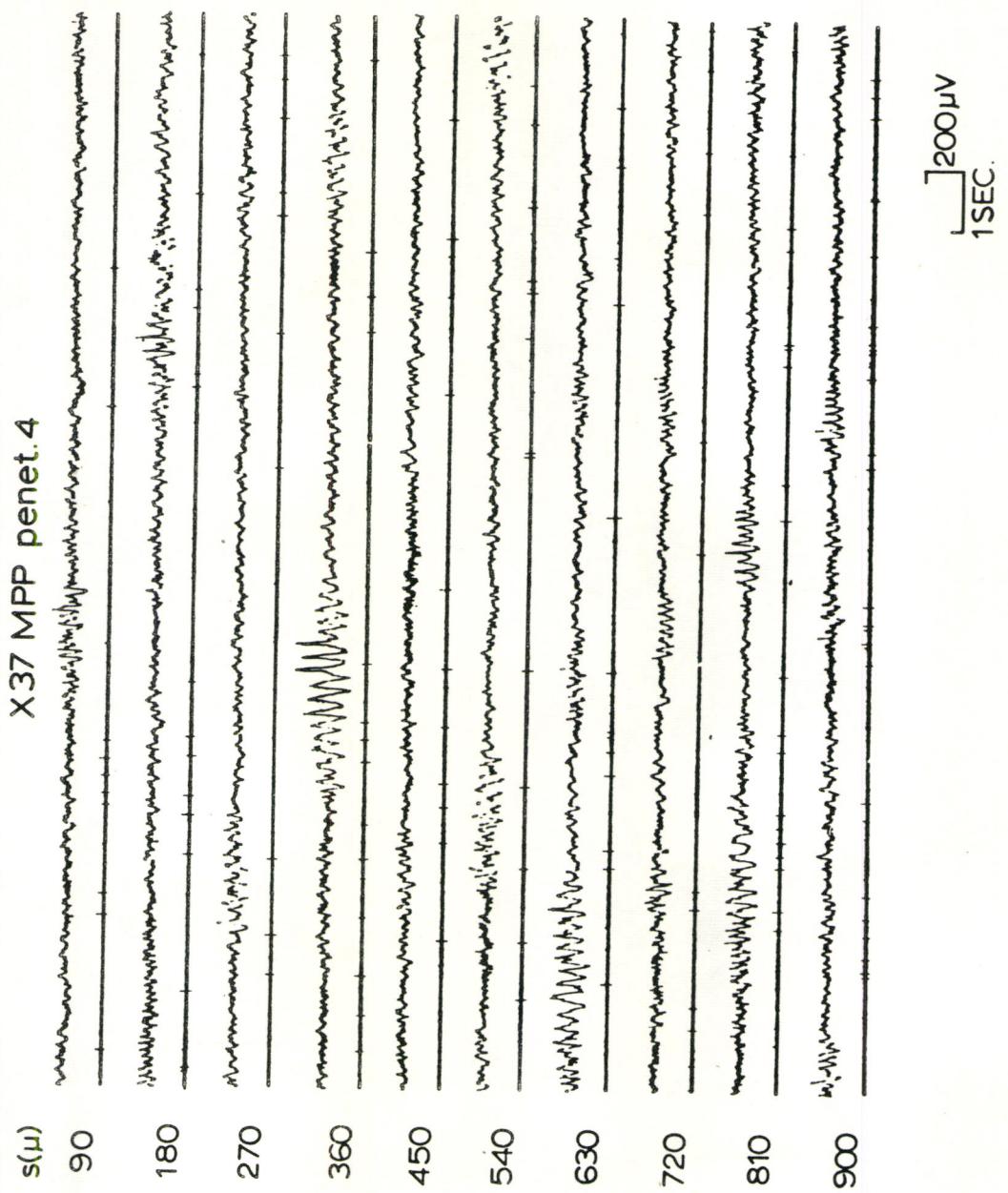

X 37 MPP penet. 4
s(μ)
90
180
270
360
450
540
630
720
810
900
200μV
1SEC.

Figure 22.

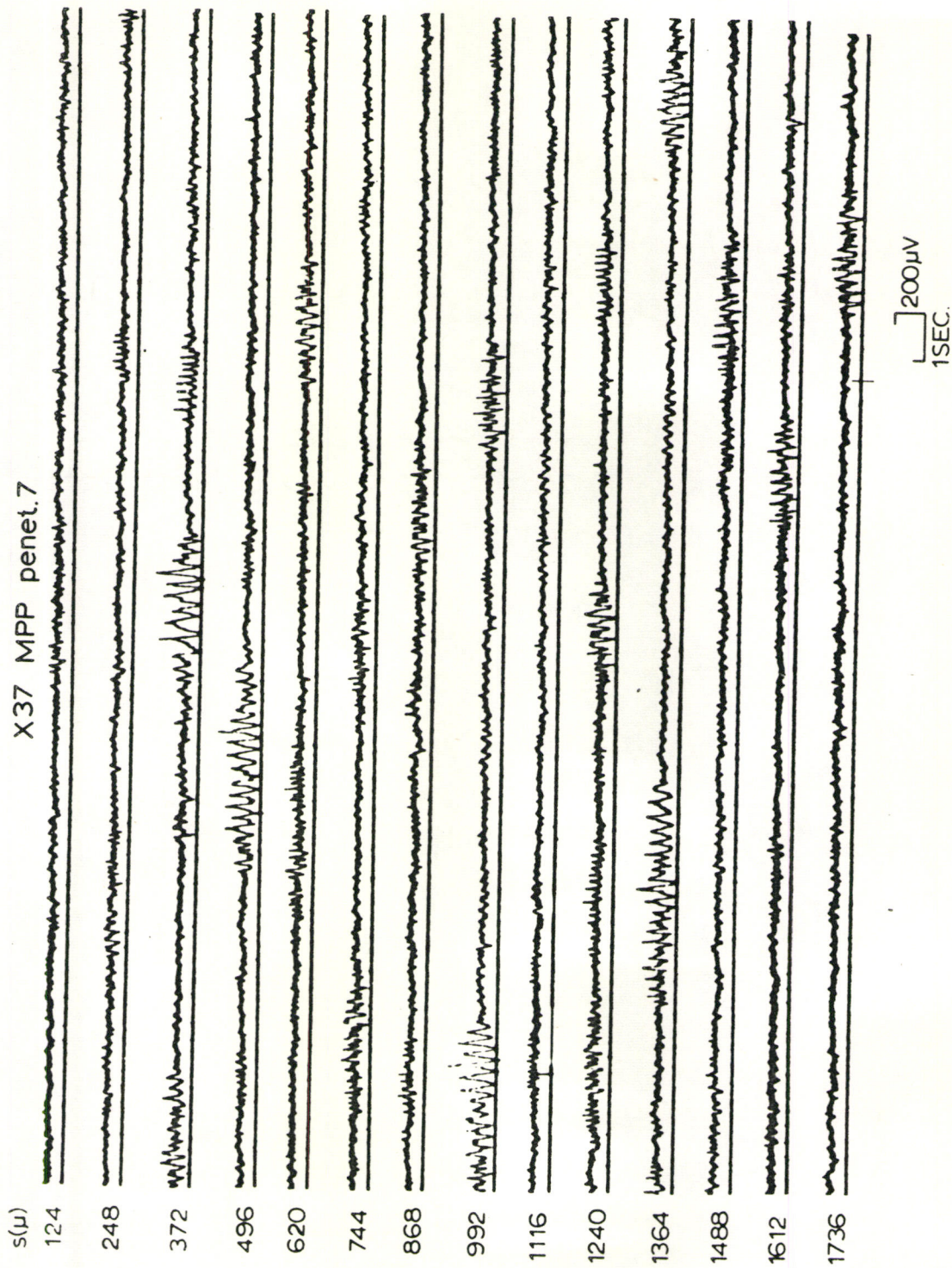

X37 MPP penet.7
s(μ)
124
248
372
496
620
744
868
992
1116
1240
1364
1488
1612
1736
200μV
1SEC.

Figure 23.

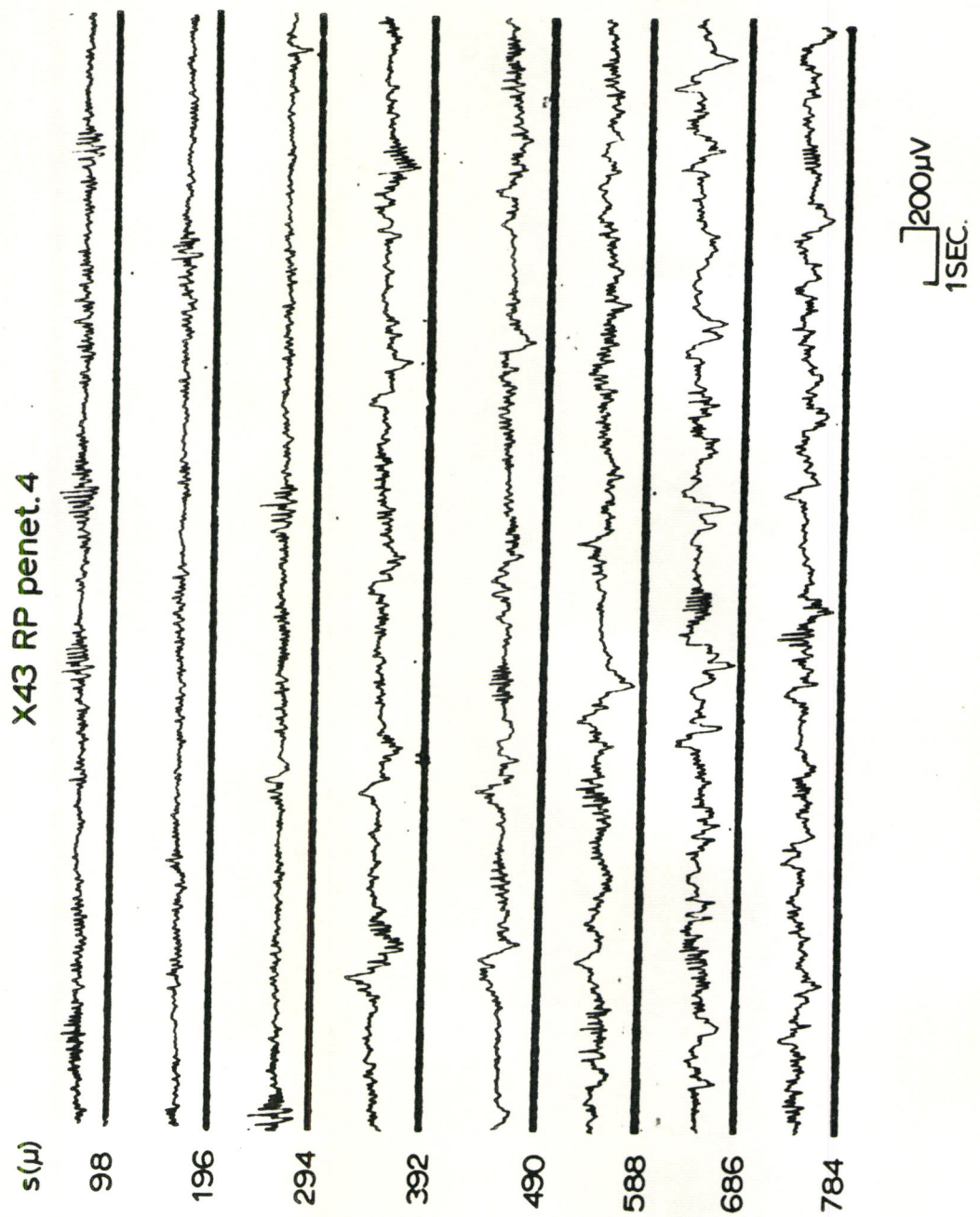


Figure 24.

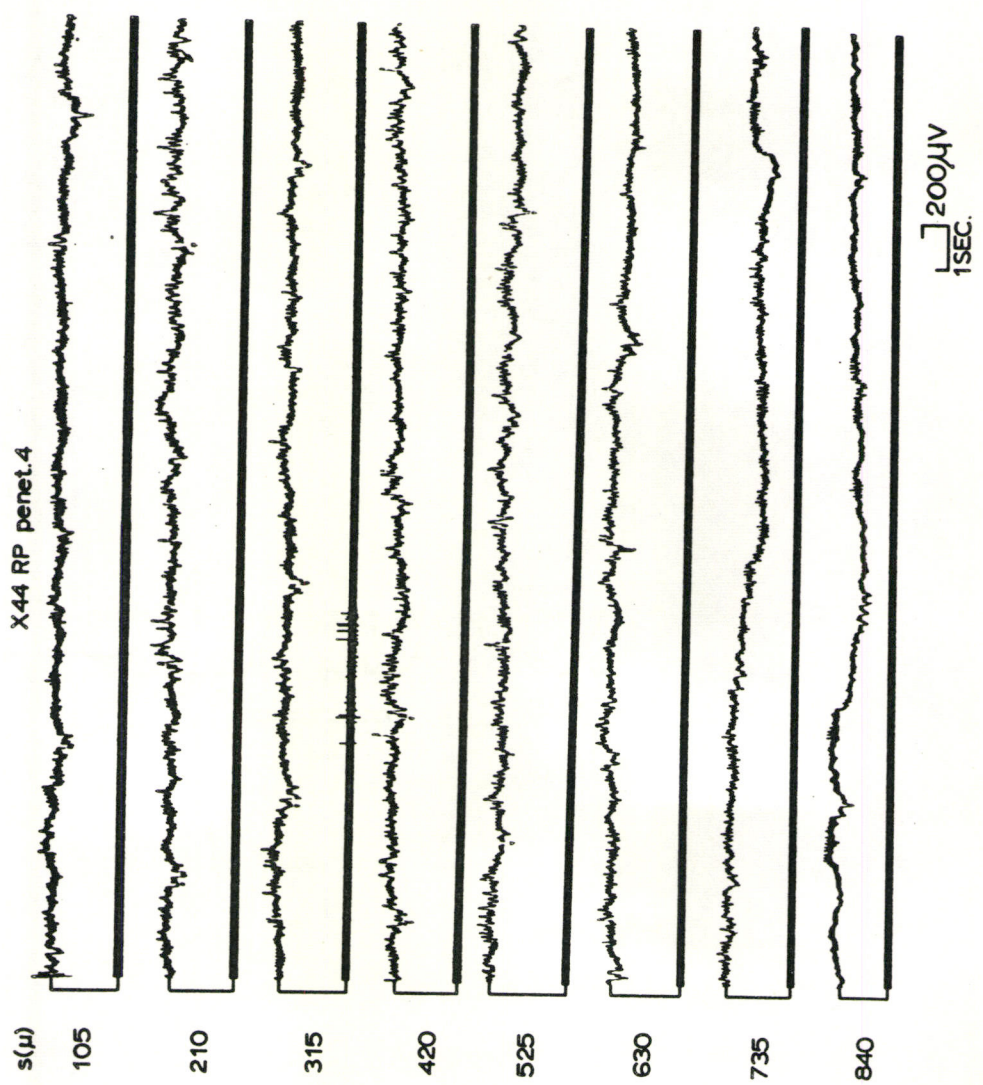


Figure 25.

duced. That the presence of active suprasylvian units did not decrease over time can be seen by comparing consecutive cell counts in MPP preparations in Table 2. Figures 20 and 21, recorded from consecutive penetrations, illustrate the relatively narrow range of low neuron activity which was recorded from pretrigeminals exhibiting either LVF (MPP) or HVS (MPP or RP) patterns of EEG.

It can be concluded that the density of spontaneous unit discharge and the likelihood of discharge due to microelectrode contact are lower in pretrigeminal (MPP or RP) than in post-trigeminal (PoT) preparations regardless of the fact that both synchronized and desynchronized EEG patterns occur in pretrigeminals. It is not true that the low activity of suprasylvian units subsequent to pretrigeminal transection determines the pattern of slow potentials derived from the cortical surface, since many EEG patterns (e.g., LVF, HVS and spindles) may accompany this low level of spike activity.

From these experiments two questions can be asked: (1) Is the relationship between EEG synchrony and lowered suprasylvian unit activity seen in Figures 22, 23, 24 and 25 an invariant one, or can synchronized surface potentials accompany the higher levels of spike activity found in post-trigeminal preparations? (2) What is the importance of fifth nerve input for the maintenance of more spike activity in PoT preparations? Both of these questions

were answered by using the same experimental methods in "gasserectomized" PoT preparations. As noted in the EEG analyses, PoT preparations with lesioned or severed trigeminal nerves (PoT + V) always exhibited synchronized EEG patterns and occasionally spindling. Cell counts from these preparations (see Table 2) are summarized in Table 3.

Table 3

	PoT + V (N=4)	MPP (N=9)	RP (N=5)
Total cells	170	110	64
Cells/penetration	9.4	2.1	2.1
s^2	6.7	3.1	3.1
S.D.	2.6	1.8	1.8

t-tests: PoT + V vs. MPP: $t = 10.90$; $p < .001$

PoT + V vs. RP: $t = 11.60$; $p < .001$

N = number of animals

A t-test comparison shows significantly more active cells in "gasserectomized" PoT preparations than in either MPP ($t = 10.90$; $p < .001$) or RP ($t = 11.60$; $p < .001$) preparations. Hence, it is clear that high excitability of suprasylvian units may accompany synchronized surface potentials. This can be seen in samples taken from preparations in which the microelectrode was lowered continuously, in Figures 26, 27 and 28 (in Figure 28 the synchronized potentials were not stored on magnetic tape and are omitted

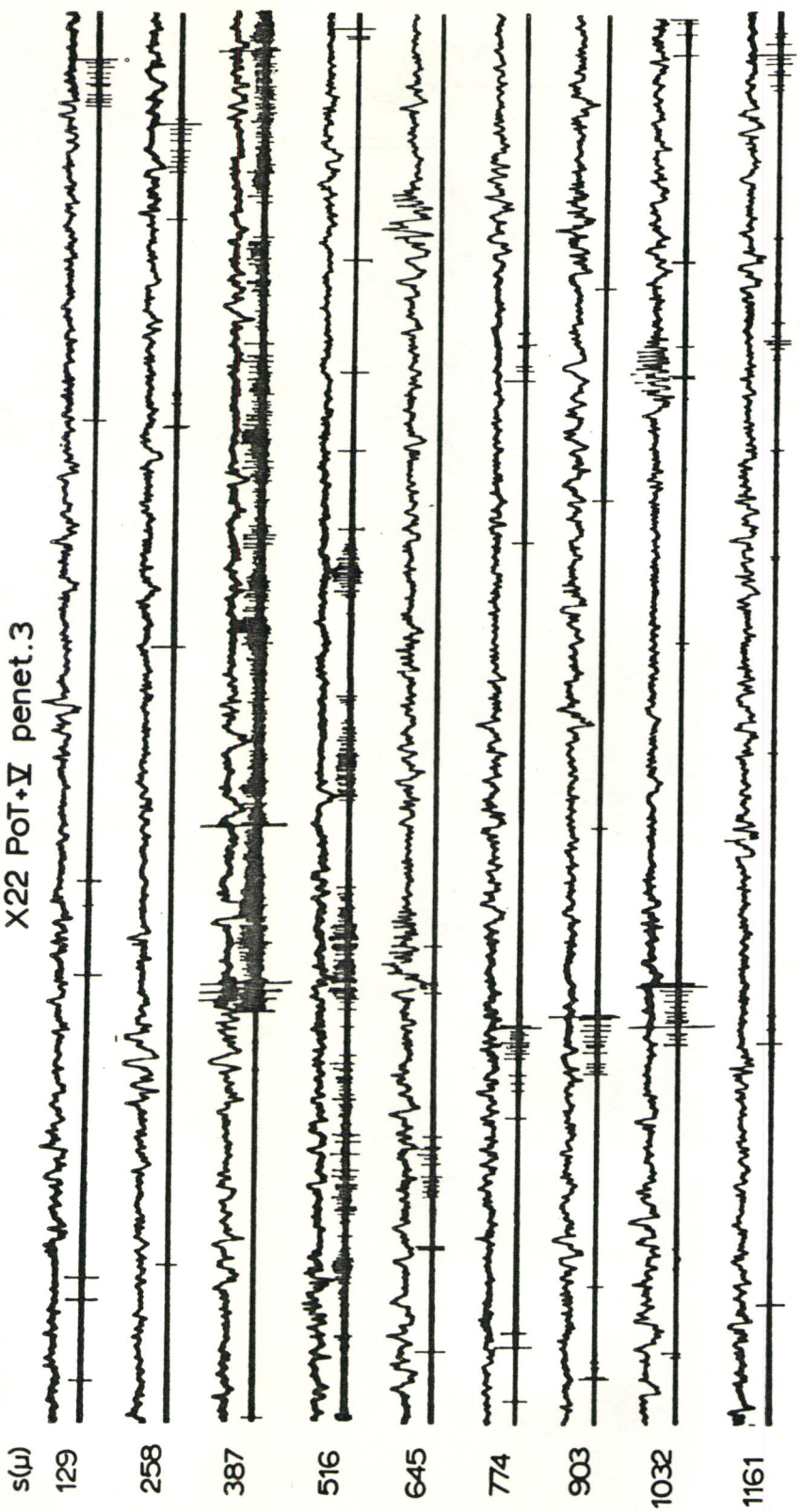

X22 POT+V penet.3
s(μ)
129
258
387
516
645
774
903
1032
1161
200μV
1SEC.

Figure 26.

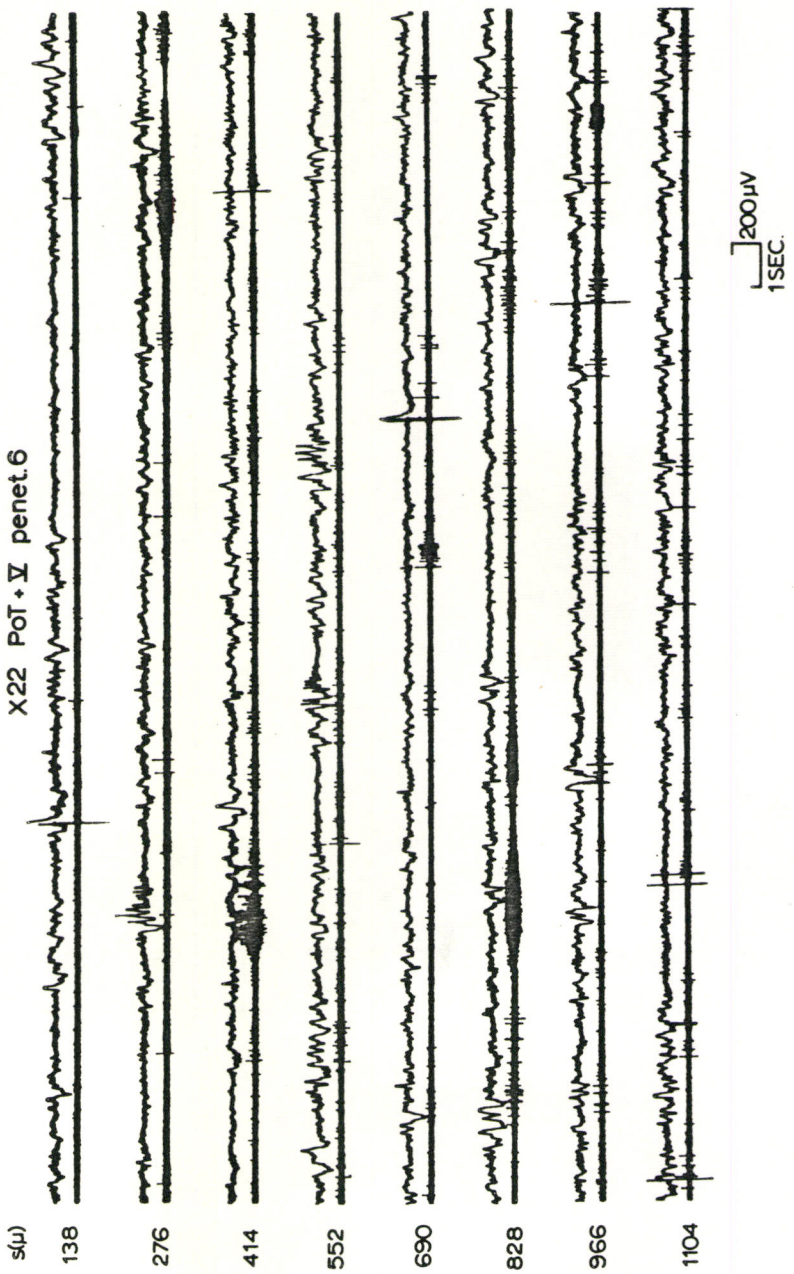


Figure 27.

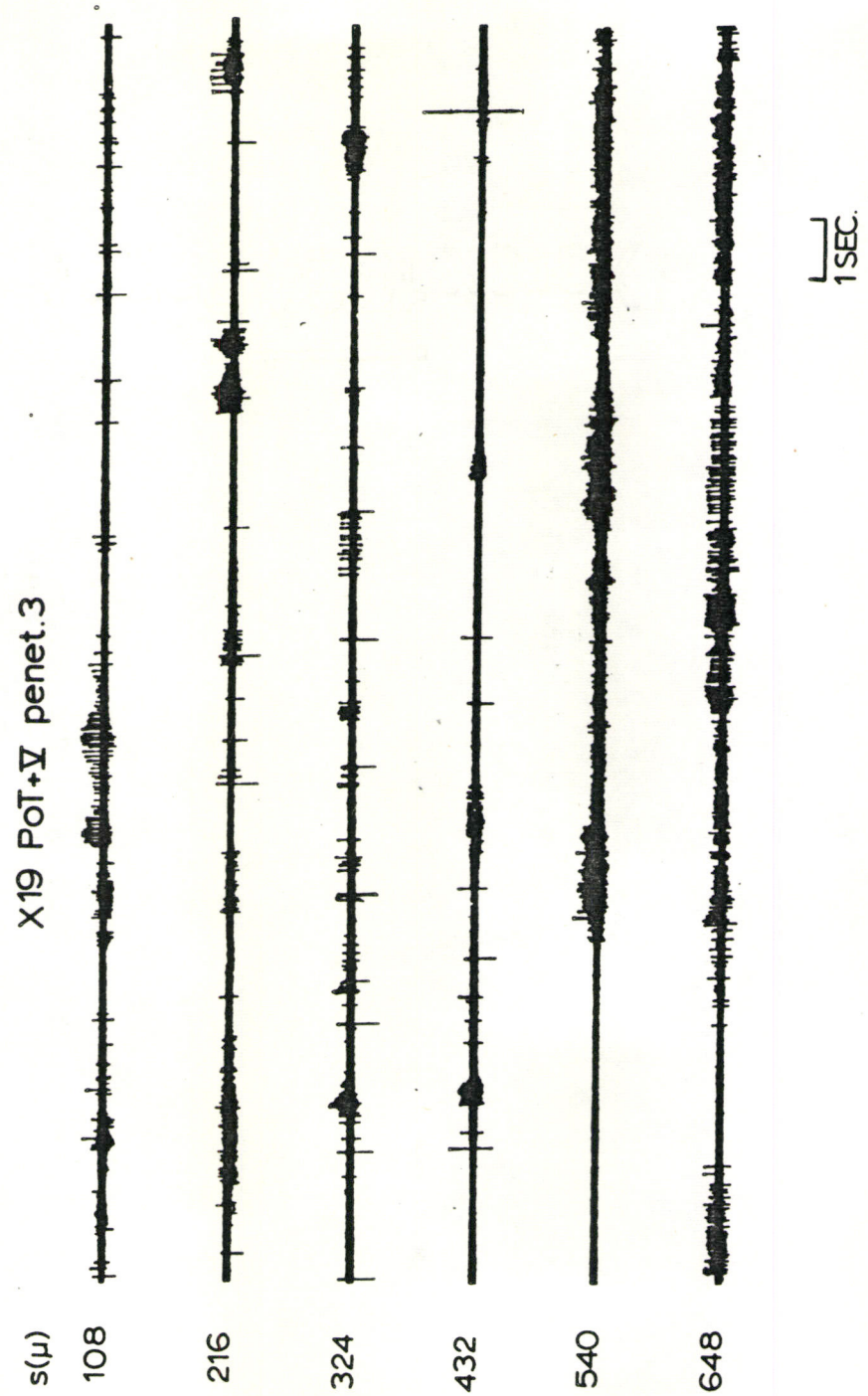


Figure 28.

from the figure).

These data, together with the previous results, lead to the conclusion that the excitability of neurons, estimated from the amount of spontaneous and injury discharge, in a population of neurons of association cortex does not determine the waveform of spontaneous surface slow potentials recorded from that population. There are relatively few spontaneously-active cells and little injury discharge both with activated (MPP) and synchronized (MPP and RP) EEG potentials; on the other hand, many spontaneously active units and more injury discharges were recorded during synchronized (PoT + V) or desynchronized (PoT) EEG patterns.

The experiments on the "gasserectomized" PoT preparations also make it clear that, although cutting the fifth nerve may alter surface potentials, the maintenance of cortical neuron activity does not depend on fifth nerve input to the PoT preparations. This would suggest that the different effects of pretrigeminal and post-trigeminal sections in influencing neuron activity in the cortex are due to the integrity of the nucleus reticularis pontis caudalis (n. r. p. c.), which is interrupted by midpontine section and spared following PoT section. On the other hand, the action of the portions of the reticular formation spared in PoT preparations may be sufficient for the maintenance of neuron activity in suprasylvian cortex. In either case, it is clear that these experiments demonstrate

the existence of a "non-specific" reticular control on the activity of suprasylvian cortex neurons. Withdrawal of this influence by pretrigeminal lesion decreases the number of neurons spontaneously discharging. Nevertheless, a small degree of spontaneous activity remained in pretrigeminals, and the patterns of this spontaneous firing in single neurons were compared with the spontaneous discharge patterns of post-trigeminals in order to discover if the reduced activity of the cortical population following pretrigeminal section, which apparently lowers the probability of occurence of spontaneous neuron discharge, may also affect the sequential probability of a series of discharges after spontaneous activity has begun.

Effect of Pontine Sections on Patterns of Spontaneous Discharge

The distribution of intervals between successive action potentials (interval distribution) was computed for 40 individual neurons recorded from both PreT and PoT preparations. These interval distribution histograms exhibit a curvilinear function which has been previously described as characteristic of spontaneous activity in pretrigeminal preparations (Smith and Smith, 1965). The authors reported that these spontaneous activity patterns could be described as bursts, i.e., sequences of random discharges separated by inactive periods of longer duration

than the relatively short random intervals occurring within each burst. The distributions of these short (within-bursts) and long (between bursts) intervals can be seen in the interval distribution histograms (Figures 29 through 34) as two slopes intersecting at approximately 200 msecs. When plotted semilogarithmically, the random distribution of short intervals (0 - 200 msec) approximates a straight line which is steeper than the slope of the distribution of long intervals (200 - 1000 msecs).

Although the sample of cells studied is too small to exclude the possibility that the pattern of spontaneous firing is never influenced by structures in the pontine reticular formation, visual inspection of the interval distributions indicates no striking differences between spontaneous discharge patterns recorded from preparations with high and low pontine sections. This implies that although pontine reticular formation (e.g., n. r. p. c.) may influence the relative number of spontaneously active cells in the cortex, it may not be important in determining the patterning of successive discharges.

If a larger number of active units is assumed to be the expression of increased neuronal excitability in PoT preparations, it might be expected that the frequency of occurrence of bursts in spontaneously active neurons would be correspondingly increased. However, visual inspection of the interval histograms indicated that the distribution

Figures 29 through 35. Interval distribution histograms. The ordinates, plotted on a logarithmic scale, are separated for different cells to allow visual inspection of the curves. The ordinates for cells with markedly different firing rates were expanded [X50 (1), X42 (1)] or compressed [X44 (1)].

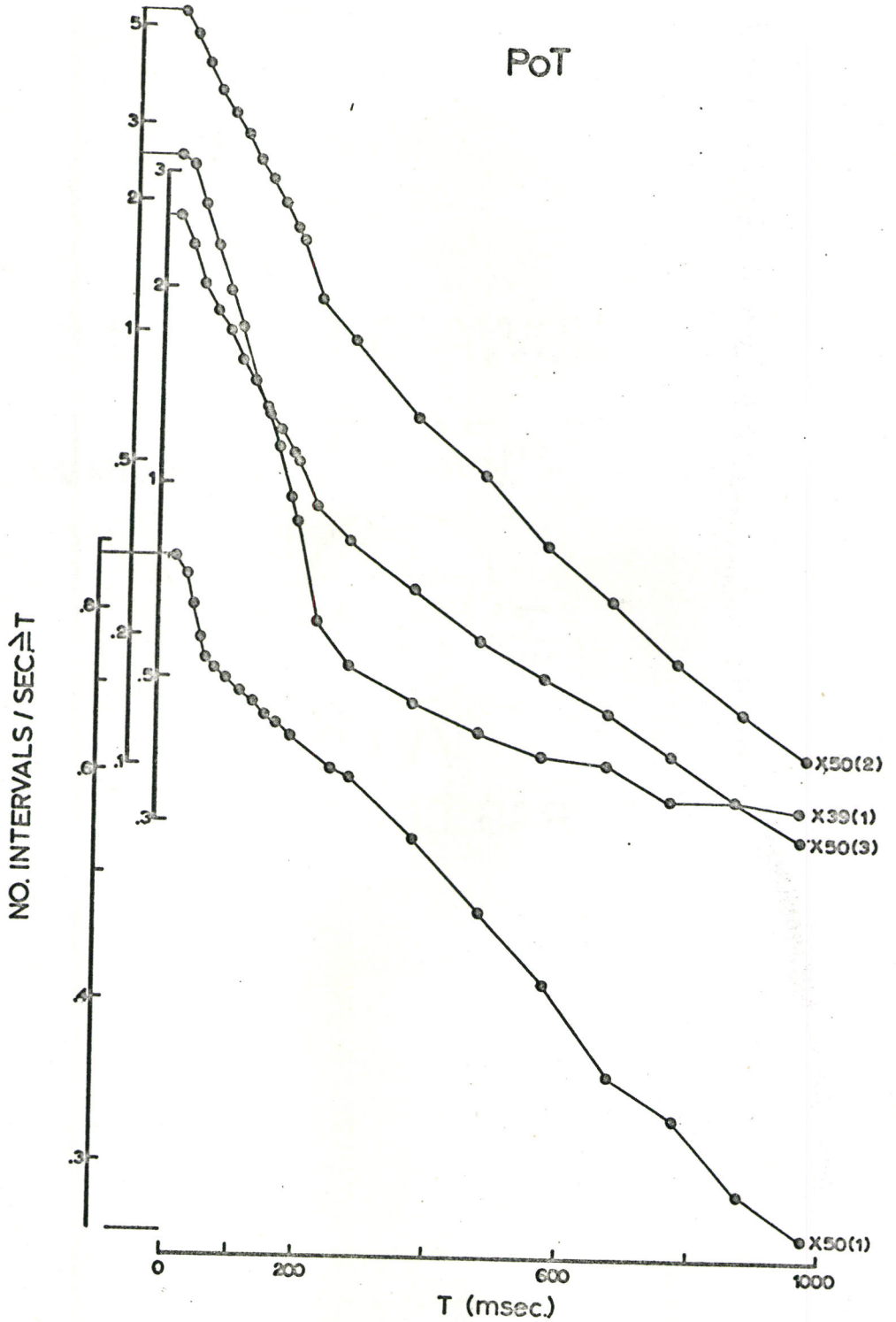

PoT
NO. INTERVALS / SEC ≥ T
T (msec.)
0
200
600
1000
X50(2)
X39(1)
X50(3)
X50(1)

Figure 29.

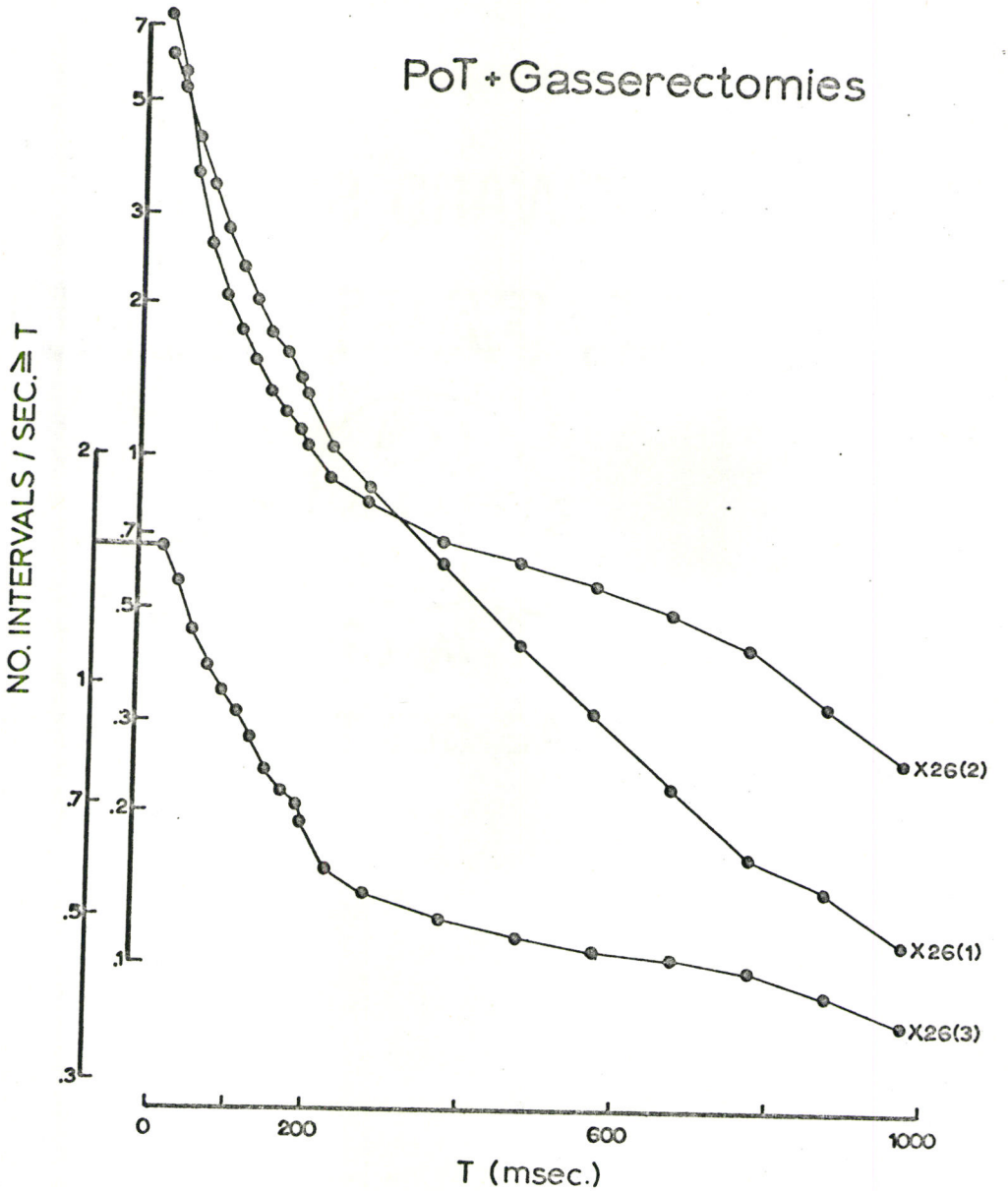

PoT + Gasserectomies
NO. INTERVALS / SEC. ≧ T
7
5
3
2
1
.7
.5
.3
.2
.1
2
1
.7
.5
.3
X26(2)
X26(1)
X26(3)
0
200
600
1000
T (msec.)

Figure 30.

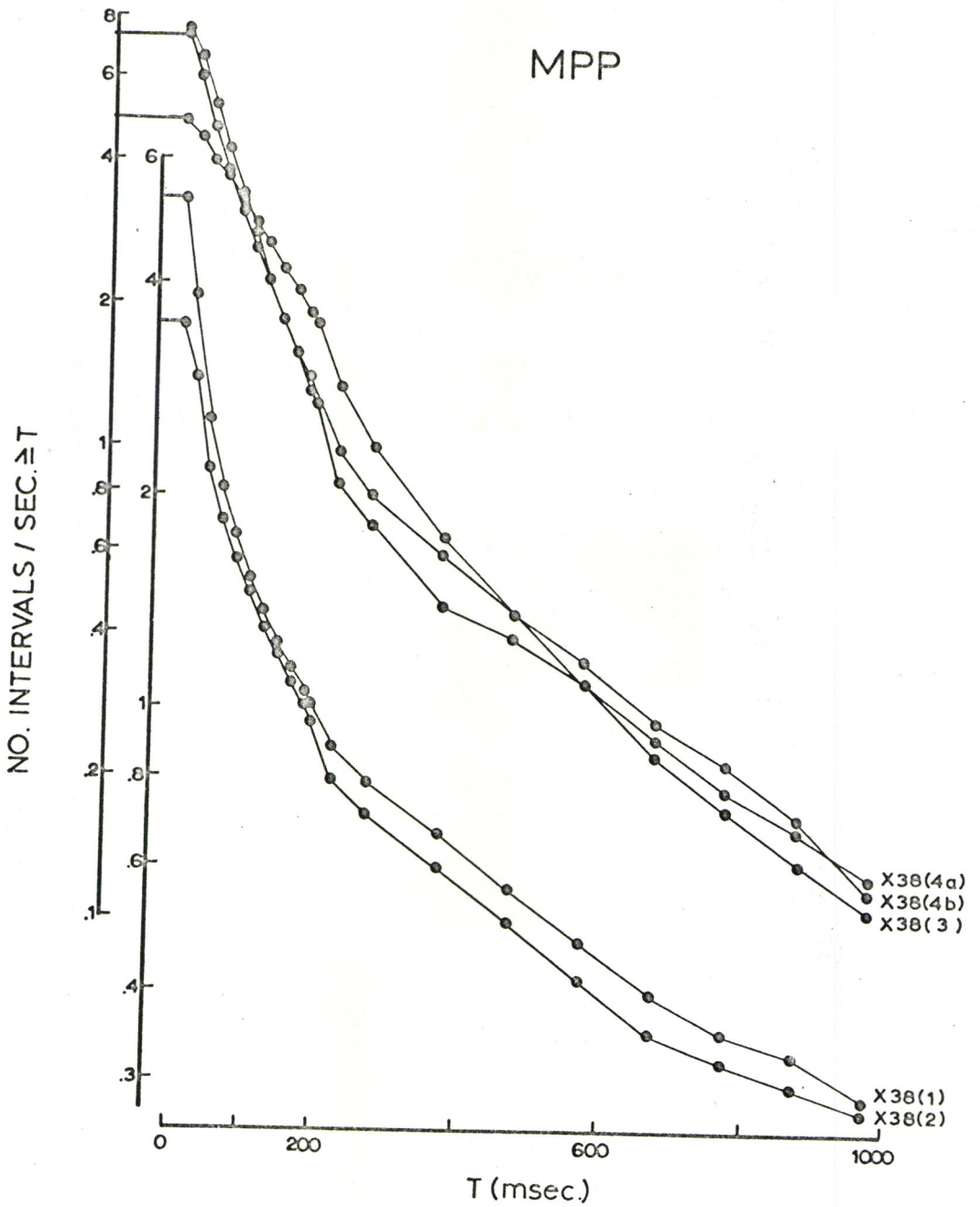

MPP
NO. INTERVALS / SEC. ≥ T
T (msec.)
0
200
600
1000
X38(4a)
X38(4b)
X38(3)
X38(1)
X38(2)

Figure 31.

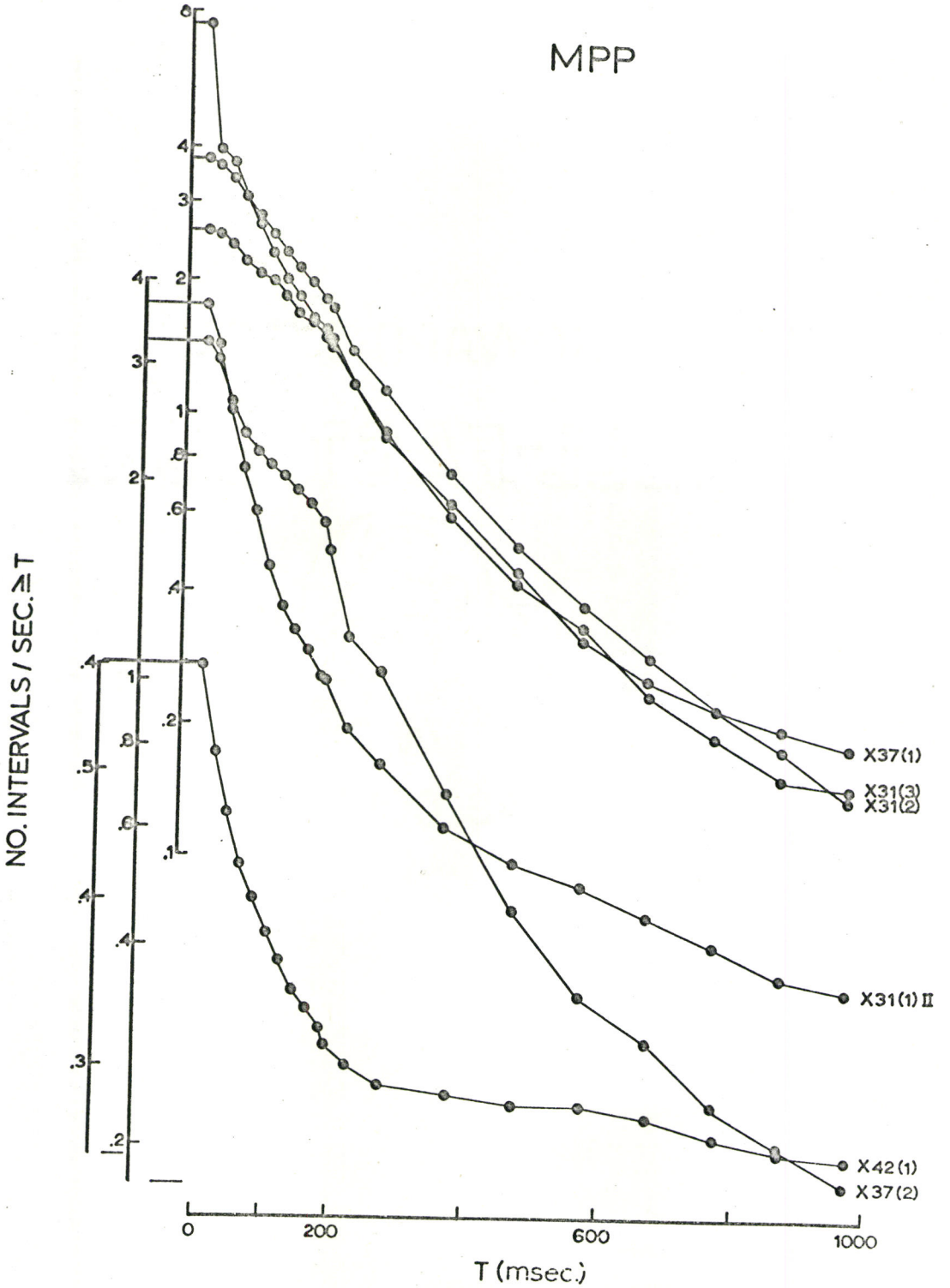

MPP
NO. INTERVALS / SEC. ≧ T
T (msec.)
0
200
600
1000
X37(1)
X31(3)
X31(2)
X31(1) II
X42(1)
X37(2)

Figure 32.

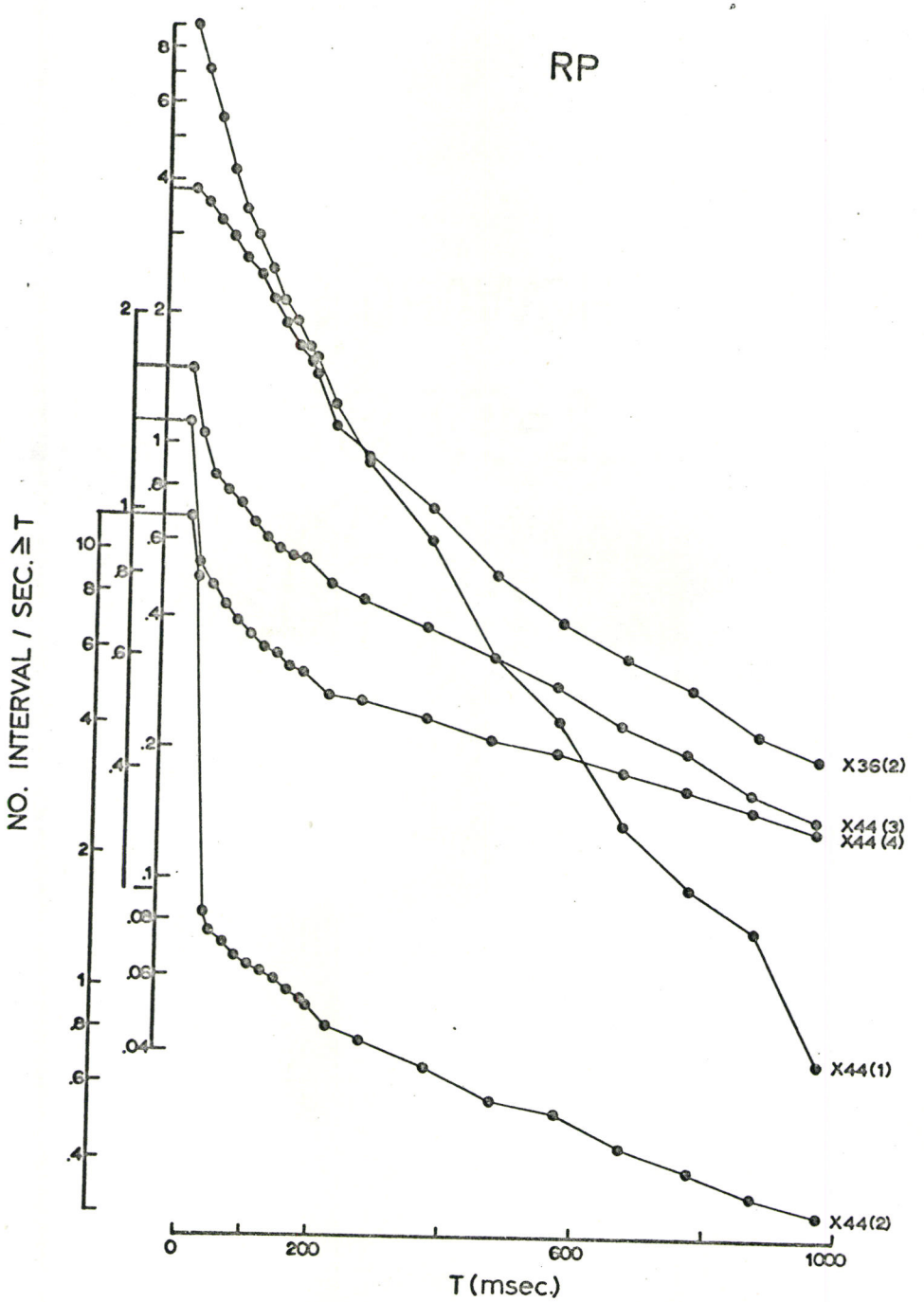

RP
NO. INTERVAL / SEC. ≥ T
T (msec.)
0
200
600
1000
X36(2)
X44 (3)
X44 (4)
X44(1)
X44(2)

Figure 33.

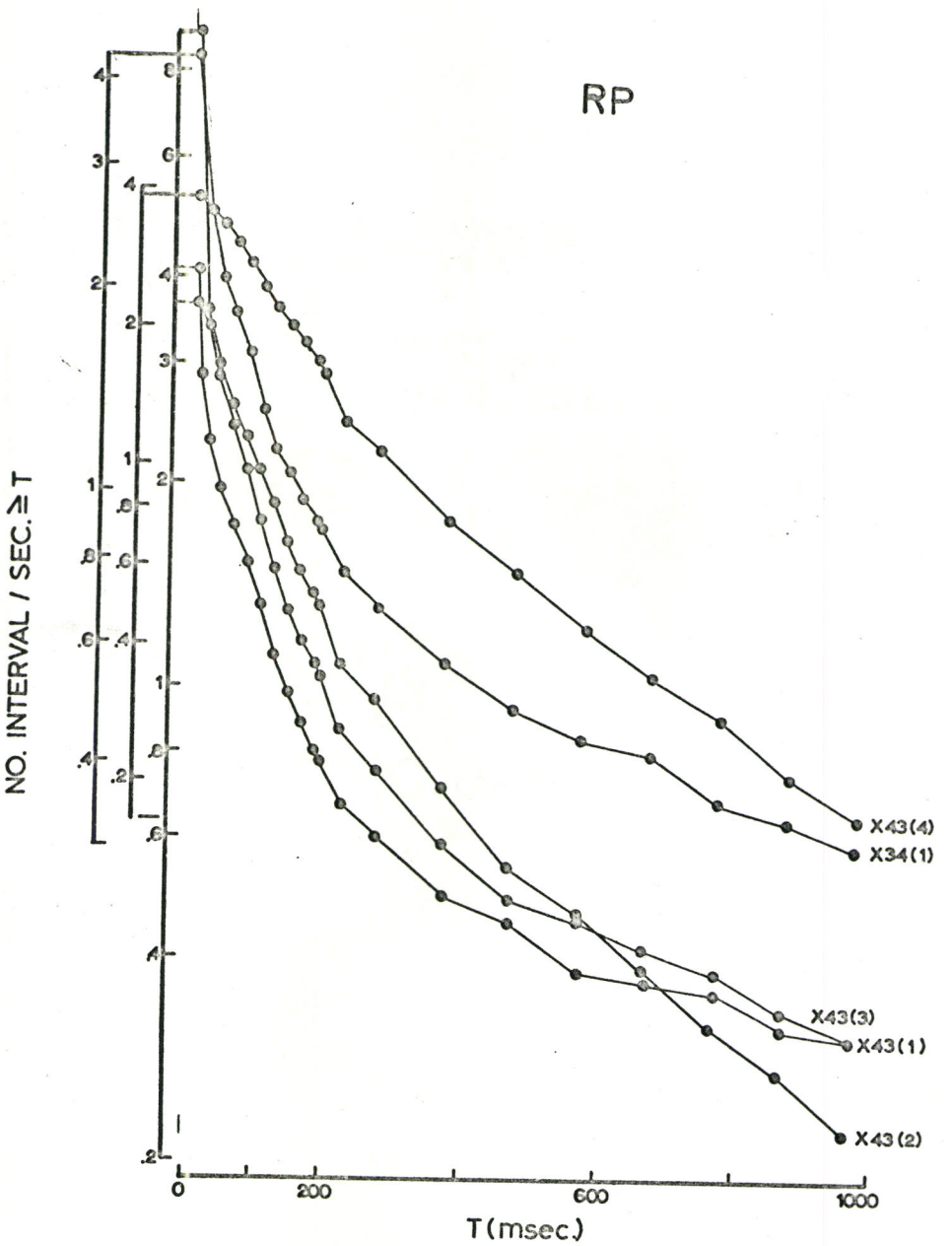

RP
NO. INTERVAL / SEC. ≥ T
T(msec)
0
200
600
1000
X43(4)
X34(1)
X43(3)
X43(1)
X43(2)

Figure 34.

of long intervals was not significantly steeper for neurons in PoT than for neurons in PreT preparations. In addition, neither mean nor median measures of average firing frequency differed between neurons from PoT or PreT preparations [mean: PoT (n = 7 cells) 4.49/sec, PreT (n = 21) 5.00/sec; median: PoT (n = 7) 3.18/sec, PreT (n = 21) 3.83/sec]. In fact, even neurons from a preparation with a virtually intact brainstem having only slight rostral pontine damage exhibited spontaneous discharge patterns which were typical of bursts (see Figure 35).

Relation Between Spontaneous Discharge and Transcortical Slow Potentials

It has been shown that the activity of a cortical neuron may be associated with surface-positive (Fromm and Bond, 1964) or deep-negative (Fox and Norman, 1968) slow potentials. Smith (1969) has demonstrated that in pretrigeminal preparations clear correlations are found between negative transients at the microelectrode tip and bursts of spikes when these tip-negative slow potentials accompany bursts roughly for the duration of the burst. In order to evaluate the importance of a pontine reticular influence on this relationship, the burst activity of the neurons just reported was compared with the simultaneous transcortical slow potentials (ECoG). It would be surprising to find this sort of correlation in preparations with a

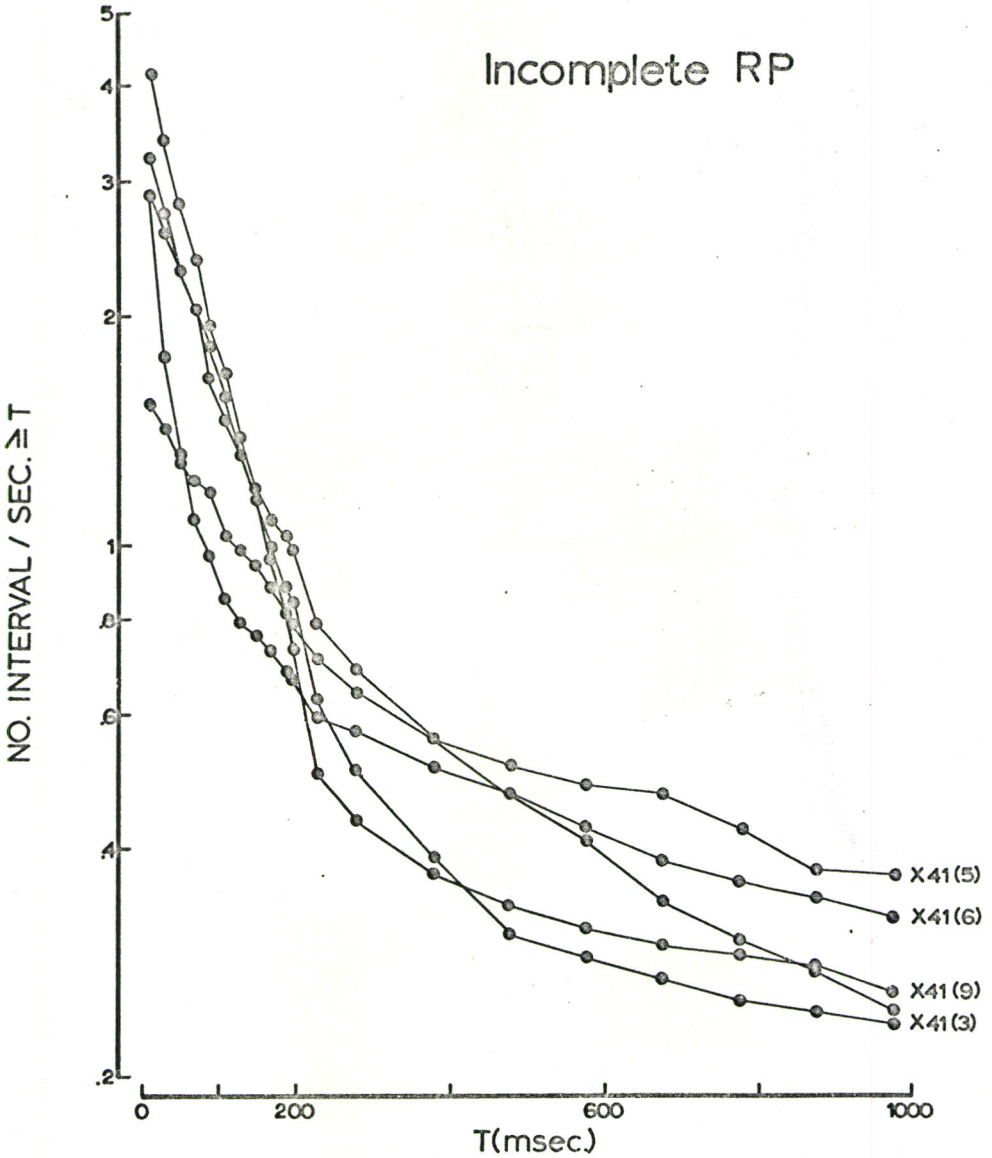

Incomplete RP
NO. INTERVAL / SEC. ≥ T
5
4
3
2
1
.8
.6
.4
.2
0
200
600
1000
T(msec.)
X41(5)
X41(6)
X41(9)
X41(3)

Figure 35.

less-synchronized ECoG (e.g., with the activated EEG of the PoT preparation). As can be seen in Figures 36 through 45, it was generally true that correlations between surface positivity and burst onsets and between surface negativity and periods following burst ends were usually better with increased ECoG synchrony. Nevertheless, in virtually every such analysis, regardless of the preparation or ECoG pattern, there was a strong relation between tip-negativity or surface-positivity and the first and last spike of each burst, (vertical bars in each histogram). This is in keeping with the finding of increasing spike likelihood with increasing D.C. deep negativity (Fox and Norman, 1968).

A relation between phase of the transcortical slow transients and rate of spontaneous discharge (i.e., during or after bursts) could be demonstrated in both PoT and PreT preparations by the use of averaging techniques. Differences observed in various correlations were due in part to the degree of ECoG synchrony present, in which large slow transients correlated well with burst activity or intervals between bursts. It was clear, however, that the slow transients in some cases (e.g., Figure 36) were not well correlated with bursts of spontaneous discharge due to the low amplitudes of the transients. Since in these cases the spontaneous unit discharges exhibited burst patterns, it must be concluded that transcortical potential shifts are

Figures 36 through 45. The average slow potential histogram relative to burst onset or end. Ordinate is the relative transcortical potential, surface-positive up. Abscissa is time relative to burst onset or end (vertical bars) and extends from -400 to +1000 msec. Note that the average surface-positivity begins prior to burst onset and remains so for roughly the average burst duration (white bar), then becomes less positive on the average. At burst end, the relatively high surface-positivity seen with the last spike in the burst gradually falls to surface-negativity after the burst has stopped and before another burst occurs. This surface-negativity can be seen prior to burst onset. Burst end histograms in the left column are always analyses in which burst durations less than 300 msec were rejected in order that short duration burst onsets would not be counted as burst ends, (the one exception is X50 cell 3, in the left column of Figure 38, which belongs in the right column). For comparison, burst end histograms in the right column had no burst duration contingency nor did any of the burst onset histograms. The number above the vertical bars in each histogram refers to the number of burst onsets or ends determining the sample. In the histograms in the right column and X50 (3) the number of burst onsets and ends are always equal. The calibration refers to the electrocorticogram tracing, surface-positive <u>down</u>. Spikes were retouched.

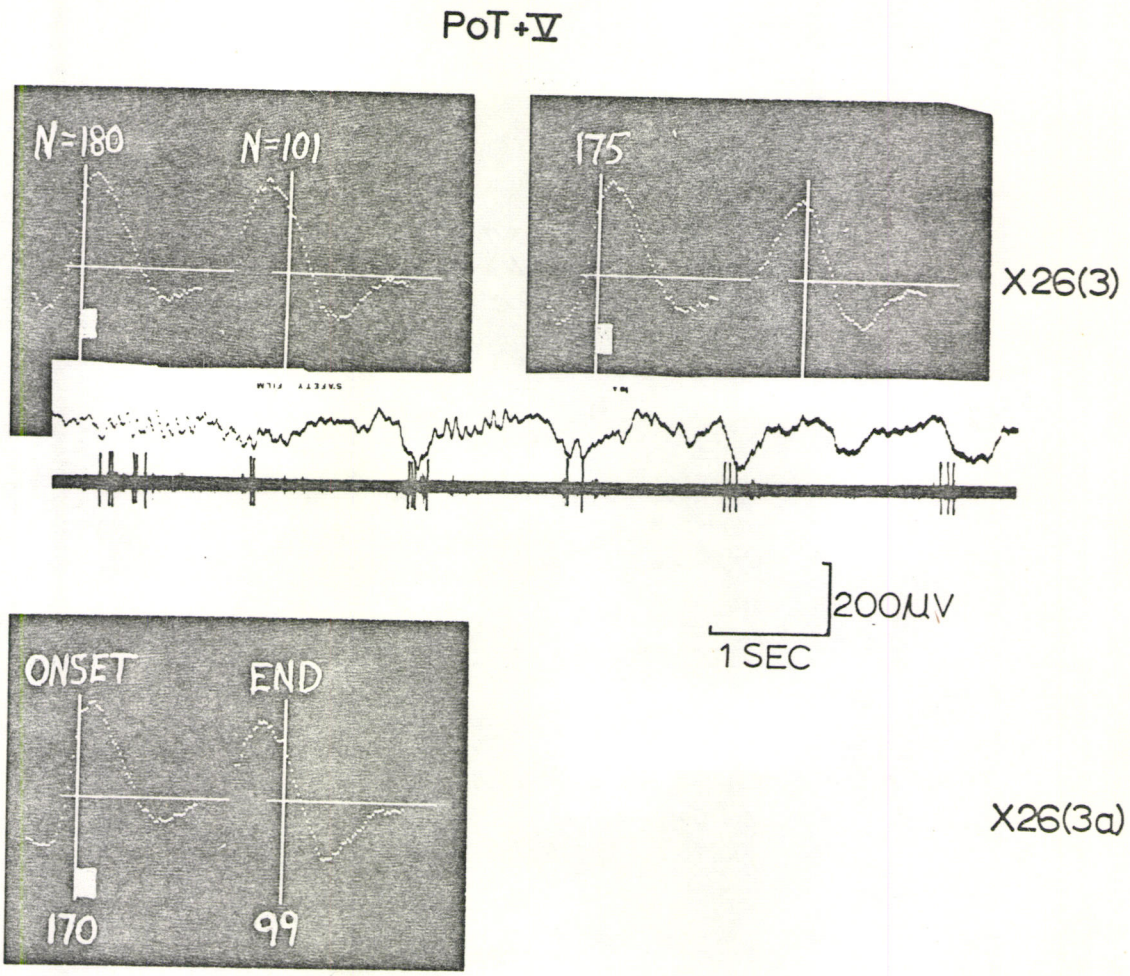


Figure 36.

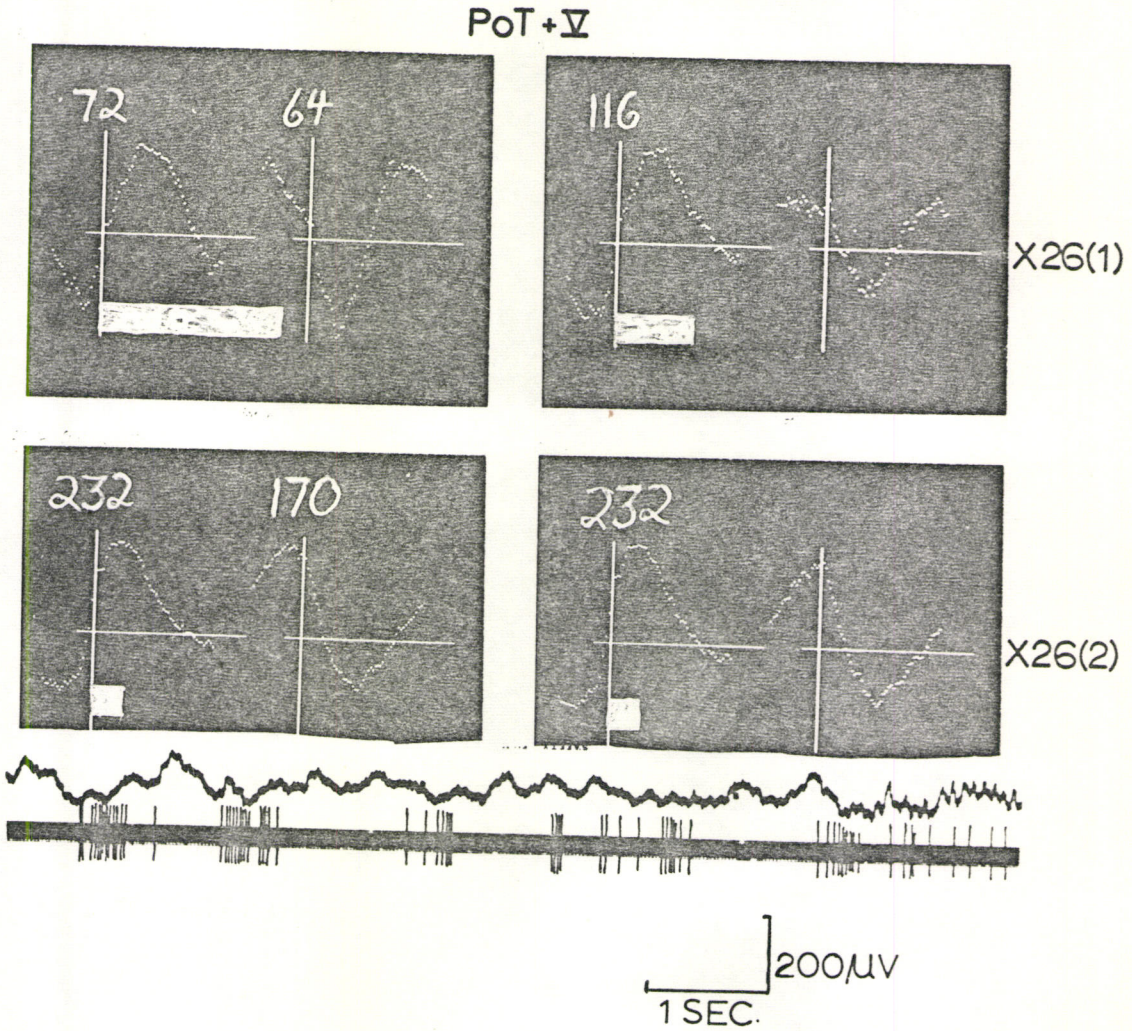


Figure 37.

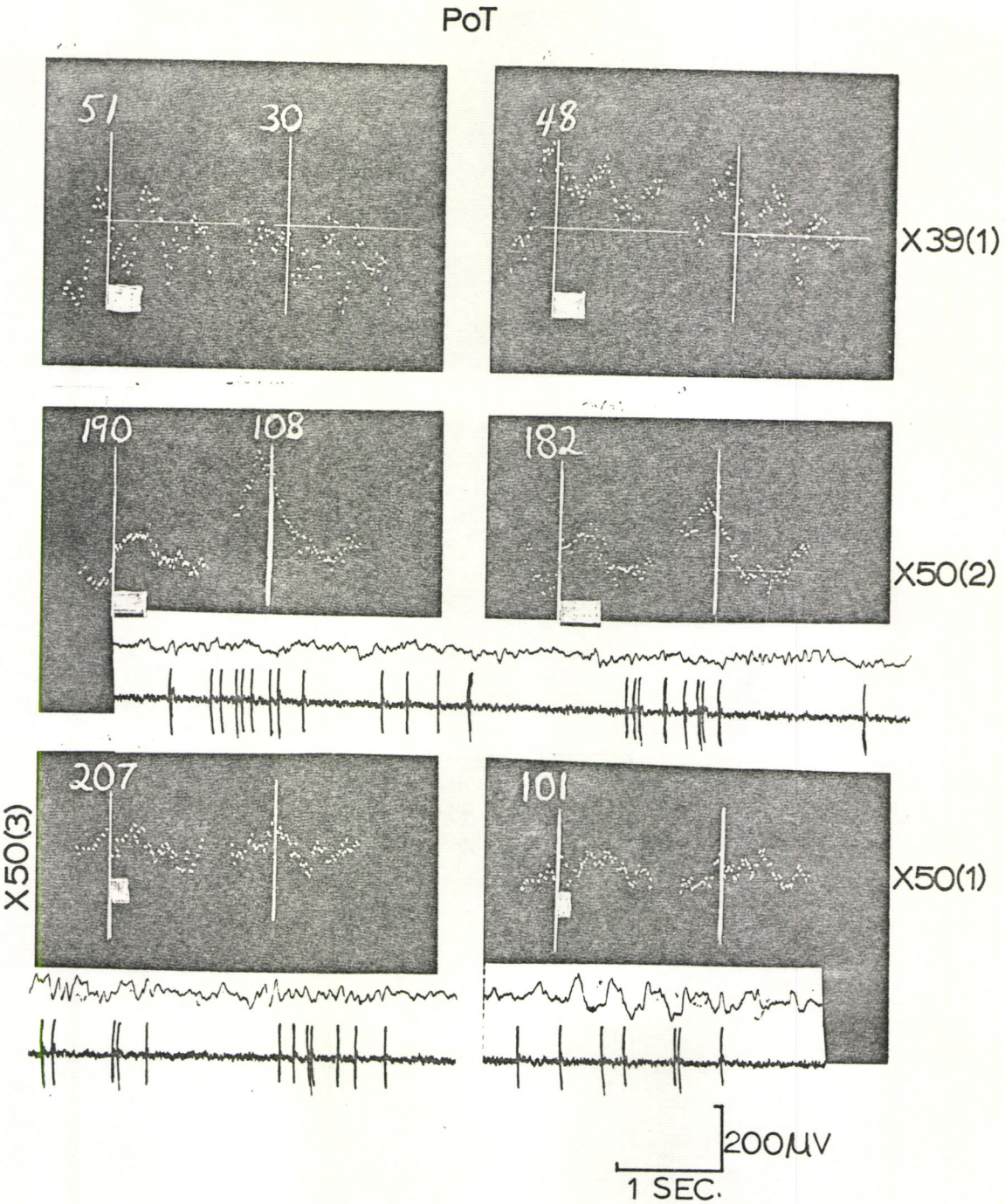


Figure 38.

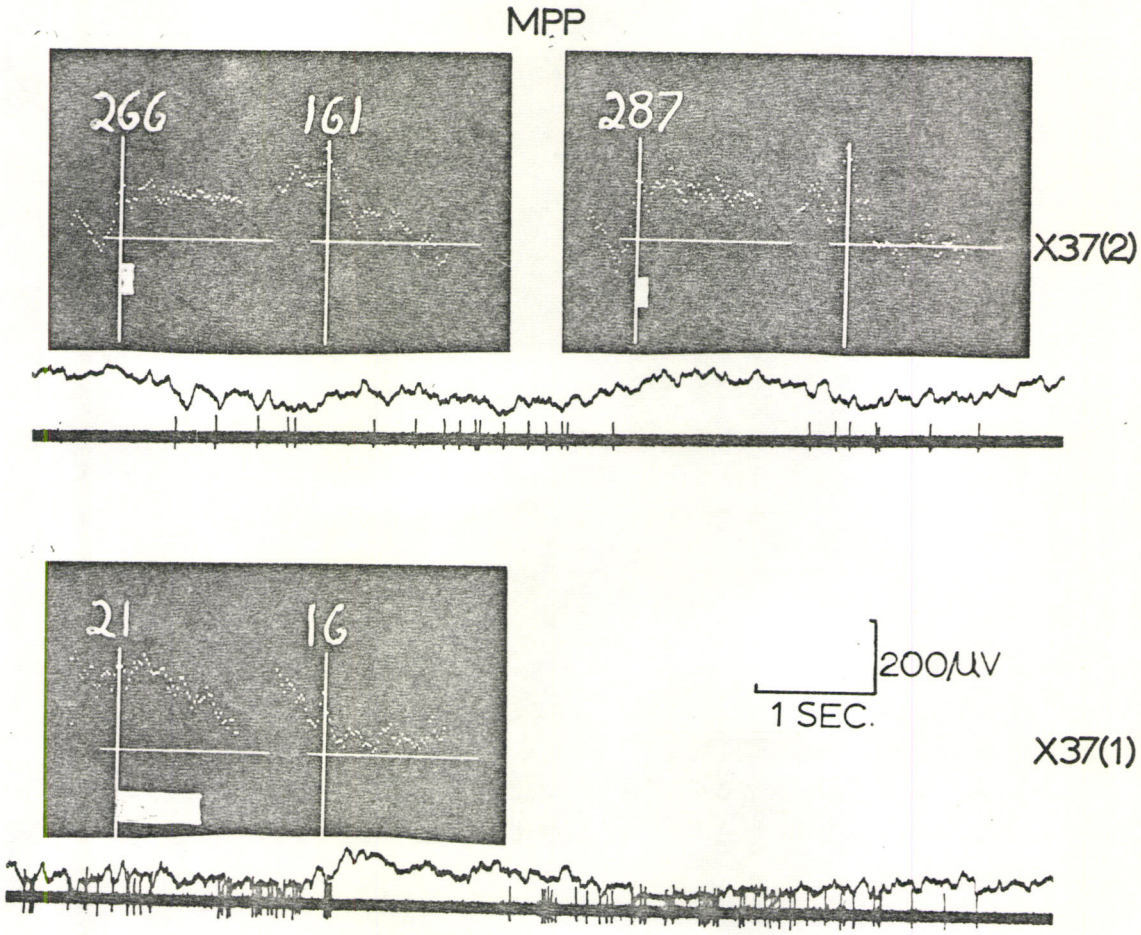


Figure 39.

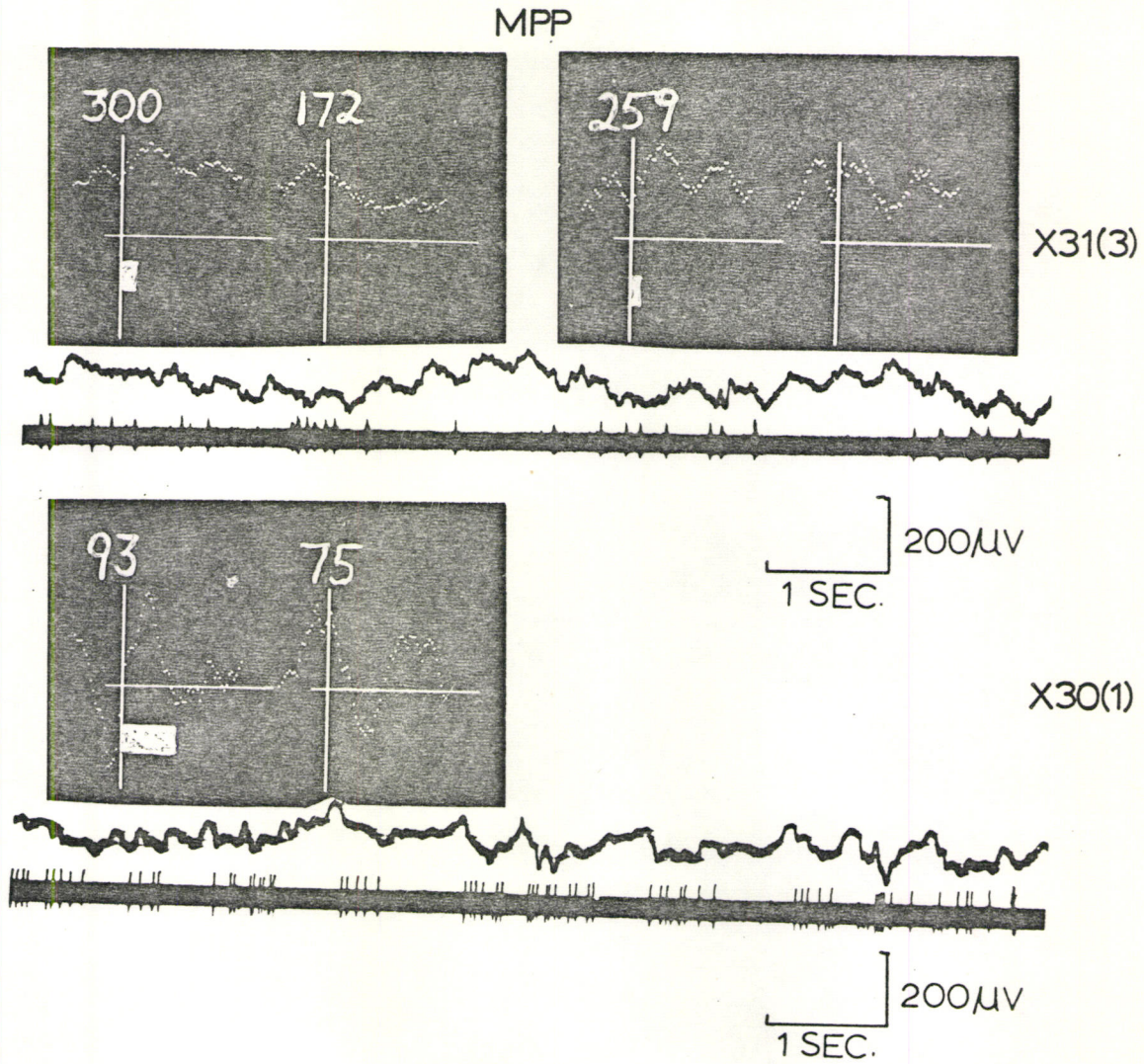


Figure 40.

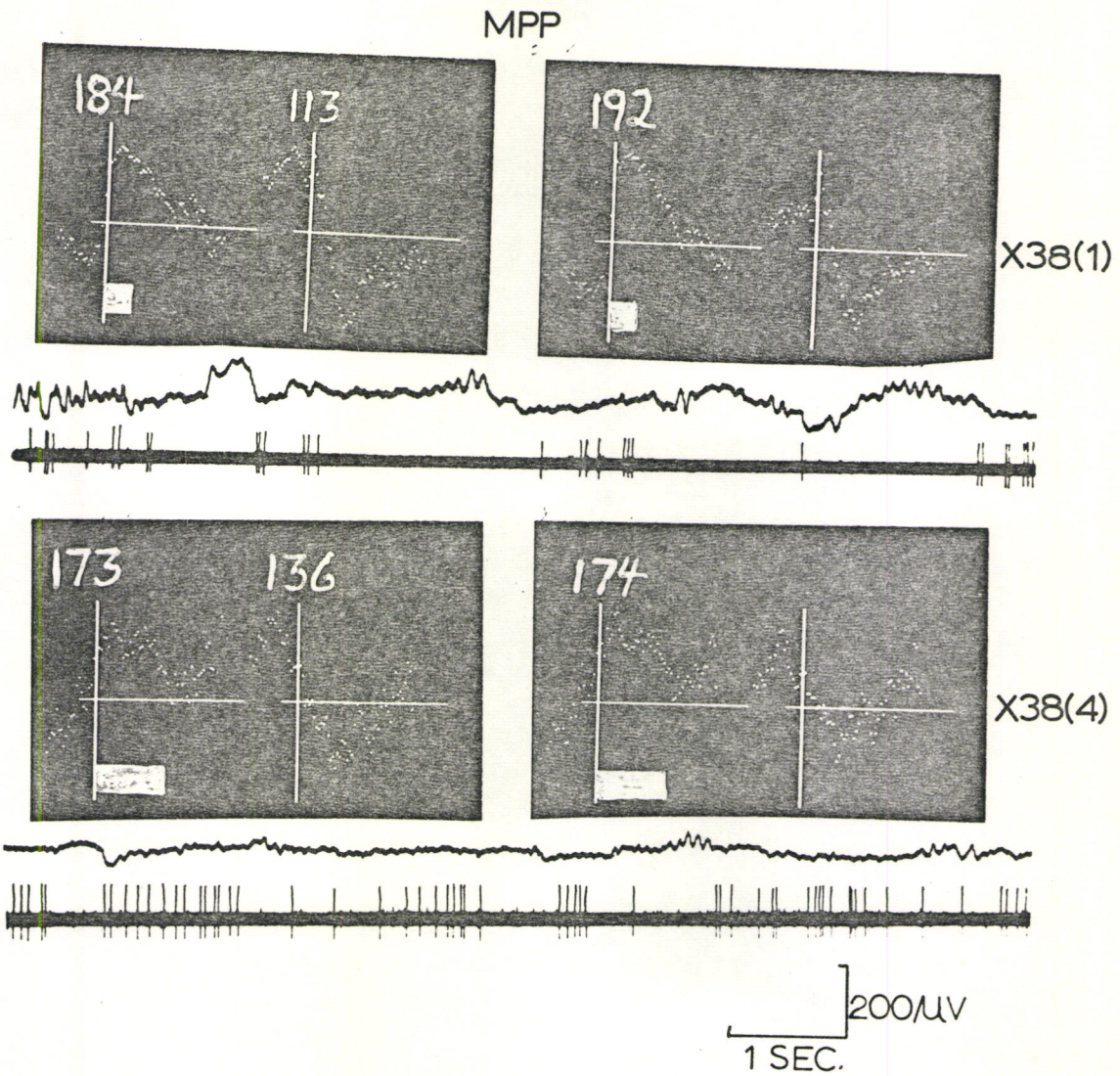


Figure 41

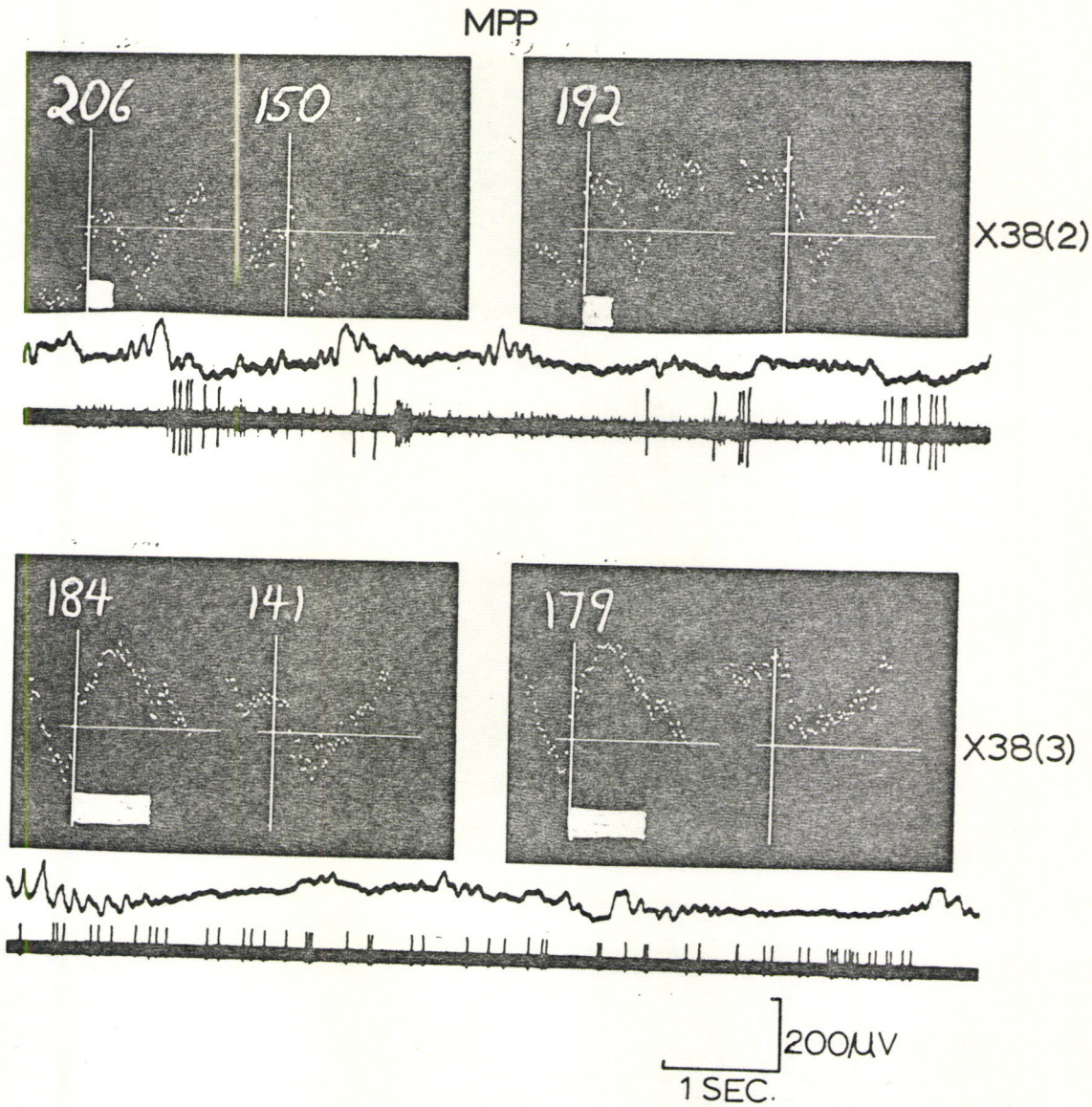


Figure 42.

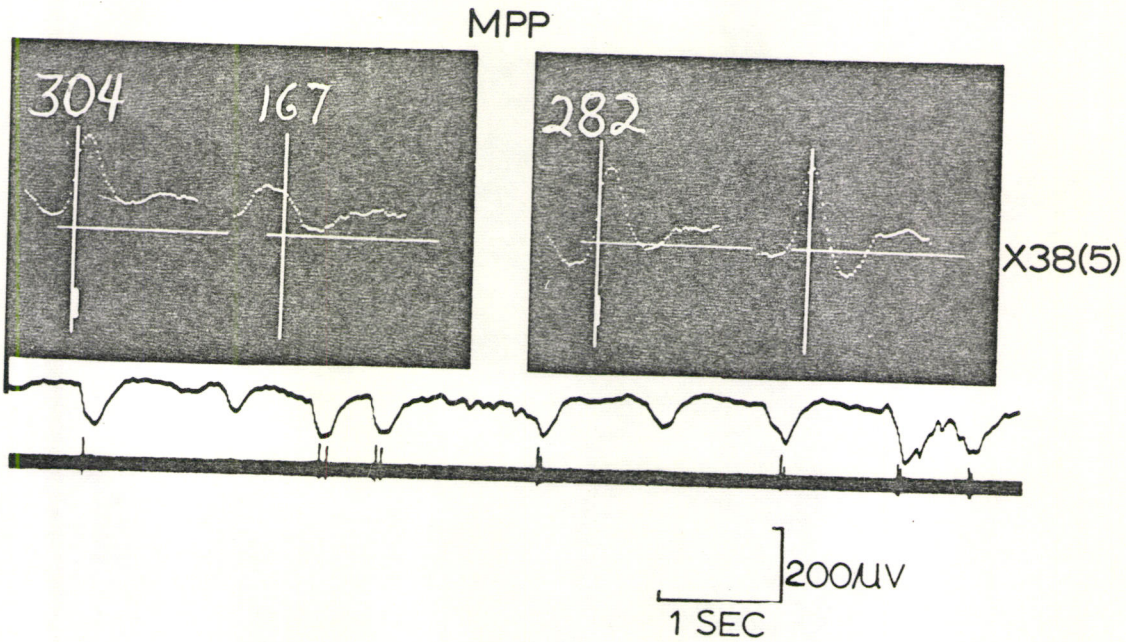


Figure 43.

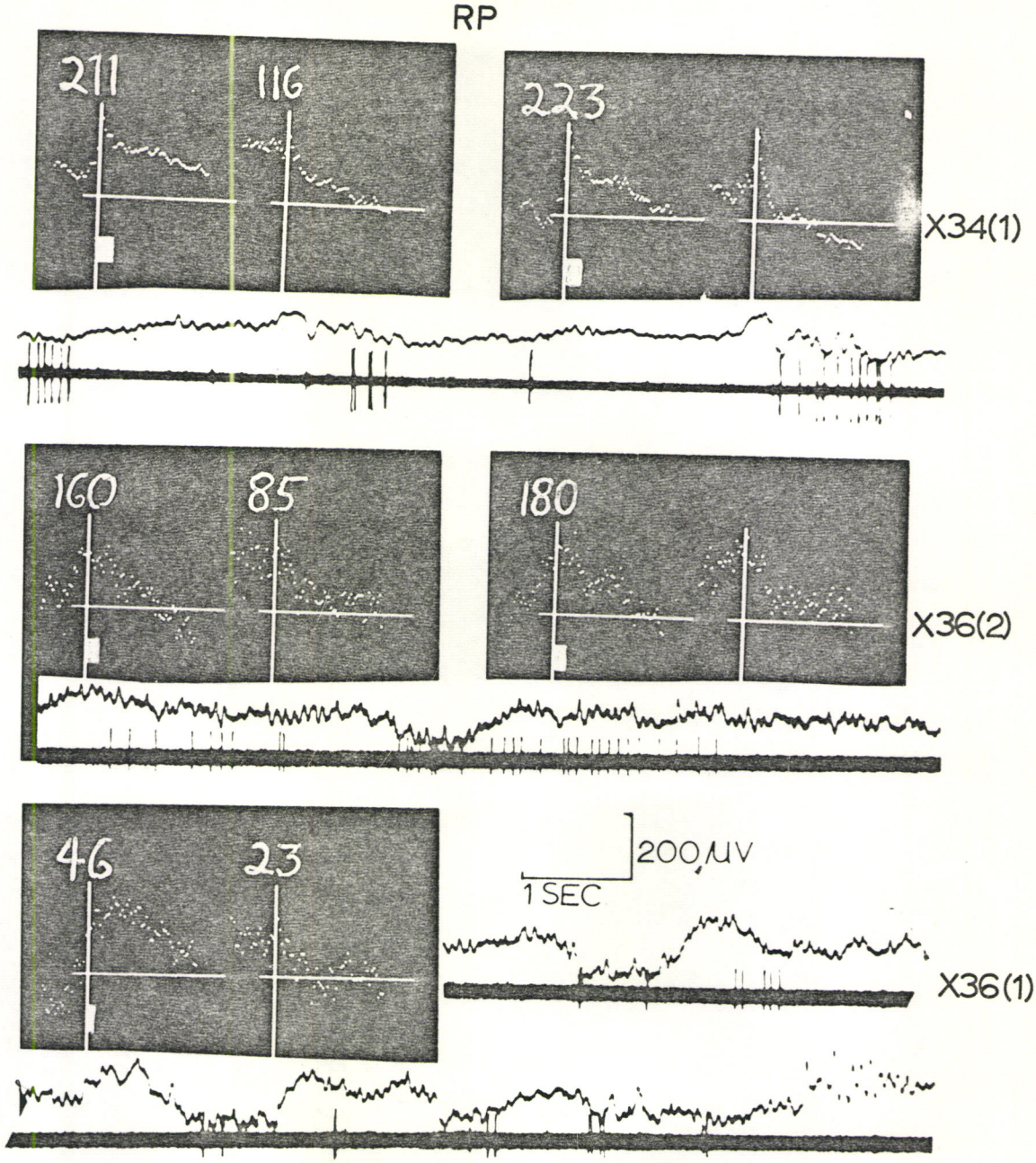


Figure 44.

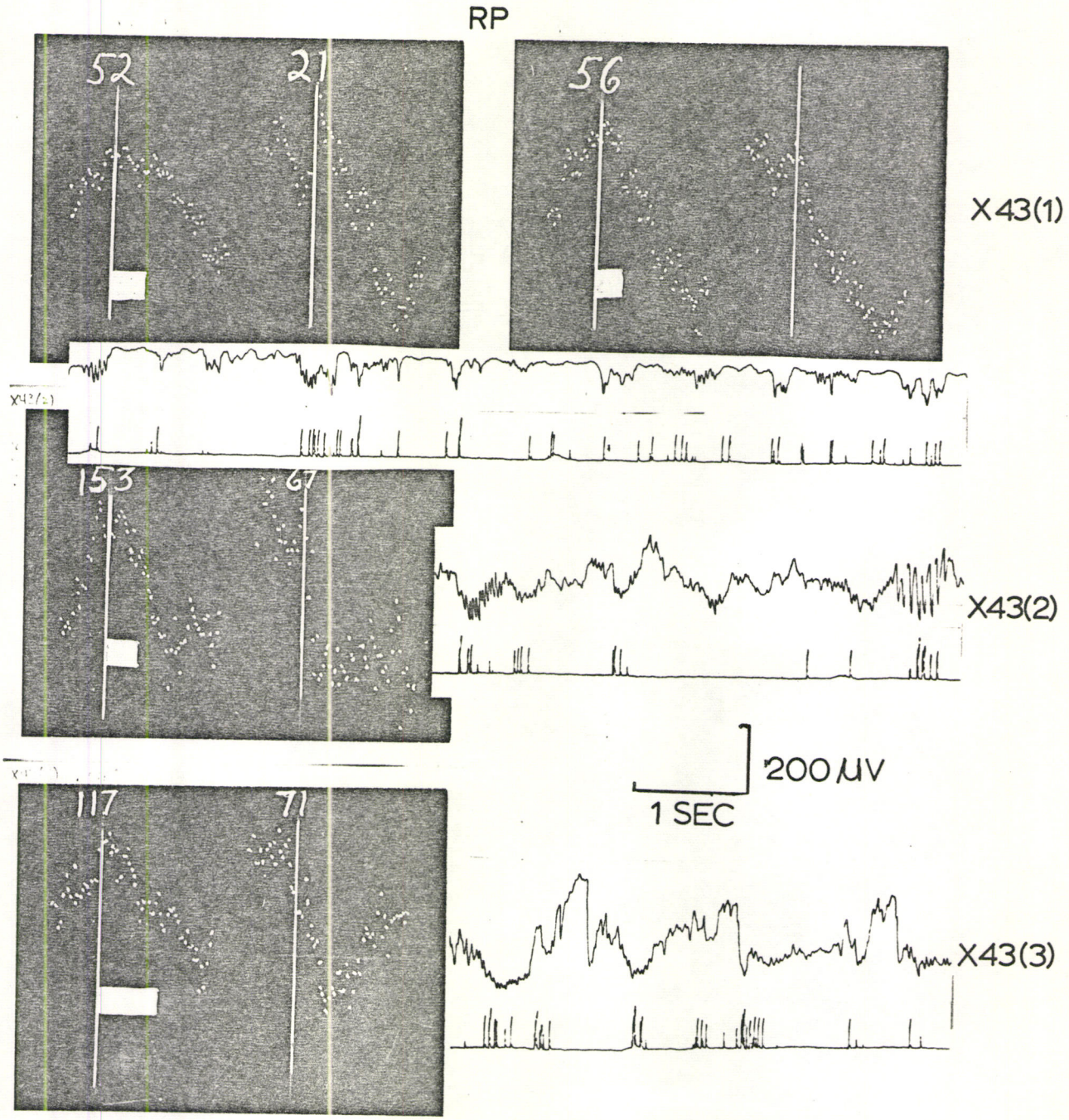


Figure 45.

not causally-related to bursts nor are individual bursts initiated by a slow potential shift in the region of the cell body. Such a spike "trigger" mechanism might be expected since in all histograms the surface-positive shift precedes burst onset. In addition, numerous experiments (e.g., Creutzfeldt, Fromm and Kapp, 1962) have shown that surface positive polarization accelerates spontaneous unit discharge. However, the notion that potential disparity between the cortical surface and the cell soma initiates or determines the pattern of spontaneous discharge is refuted by the experiments of Langsam and Smith (1967) which showed that neither stabilization (i.e., voltage clamp) nor systematic modulation of transcortical potentials affected the rate or pattern of spontaneous discharge.

Summary of Experiment I

The findings of Experiment I lead to the conclusion that a "non-specific" reticular brainstem influence (perhaps from n. reticularis pontis caudalis) contributes to the maintenance of activity in neuron populations of the suprasylvian gyrus, as evidenced by a greater incidence of spontaneous and injury discharges in post-trigeminal as compared with pretrigeminal preparations. Following pretrigeminal lesions, the activity of suprasylvian neurons is greatly reduced (i.e., fewer units spontaneously active or discharging upon contact), presumably because this lower

pontine reticular influence is abolished by midpontine section. Nevertheless, with either state of activity a variety of cortical surface potentials were recorded, (i.e., synchronized, desynchronized, and/or spindles), indicating that the total number of discharging neurons in a population does not determine the waveforms of surface potentials recorded from the vicinity of that population.

It was also found that although the density of units spontaneously discharging within a neuron population was decreased following pretrigeminal lesion, spontaneous discharge patterns which did occur in cortex following pretrigeminal section were not grossly different from patterns recorded after post-trigeminal section. This result suggested that the function of the lower pontine reticular influence was simply to control the number of spontaneously discharging units in the suprasylvian gyrus. The patterns of spontaneous discharge are presumably determined by a mechanism which does not depend on the number of spontaneously active neurons in surrounding cortex. Such a conclusion would provide evidence against the notion of a self re-exciting network of neurons as the basis for the maintenance of spontaneous activity (Burns, 1958), since it would be expected that the decreased density of active units subsequent to pretrigeminal lesion would likely result in one or more neurons in the network being unresponsive and thereby interrupt the chain.

Slow potentials derived from the region of a spontaneously discharging neuron and referred to the overlying cortical surface on the average exhibited a phase relation to bursts of neuron discharge, regardless of the level of pontine lesion. However, occasionally this relation was obscured, indicating that these fast and slow transient phenomena are not interdependent.

Experiment II was performed in order to repeat some of these results, obtained in Experiment I by comparing between animals with different lesions, in individual animals with cryogenic blockade of the midpontine reticular formation. This method of blocking a lower pontine reticular influence avoids the possible variability in long-term effects of different brainstem lesions, (e.g., cortical trauma or coma, circulatory collapse, or decreased cerebral blood flow) as well as providing a within-subject comparison of electrocortical activity. In brief, Experiment II demonstrated that the activity of suprasylvian neurons in a post-trigeminal preparation was reduced by cryogenic blockade of the mid-pontine brainstem and that this activity recovered when the brainstem was warmed.

CHAPTER FOUR

EXPERIMENT II: RESULTS

Reversible Cryogenic Blockade of Brainstem Conduction

Since Experiment I demonstrated that the pontine reticular nuclei affects the activity of neurons in suprasylvian cortex, it seemed reasonable to assume that temporarily blocking the pathways from this reticular region would result in a temporary reduction in neuron activity, and that this level of activity would return to normal after restoration of pontine conduction. This experiment is an improvement over the experiments which relied on comparisons between animals with different brainstem lesions since it may be argued that pretrigeminal tissue damage eventually leads to greater deterioration of the preparation (e.g., forebrain coma or decreased cerebral blood flow) than is seen following post-trigeminal sections. Although in Experiment I there was no evidence of a lower physiological status for pretrigeminals than for post-trigeminals, control of the amount and position of brainstem tissue damage was necessary in order to unambiguously demonstrate that the results obtained in Experiment I could be due to the withdrawal of pontine conduction per se.

In order to demonstrate that it was possible to

reversibly block conduction through the brainstem, cooling probes were inserted bilaterally into the brainstem at the midpontine level in a nembutalized cat (33 mg/kg). Evoked potentials were recorded from the posterior sigmoid gyrus during electrical stimulation (5 V, 2 msec, 1/sec) of the forepaw. As can be seen in Figure 46, the latency of the evoked response increased with cooling (COLD 3 MINS) until medial lemniscal conduction through the brainstem was abolished (COLD 5 MINS). The response gradually recovered after cooling was stopped.

In order to verify that the effect of the cooling was due to localized blockade of conduction in the vicinity of the cryoprobe and that the result was not due to a lack of responsiveness in the sigmoid gyrus, the experiment was repeated in a cat with a stimulating electrode in the medial lemniscus <u>rostral</u> to the level of the cryoprobes (see tissue section in Figure 47 for probes and stimulating electrode placements). The evoked response to electrical (6 V, 2 msec, 1/sec) lemniscal stimulation was unaffected by brainstem cooling, while simultaneous lemniscal conduction through the brainstem was blocked, (see Figure 47).

As can be seen from the horizontal tissue section in Figure 47, the effectiveness of the cooling by the laterally situated cryoprobes extended medially for about 6 mm. to the medial lemniscus but obviously not as far rostrally as the point of lemniscal electrical stimulation.

Figure 46. Reversible effects of midpontine cooling on a somatically evoked potential in the posterior sigmoid gyrus of the cat. See text for explanation.

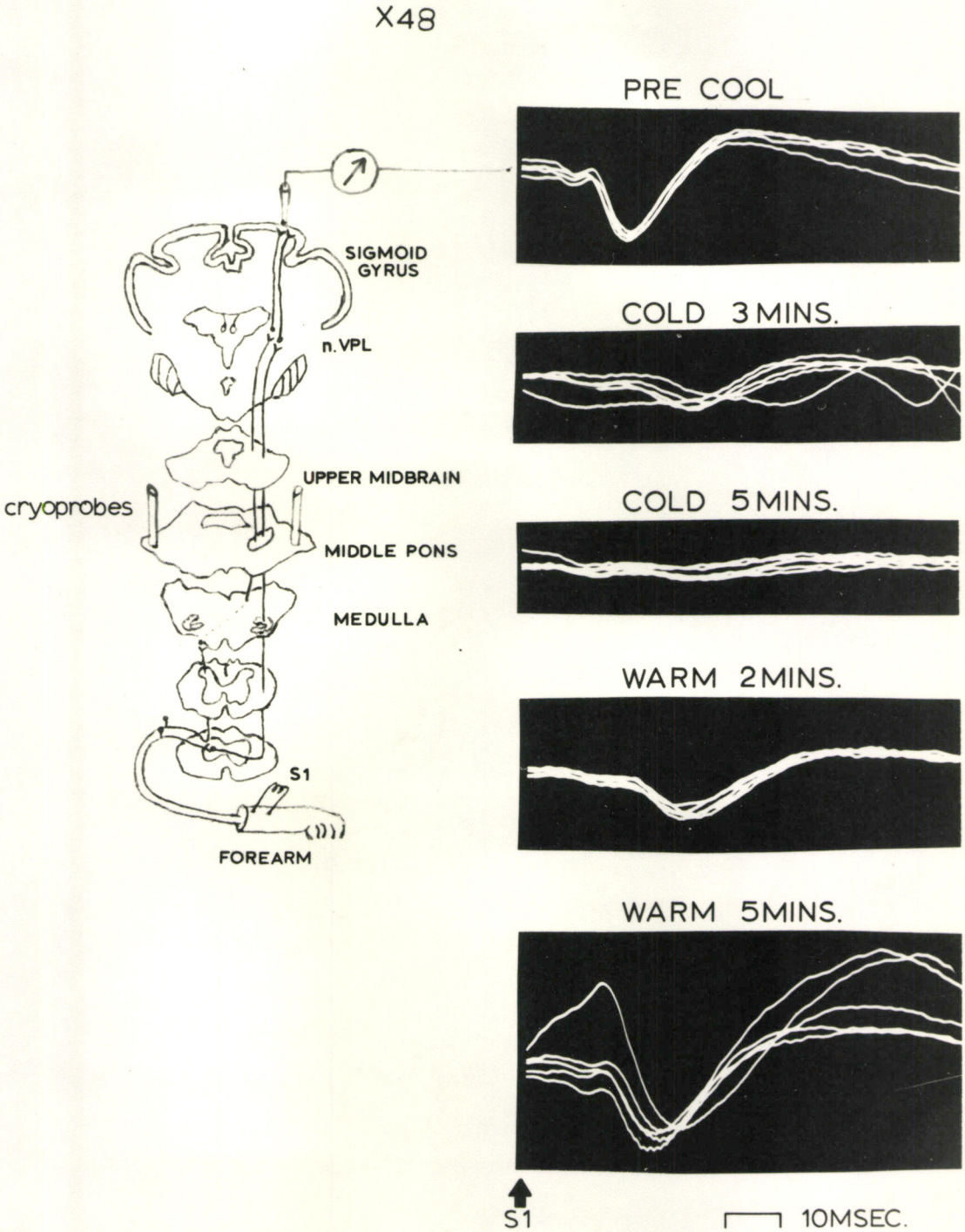

X48
PRE COOL
SIGMOID GYRUS
n.VPL
COLD 3MINS.
UPPER MIDBRAIN
cryoprobes
COLD 5MINS.
MIDDLE PONS
MEDULLA
WARM 2MINS.
S1
FOREARM
WARM 5MINS.
S1
10MSEC.

Figure 46.

Figure 47. Horizontal brainstem section (100 u) at bottom left shows the locus of the lemniscal stimulating electrode (S2) and positions of the cryoprobes. See text for further explanation.

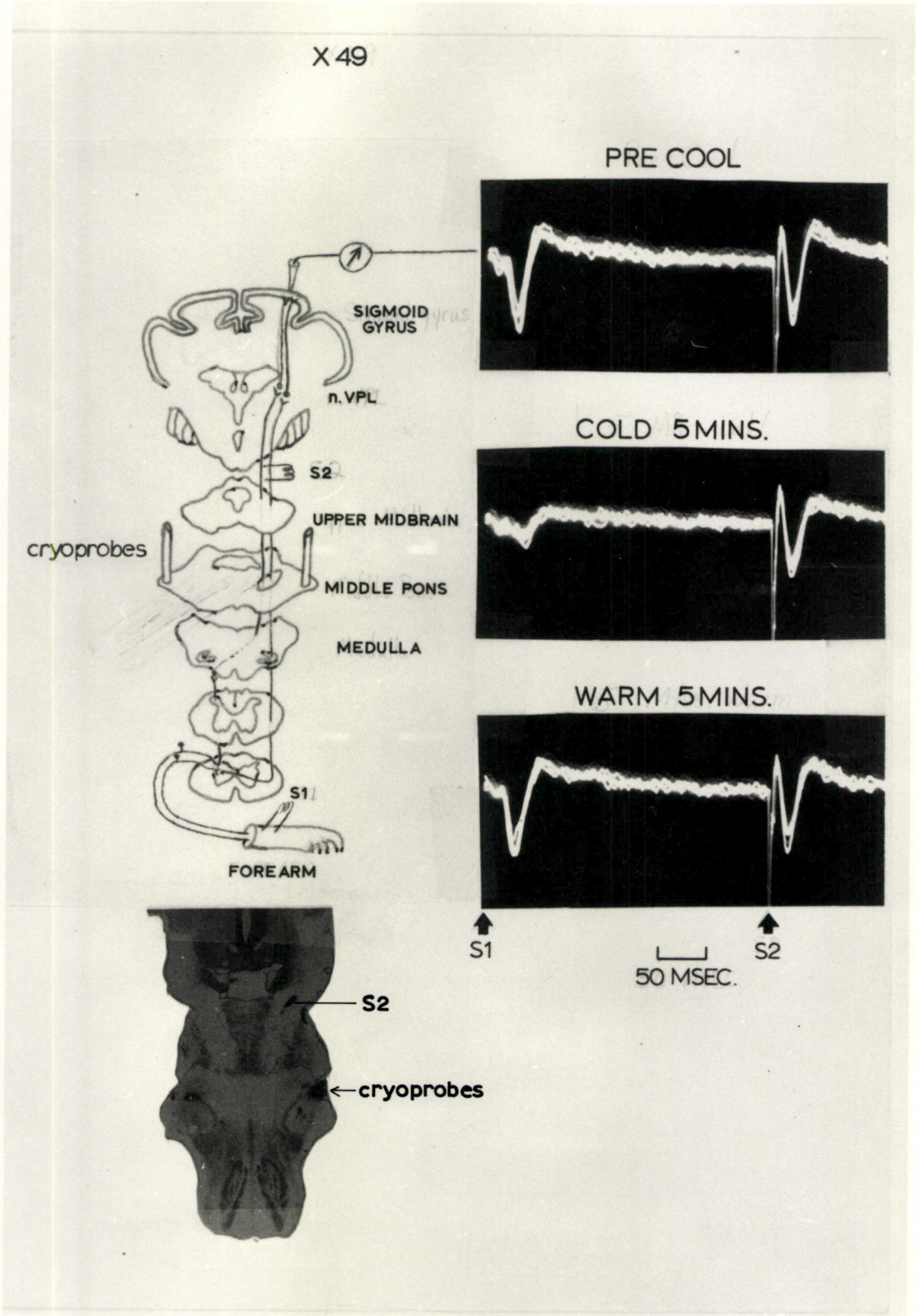

X 49
PRE COOL
SIGMOID GYRUS
n. VPL
S2
UPPER MIDBRAIN
cryoprobes
MIDDLE PONS
MEDULLA
S1
FOREARM
COLD 5 MINS.
WARM 5 MINS.
S1
S2
50 MSEC.
S2
cryoprobes

Figure 47.

The coolant flow rate (one ml/sec) used in this experiment was never exceeded in all subsequent cooling experiments.

Cooling at the midpontine level produced a significant drop in blood pressure when the brainstem was intact. This temporary effect on blood pressure was eliminated by a post-trigeminal section (see Figure 48).

Effect of Pontine Cooling on Cortical Surface Potentials

Four cats (X58, X59, X60, X61) were prepared with PoT transections and cryoprobes inserted at the midpontine level (see Figure 49 for horizontal tissue sections). Only extracellular action potentials and slow surface potentials referred to killed muscle (EEG derivation) were recorded in these four experiments; no transcortical potentials (ECoG) were recorded.

Prior to cooling, all preparations exhibited predominantly activated EEG patterns characteristic of PoT preparations, but synchronized episodes were evident (see Figure 50, PRE-COOL column). It was consistently found that the initial effect of coolant flow through the cryoprobes was an excitatory influence lasting for about the first 30 seconds of the coolant flow. This excitatory influence was seen in the evoked potential experiments just reported as a temporary increase in the size of the evoked potential. As will be seen later, this transient reticular excitation desynchronized surface potentials and occasionally

Figure 48. The effects of midpontine cooling on femoral artery blood pressure before and after post-trigeminal transection of the brainstem.

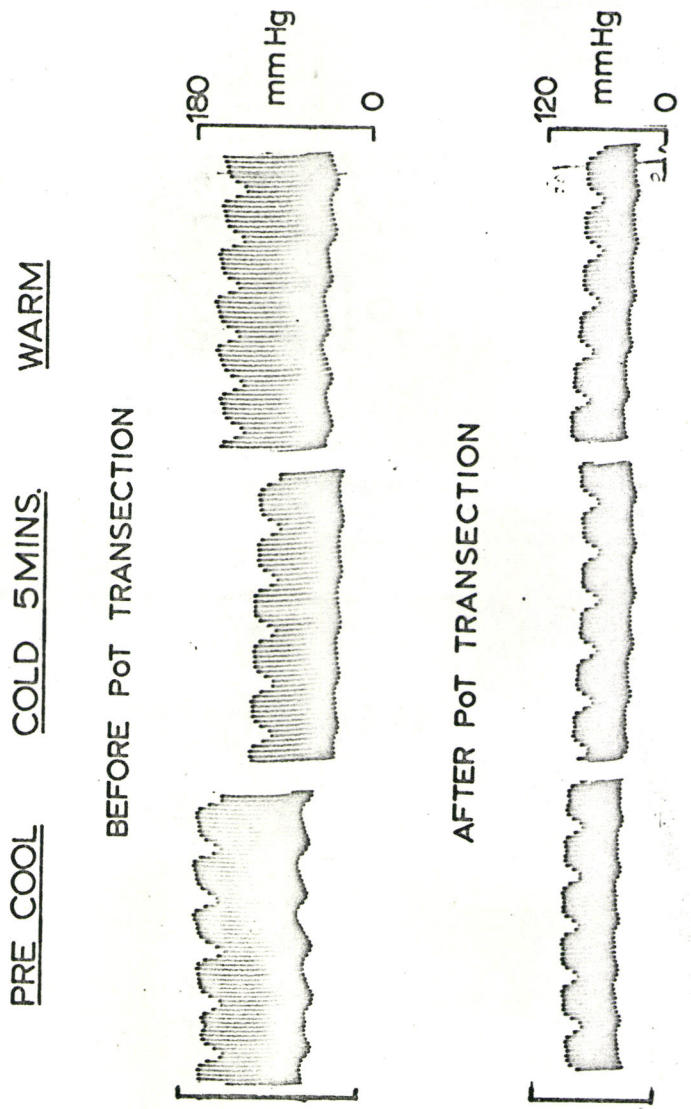

PRE COOL
COLD 5MINS.
WARM
BEFORE PoT TRANSECTION
180
mm Hg
0
AFTER PoT TRANSECTION
120
mmHg
0

Figure 48.

Figure 49. Horizontal sections (100 u) of the brainstem in post-trigeminal preparations showing the positions of the cooling probes (white circles).

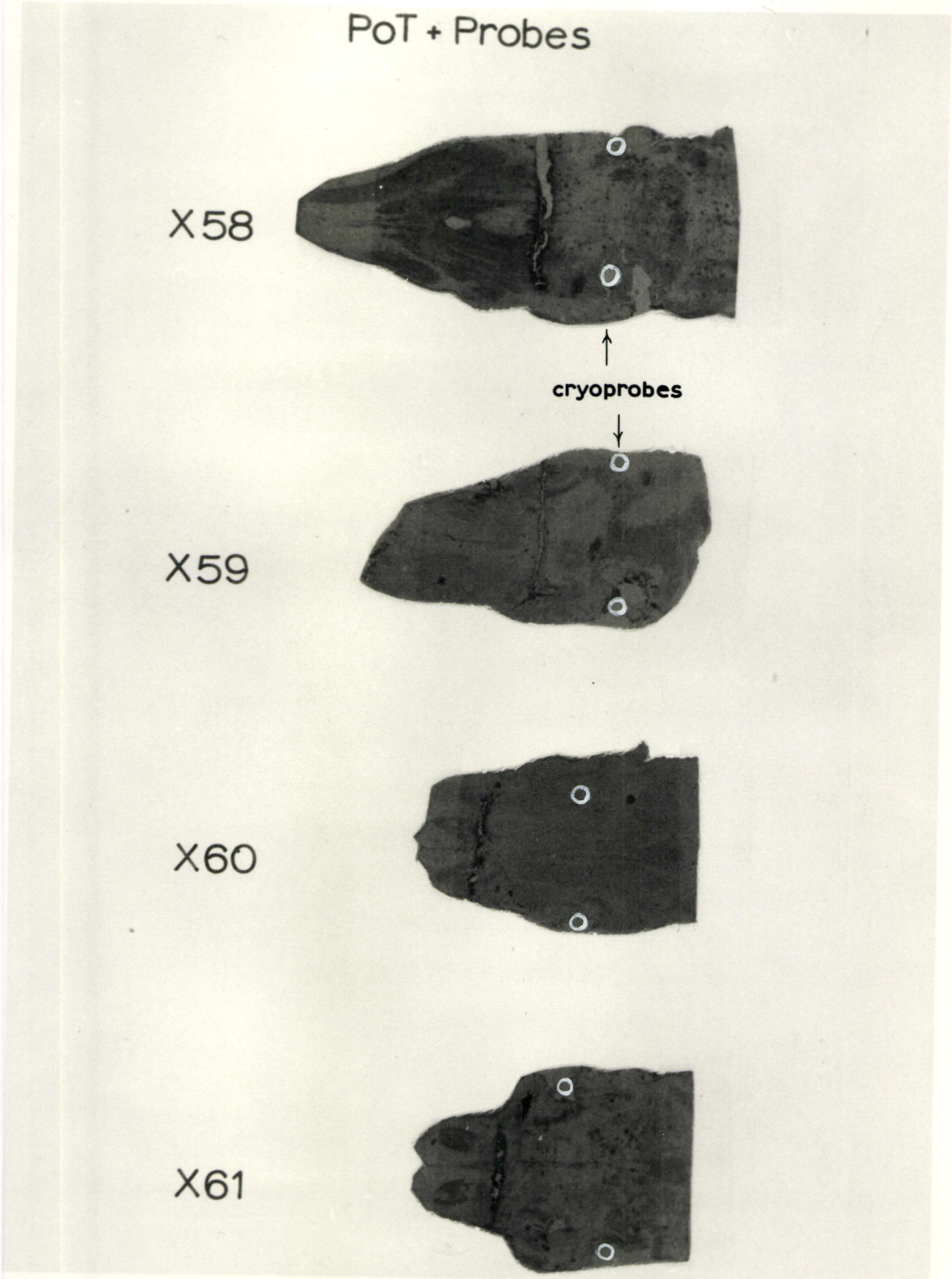


Figure 49.

Figure 50. Effects of midpontine cooling on cortical EEG. EEG tracings were recorded with 3 db attenuation at 0.1 and 100 Hz. For power spectral estimates, these signals were additionally filtered for 3 db attenuation at 1.6 and 35 Hz. The ordinate is arbitrary and in each histogram the amplitude of individual points is proportional to the square of the voltage at that frequency. The calibration is 200 uV and refers to the waveform tracings, surface negative up.


PRE-COOL
EARLY COLD
(3-4 MINS)
LATE COLD (6-7 MINS)
WARM
X58
EEG
X59
EEG
X60
EEG
X61
EEG
1SEC
0
5
10
15
20
25
FREQUENCY (Hz)

Figure 50.

accelerated unit firing. With continued cooling, the first significant change in the spontaneous EEG was an increase in low frequencies (0 - 2.5 Hz) as compared to the rest of the spectrum (see Figure 50, EARLY COLD column). The power spectral estimates show that EEG frequencies above 12 Hz were virtually abolished in all animals except X61 where there were some high frequencies as well as a significant spindle component (11 - 12 Hz).

Toward the end of the cooling period, high frequency components were abolished in all animals, and high amplitude spindles (11 - 12 Hz) became a more prominent feature of the EEG (see Figure 50, LATE COLD column). Five to ten minutes after the coolant flow was stopped, the cold-induced spindles had nearly disappeared but the EEG pattern did not recover spontaneous patterns similar to those observed prior to cooling. However, recovery of relative amplitudes of frequency components occurred to some extent in X61. In all others, synchronized or slow waves (1 - 3 Hz) predominated and the high frequency components seen prior to cooling were less evident (see Figure 50, WARM column). These synchronized patterns are reminiscent of the effect of "gasserectomies" on the cortical EEG. Since the cryoprobes were situated among the fiber bundles of the trigeminal nerves as they enter the brainstem, it is possible that their proximity to the cryoprobes, where temperatures would be the lowest, resulted in irreversible damage. Hence,

after the first cooling the preparation may have been equivalent to a "gasserectomized" PoT (PoT + V) preparation.

Activity of Cortical Neurons During Cryogenic Pontine Blockade

In the experiments just mentioned (X58, X59, X60 and X61), samples were taken of the amount of spontaneous and injury discharges of neurons in the suprasylvian cortex from which the EEG recordings were taken. Using the method of constant penetration, in which the microelectrode is moved through the cortex from an arbitrary point beneath the pia at a fixed rate of five microns per second, extracellular action potentials were recorded before (PoT preparation) during (equivalent to a pretrigeminal preparation) and after (PoT again) cooling. In all four animals and with repeated cooling episodes in each, it was shown that the activity of suprasylvian neurons was reduced by midpontine cooling and that the activity returned after warming despite the fact that synchronized EEG patterns induced by the cooling procedure persisted. These results again indicate pontine reticular control of suprasylvian neuron activity. Also, they confirm the dissociation between this activity and the spontaneous patterns of the cortical EEG.

Figures 51 through 55 demonstrate reversible effects of cooling on the density of neuron discharges in successive penetrations through suprasylvian cortex. Prior to cooling

Figures 51 through 67. Neuron activity (second channel) sampled by continuous penetration of the microelectrode at a fixed rate of 5 microns per second. The coincident surface EEG is recorded in channel one. Numbers on the scale refer to the depth, in microns, of the microelectrode tip from an arbitrary zero point under the pia at the end of each recording strip. The calibration is 200 uV for all figures and refers to the EEG, surface negative up. Spike amplitudes are always arbitrary.

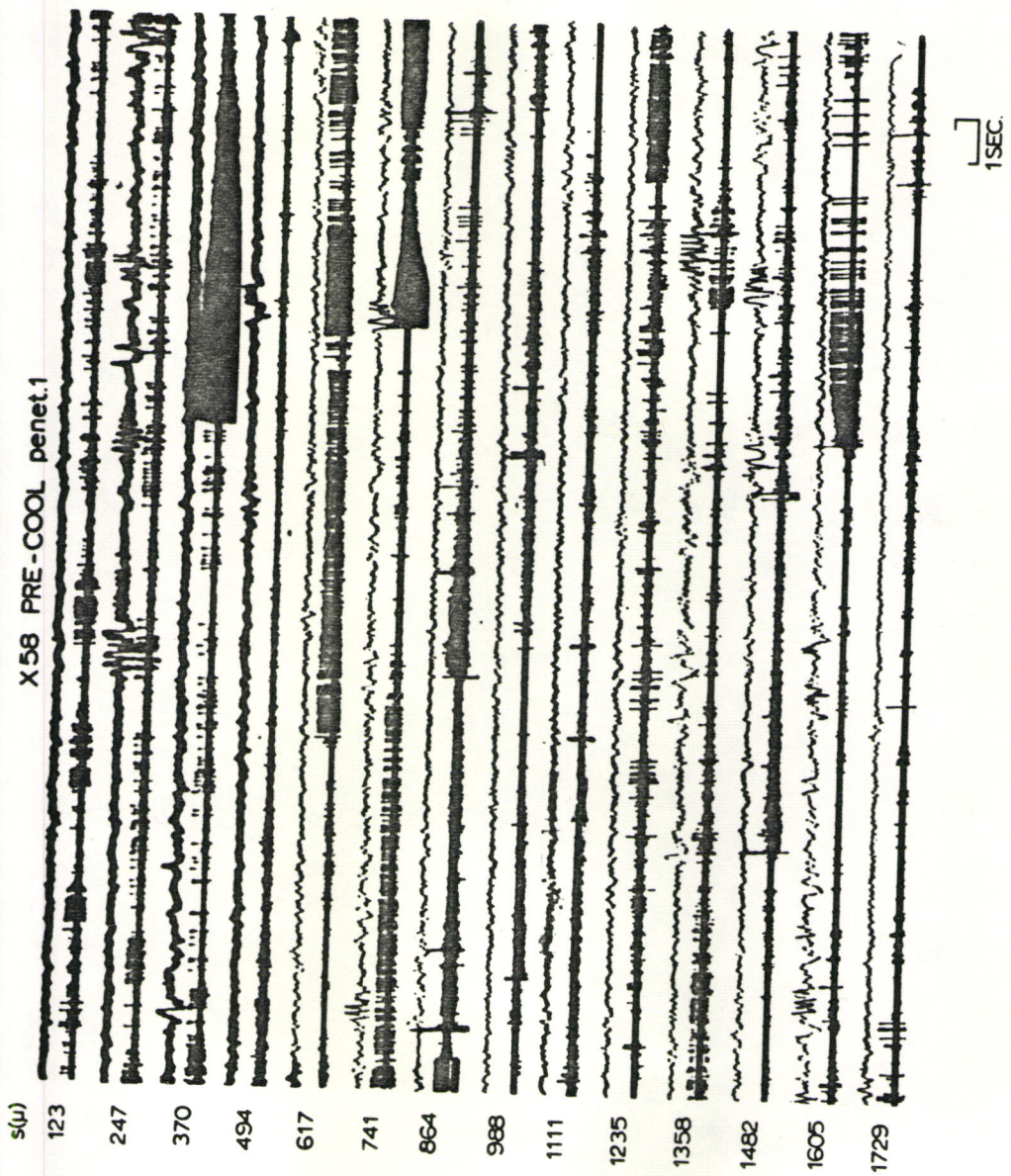

X58 PRE-COOL penet.1
s(μ)
123
247
370
494
617
741
864
988
1111
1235
1358
1482
1605
1729
1SEC.

Figure 51.

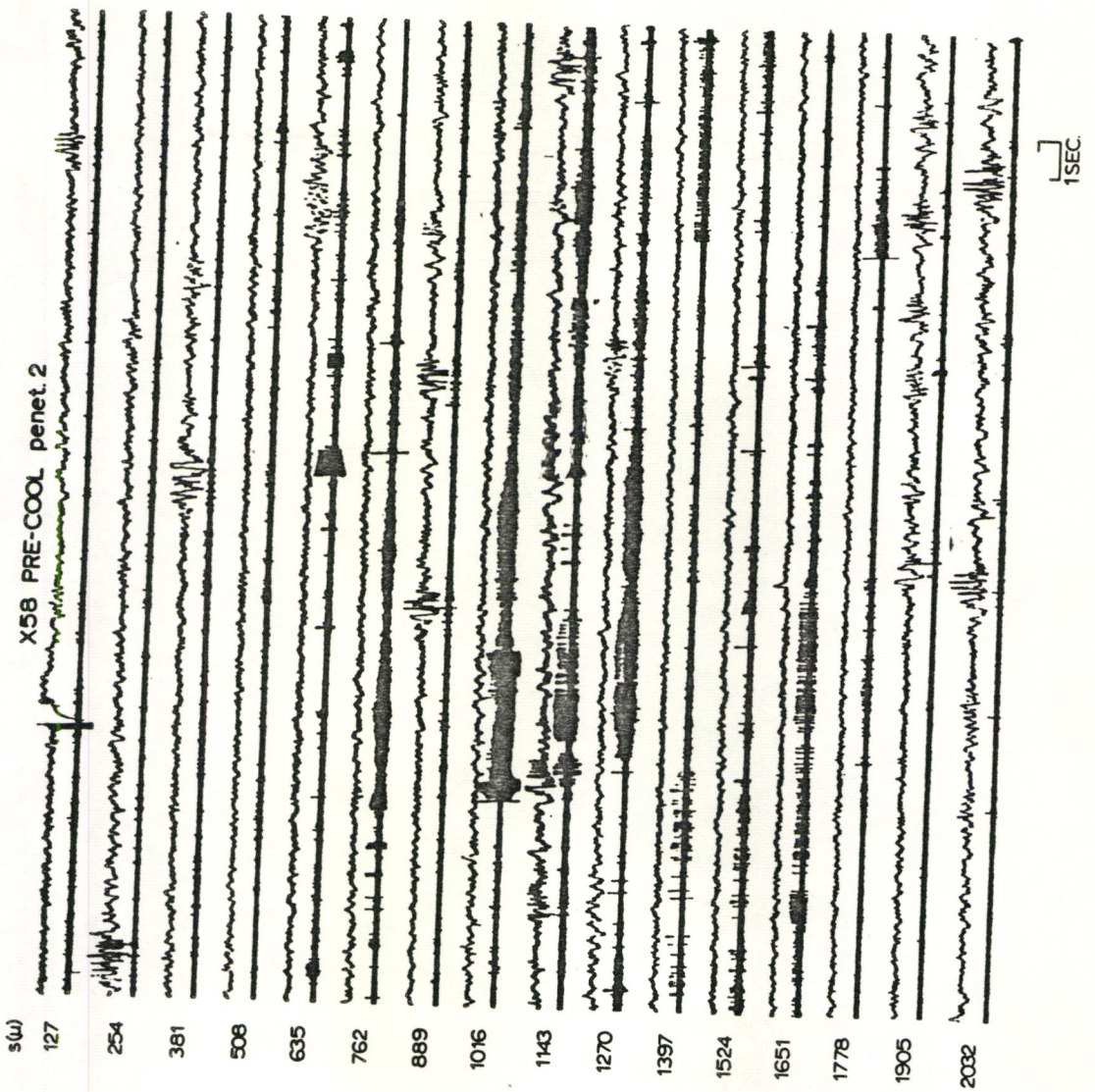

X58 PRE-COOL penet. 2
s(u)
127
254
381
508
635
762
889
1016
1143
1270
1397
1524
1651
1778
1905
2032
1SEC.

Figure 52.

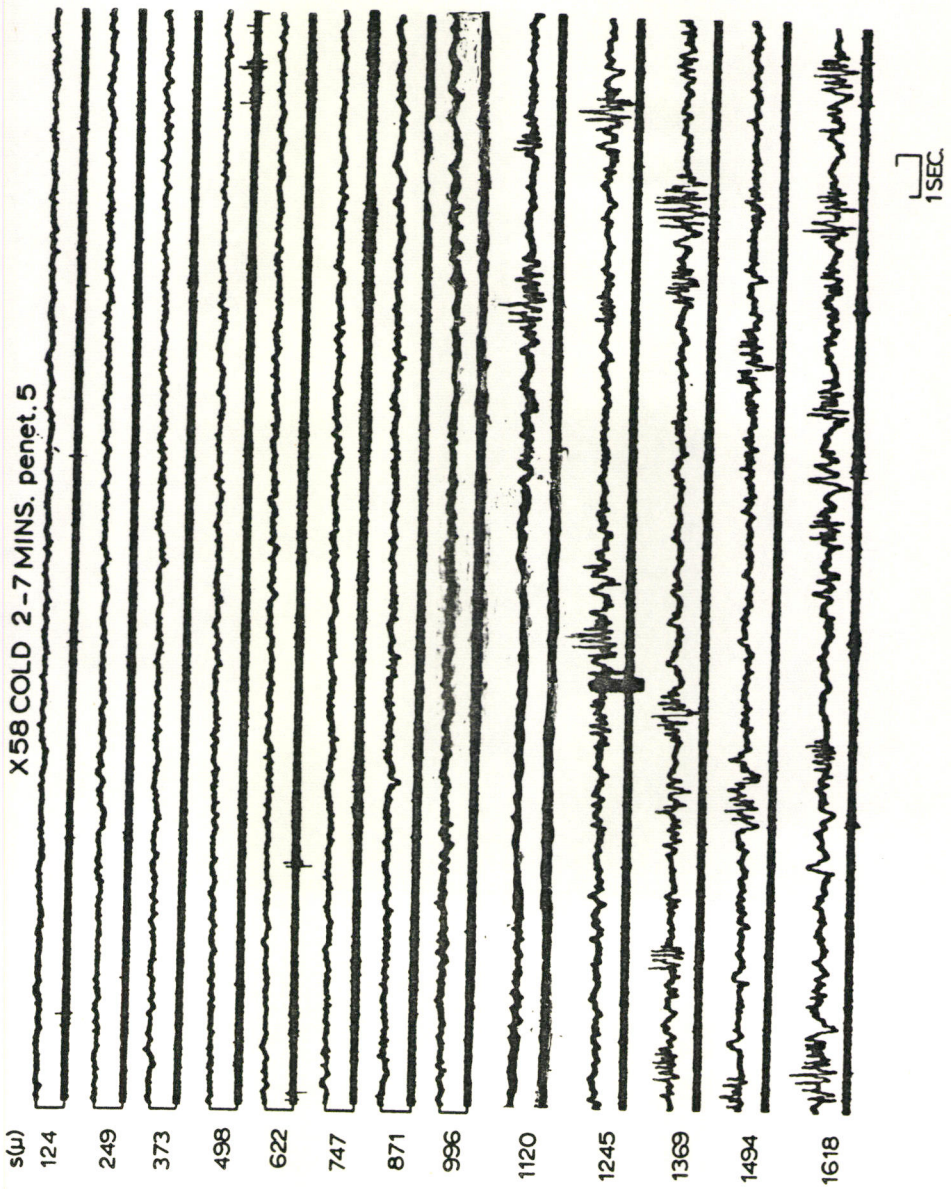

X58 COLD 2-7 MINS. penet.5
s(u)
124
249
373
498
622
747
871
996
1120
1245
1369
1494
1618
1SEC.

Figure 53.

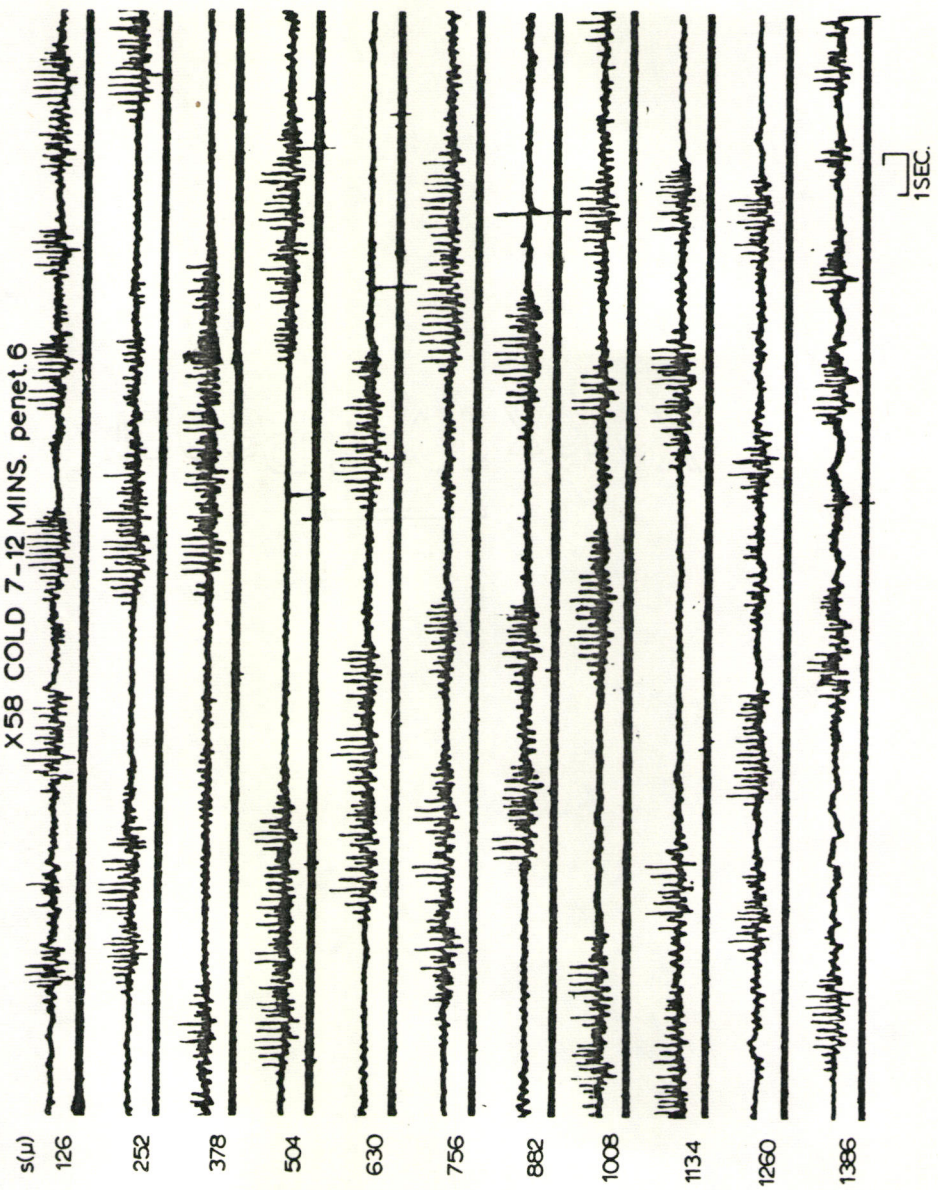

X58 COLD 7-12 MINS. penet.6
s(μ)
126
252
378
504
630
756
882
1008
1134
1260
1386
1SEC.

Figure 54.

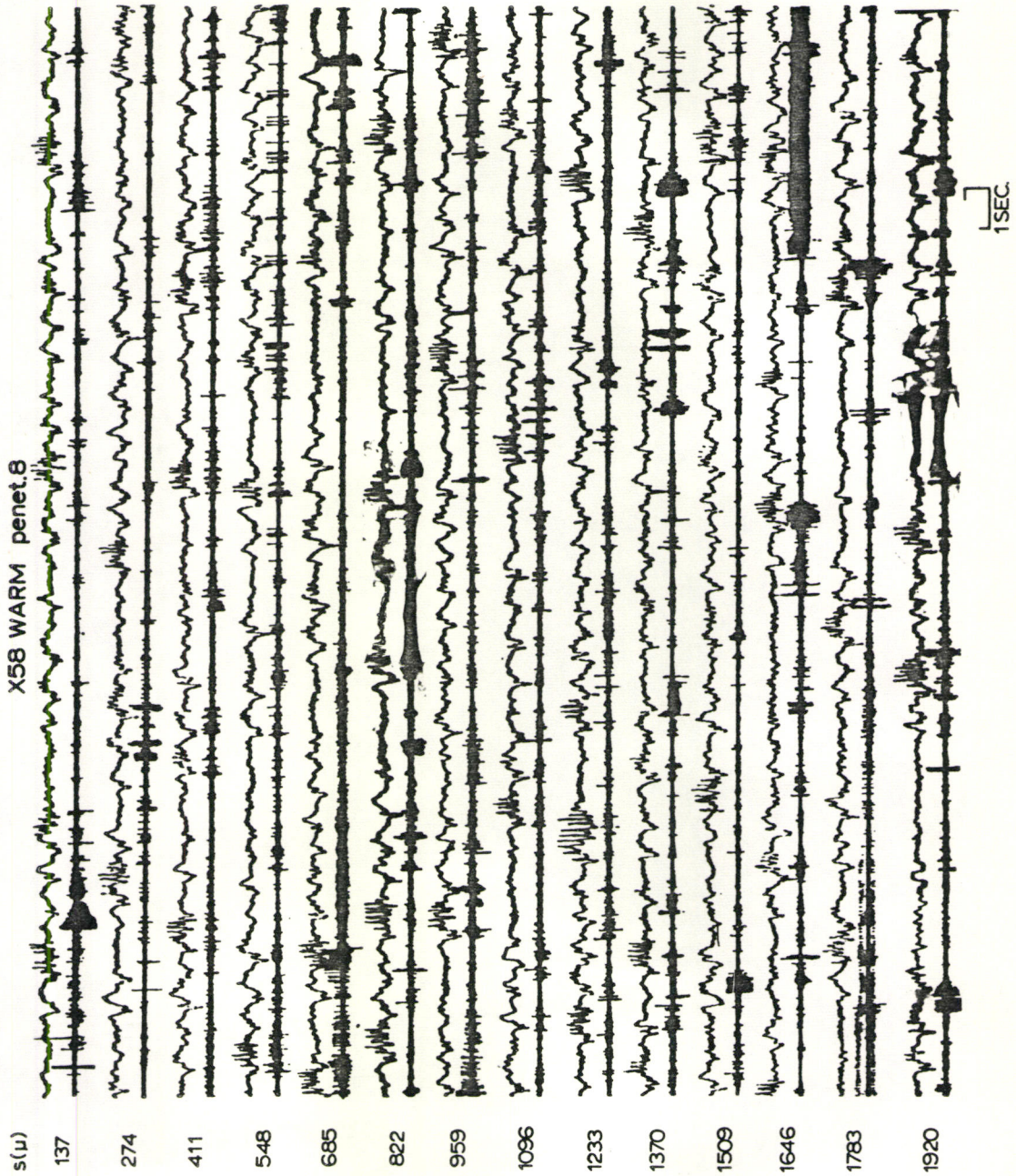

X58 WARM penet.8
s(μ)
137
274
411
548
685
822
959
1096
1233
1370
1509
1646
1783
1920
1SEC.

Figure 55.

(Figures 51 and 52), the neuron population sampled exhibited high activity. After two minutes of cooling (Figure 53) and later when surface-negative spindles had developed (Figure 54), the activity of neurons in both of the samples was greatly reduced. Fifteen minutes after the coolant flow was stopped, however, the activity of suprasylvian neurons had returned to a high level, despite persistent EEG synchrony (Figure 55).

In these particular samples, it may be noted that the number of discharges recorded after the brainstem had warmed was perhaps a little less than the number recorded prior to cooling. In addition, the fact that the EEG patterns remained somewhat altered suggested that the cooling partially damaged parts of the mid-pons. That may have been true; however, it was possible to demonstrate reversible effects of cooling on neuron activity more than once in each preparation without a necessary decrease in activity after the first cooling episode. For example, Figures 56 through 60 demonstrate two cooling episodes and the diminution and recovery of neuron activity accompanying the cooling (Figures 57 and 59) and warming (Figures 58 and 60) respectively of the pons of cat X60. Note that following the second cooling (Figure 60 WARM) neuron population activity remained as high as that following the first cooling (Figure 58); although synchrony in the EEG was more prominent than it was following the first cold block.

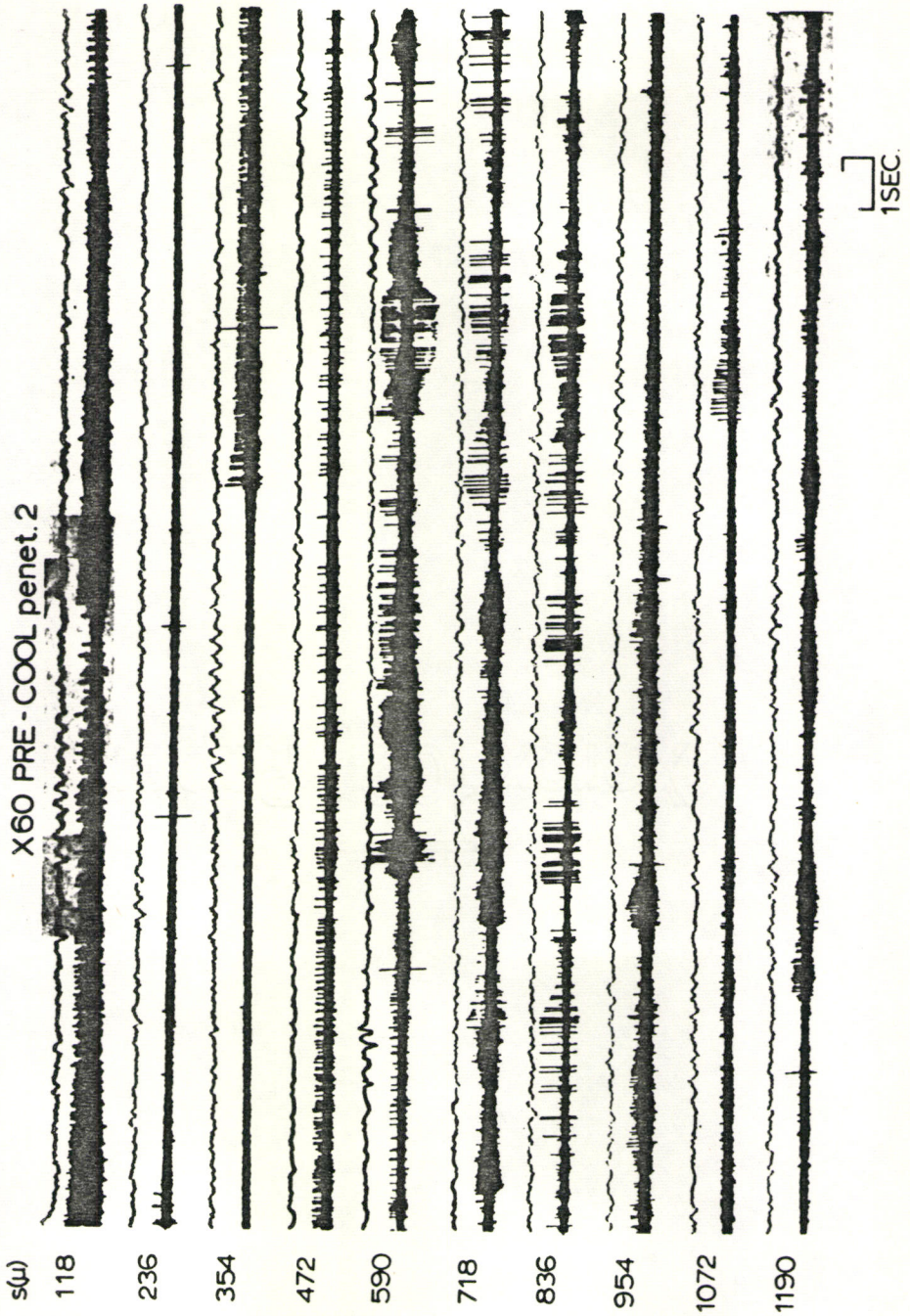

X60 PRE-COOL penet.2
s(μ)
118
236
354
472
590
718
836
954
1072
1190
1SEC

Figure 56.

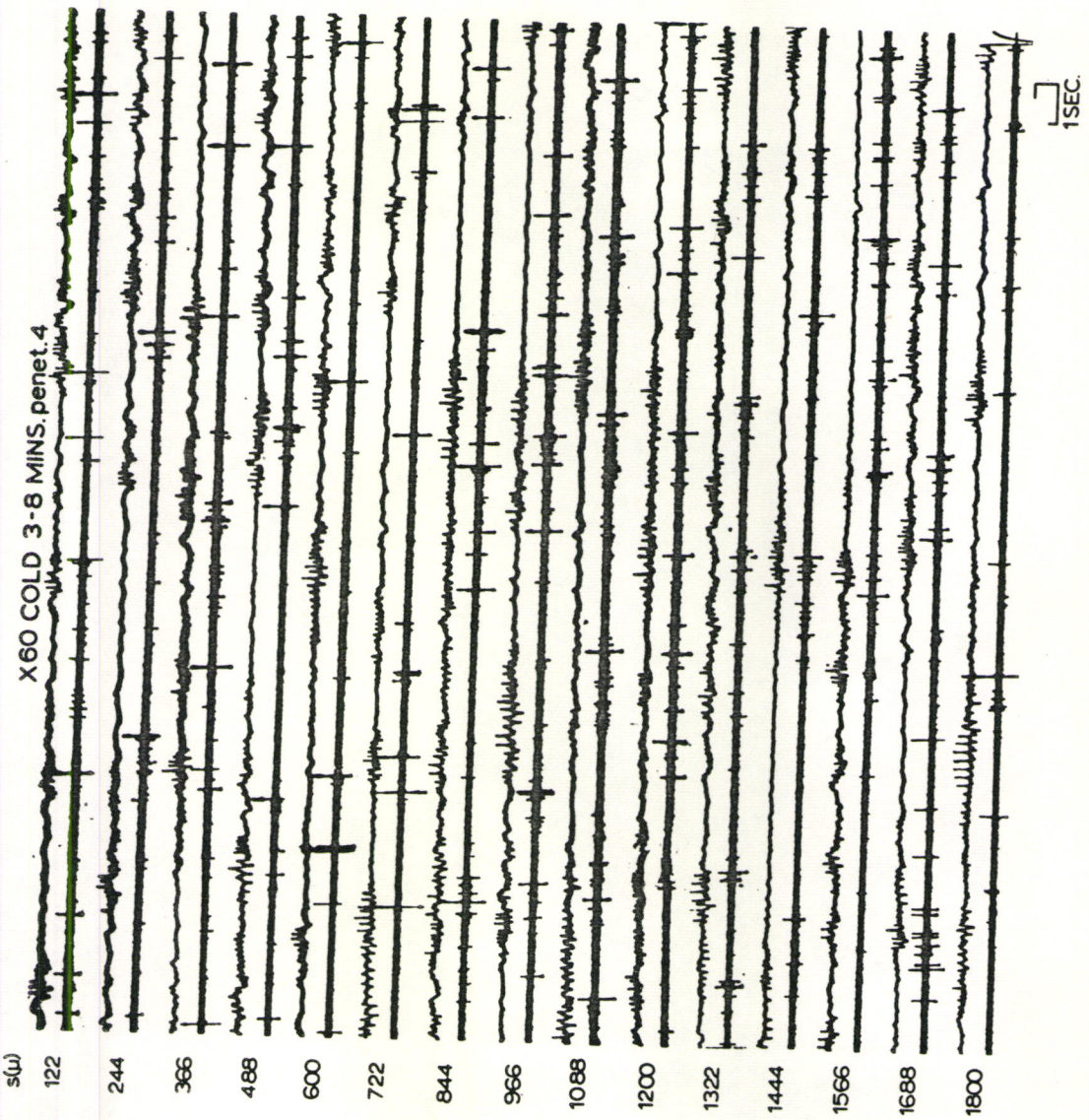

X60 COLD 3-8 MINS. penet.4
s(ω)
122
244
366
488
600
722
844
966
1088
1200
1322
1444
1566
1688
1800
1SEC.

Figure 57.

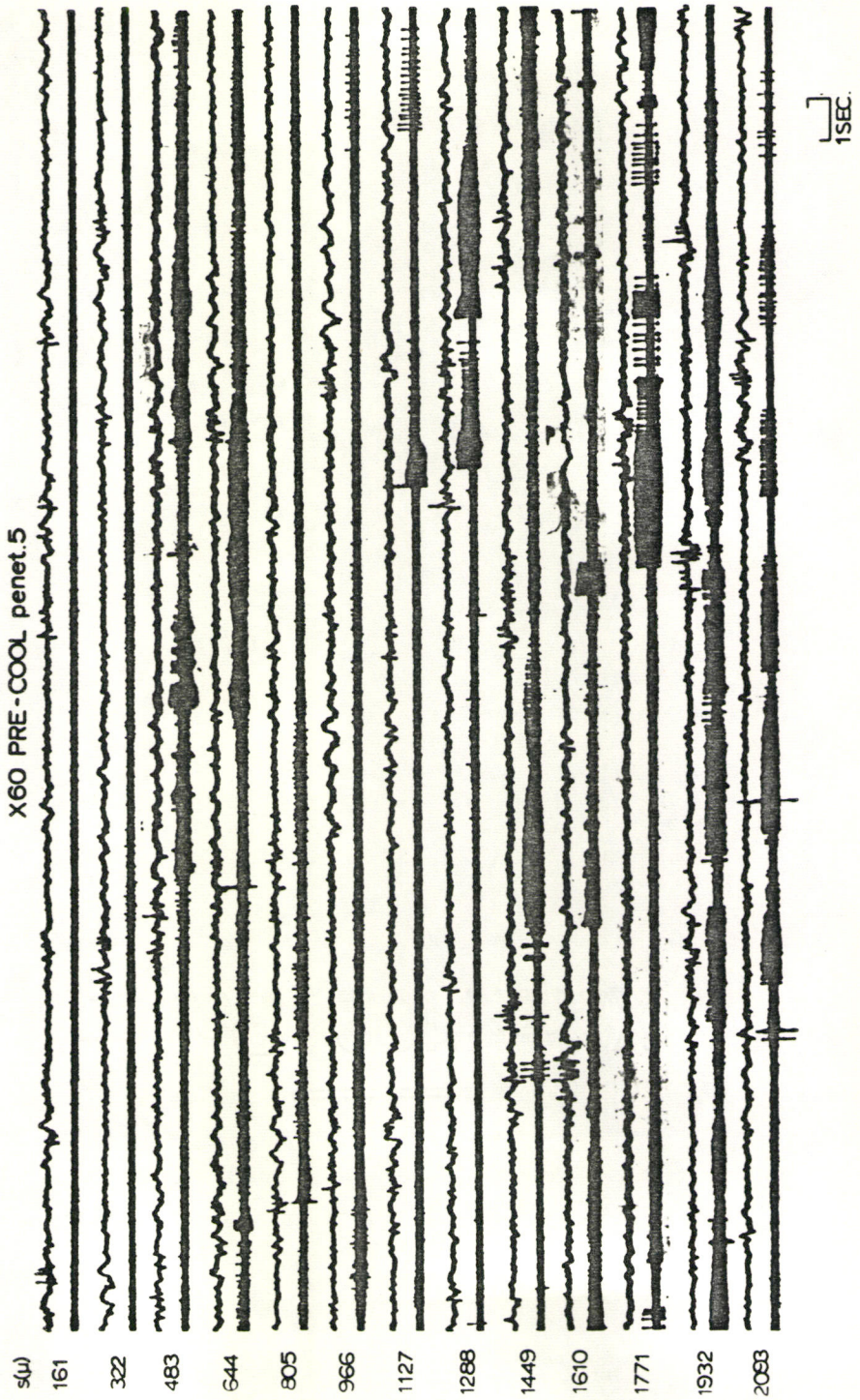

X60 PRE-COOL penet.5
s(μ)
161
322
483
644
805
966
1127
1288
1449
1610
1771
1932
2083
1SEC.

Figure 58.

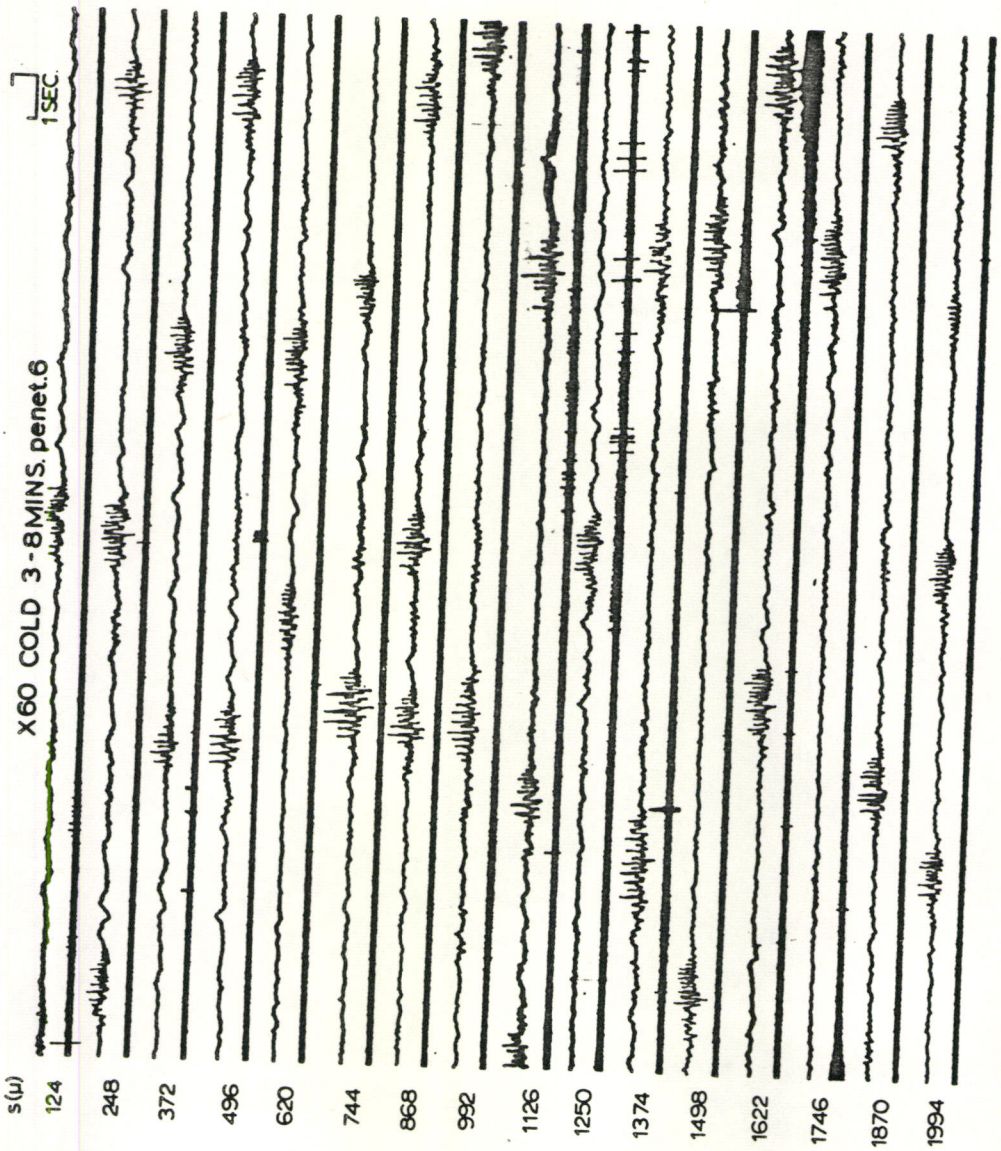

X60 COLD 3-8MINS. penet.6
1SEC.
s(μ)
124
248
372
496
620
744
868
992
1126
1250
1374
1498
1622
1746
1870
1994

Figure 59.

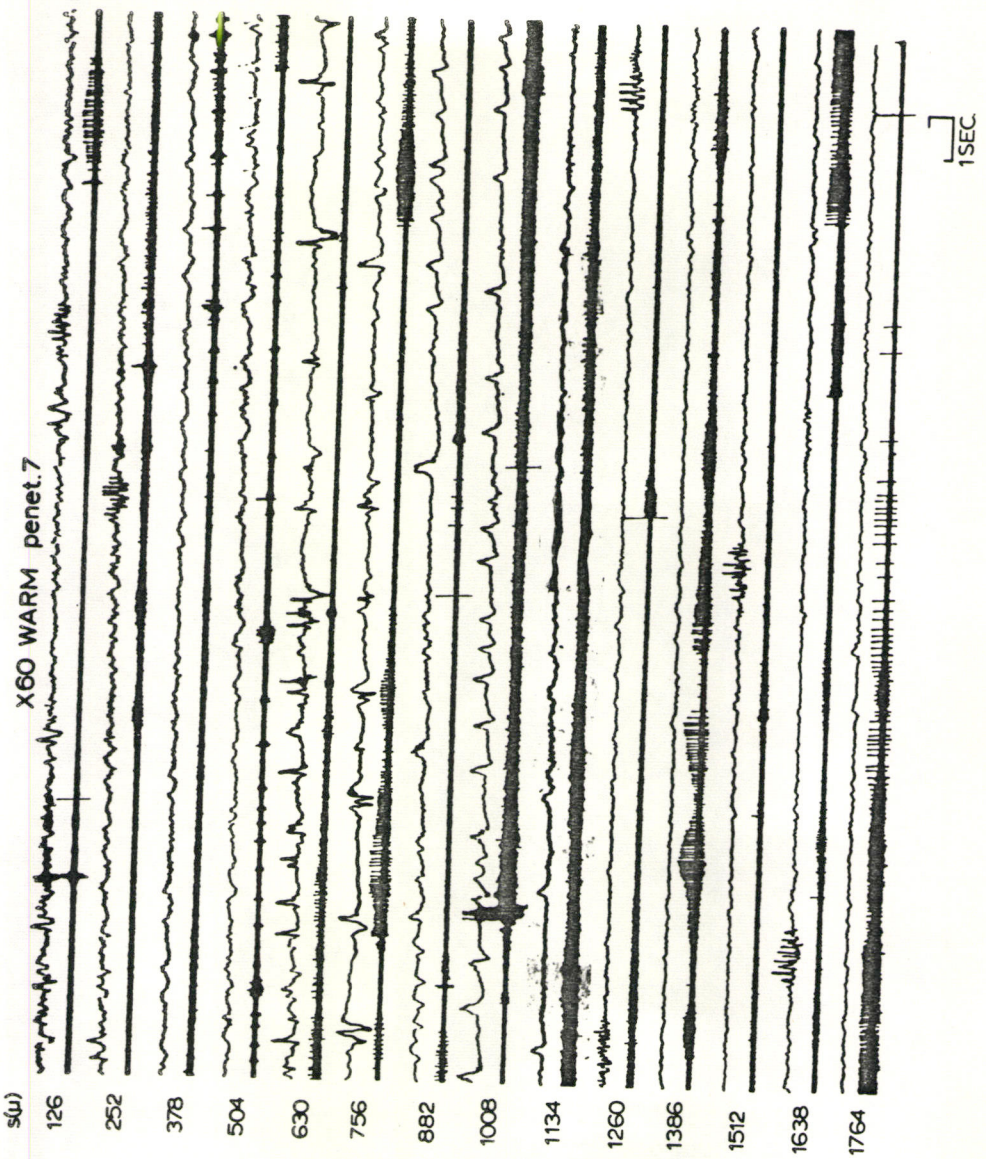

X60 WARM penet.7
s(ω)
126
252
378
504
630
756
882
1008
1134
1260
1386
1512
1638
1764
1SEC

Figure 60.

Figures 61 through 64 show a second cooling episode in cat X61, where both EEG pattern and suprasylvian neuron activity are altered by cryogenic pontine blockade and recover after warming to roughly the same activity patterns seen prior to cooling. Figures 65 through 67 show another example, from the first cooling of cat X59.

Activity Patterns of Single Cortical Neurons During Cryogenic Pontine Blockade

Since it was shown in Experiment I and Experiment II that a pontine reticular influence maintained the number of spontaneously active suprasylvian neurons, it was thought that withdrawal of that influence by cryogenic pontine blockade would inhibit the on-going spontaneous discharge of a high percentage of individual suprasylvian cells and that this spontaneous activity would resume when pontine conduction recovered. In two experiments (X52 and X54), seven neurons were recorded from paralyzed, locally-anesthetized cats without brainstem transection. In every case, during mid-pontine cooling the spontaneous activity of the neuron under observation was diminished gradually until no spikes were observed. After an arbitrarily-determined length of time, depending upon the rate of firing which was observed prior to cooling, cooling was discontinued. However, spontaneous activity never returned in any of the cells. For as long as fifteen minutes after the coolant

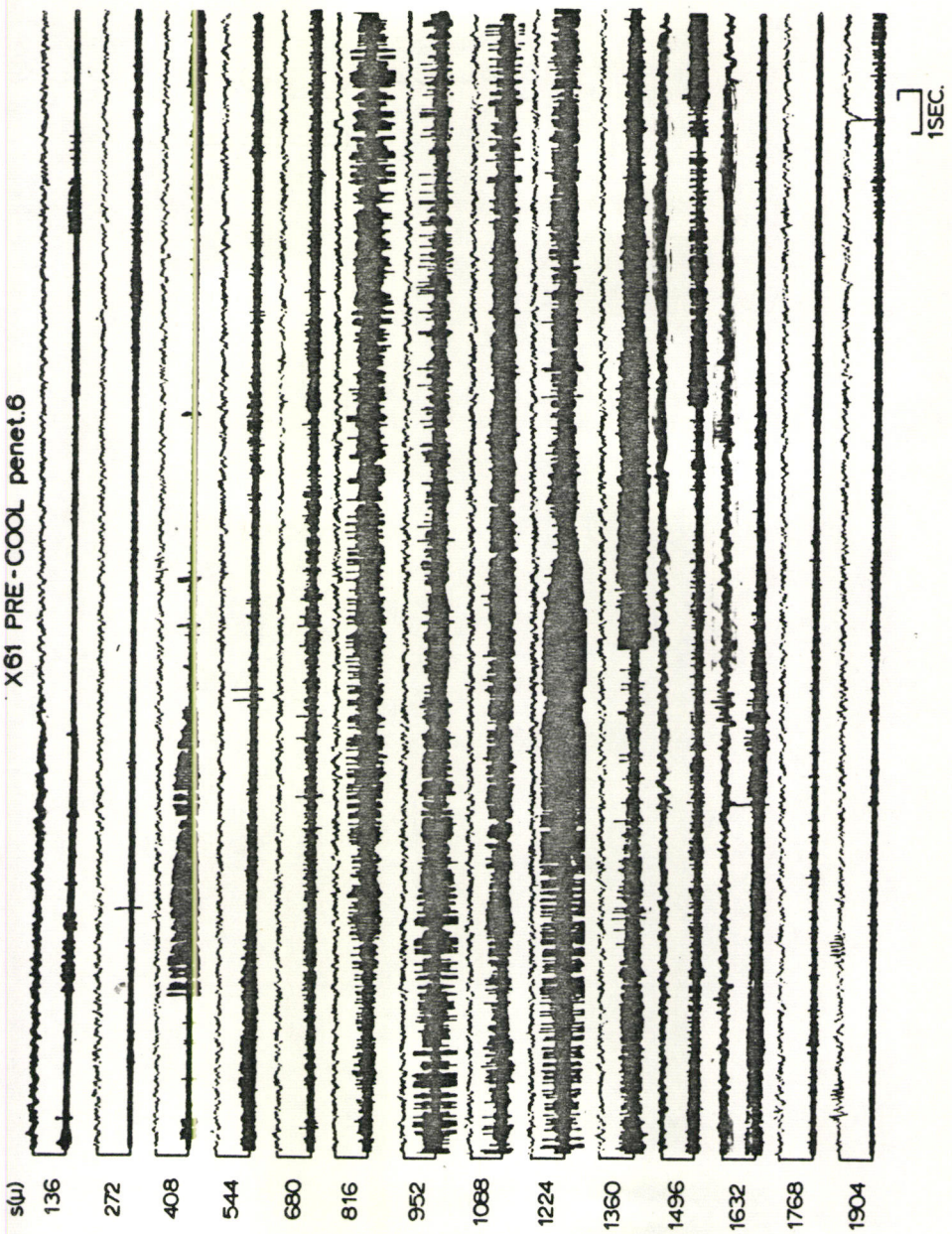

X61 PRE-COOL penet.6
s(μ)
136
272
408
544
680
816
952
1088
1224
1360
1496
1632
1768
1904
1SEC.

Figure 61.

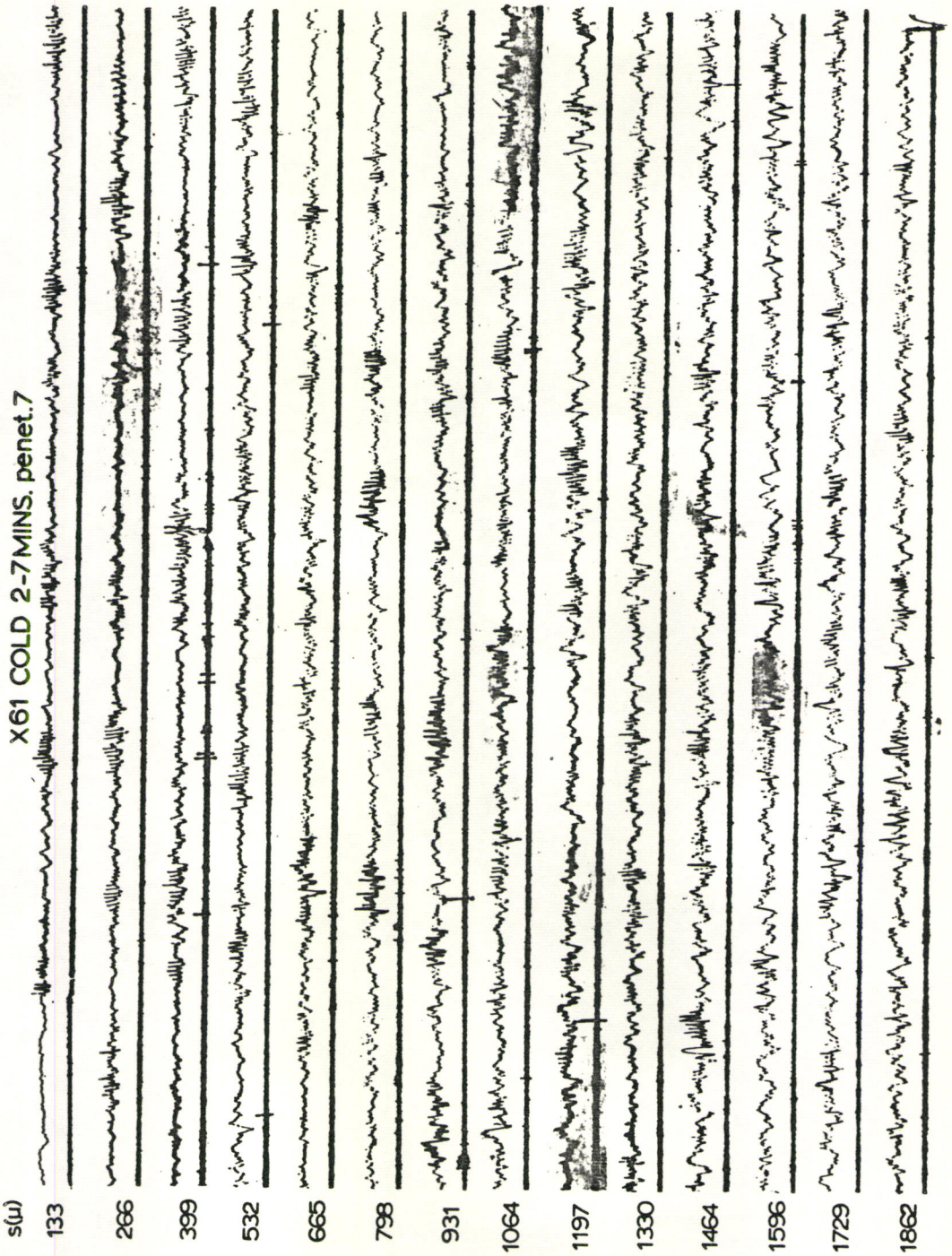

X61 COLD 2-7MINS. penet.7
s(u)
133
266
399
532
665
798
931
1064
1197
1330
1464
1596
1729
1862
1SEC.

Figure 62.

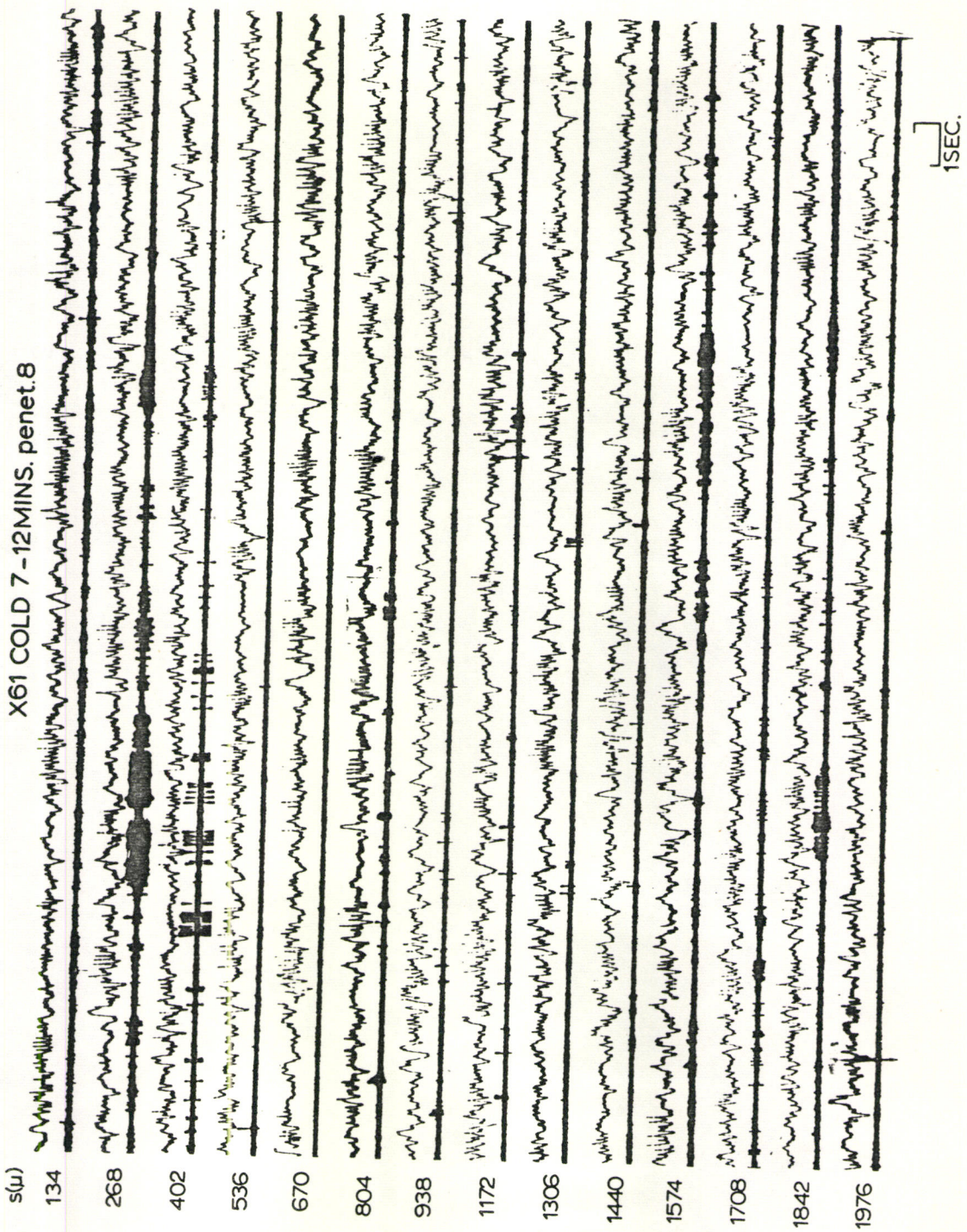

X61 COLD 7-12MINS. penet.8
s(μ)
134
268
402
536
670
804
938
1172
1306
1440
1574
1708
1842
1976
1SEC.

Figure 63.

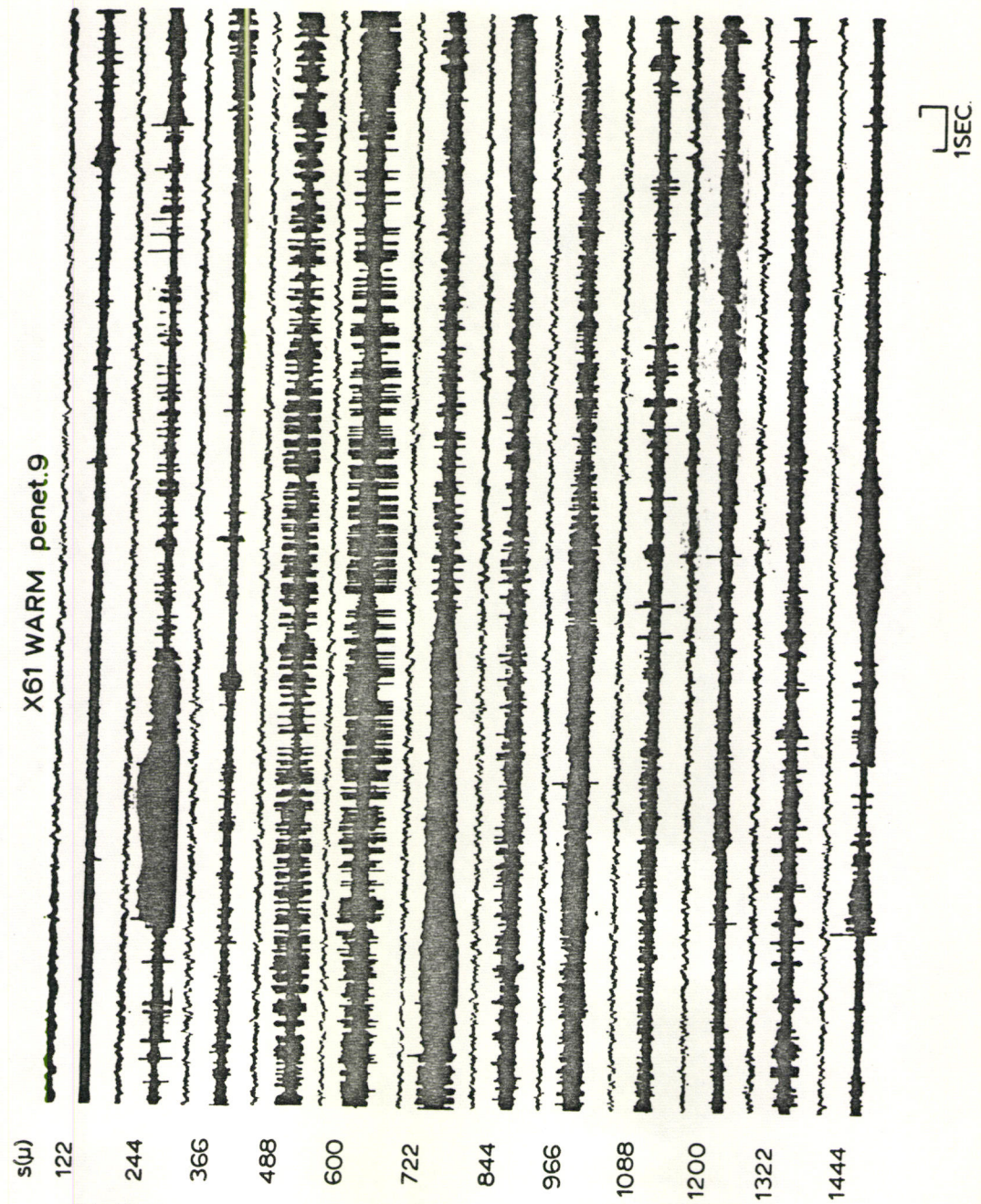

X61 WARM penet.9
s(μ)
122
244
366
488
600
722
844
966
1088
1200
1322
1444
1SEC

Figure 64.

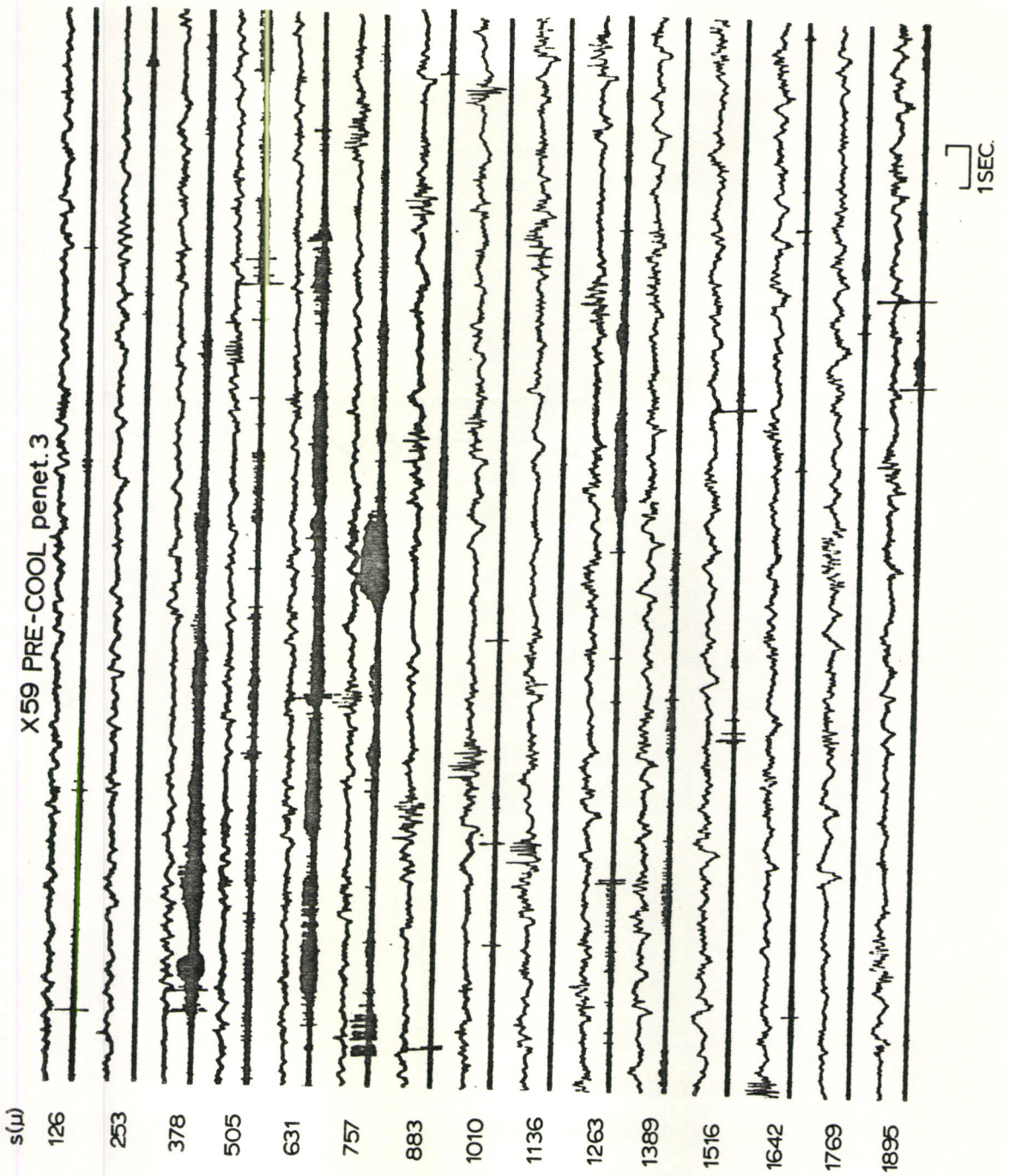

X59 PRE-COOL penet.3
s(μ)
126
253
378
505
631
757
883
1010
1136
1263
1389
1516
1642
1769
1895
1SEC.

Figure 65.

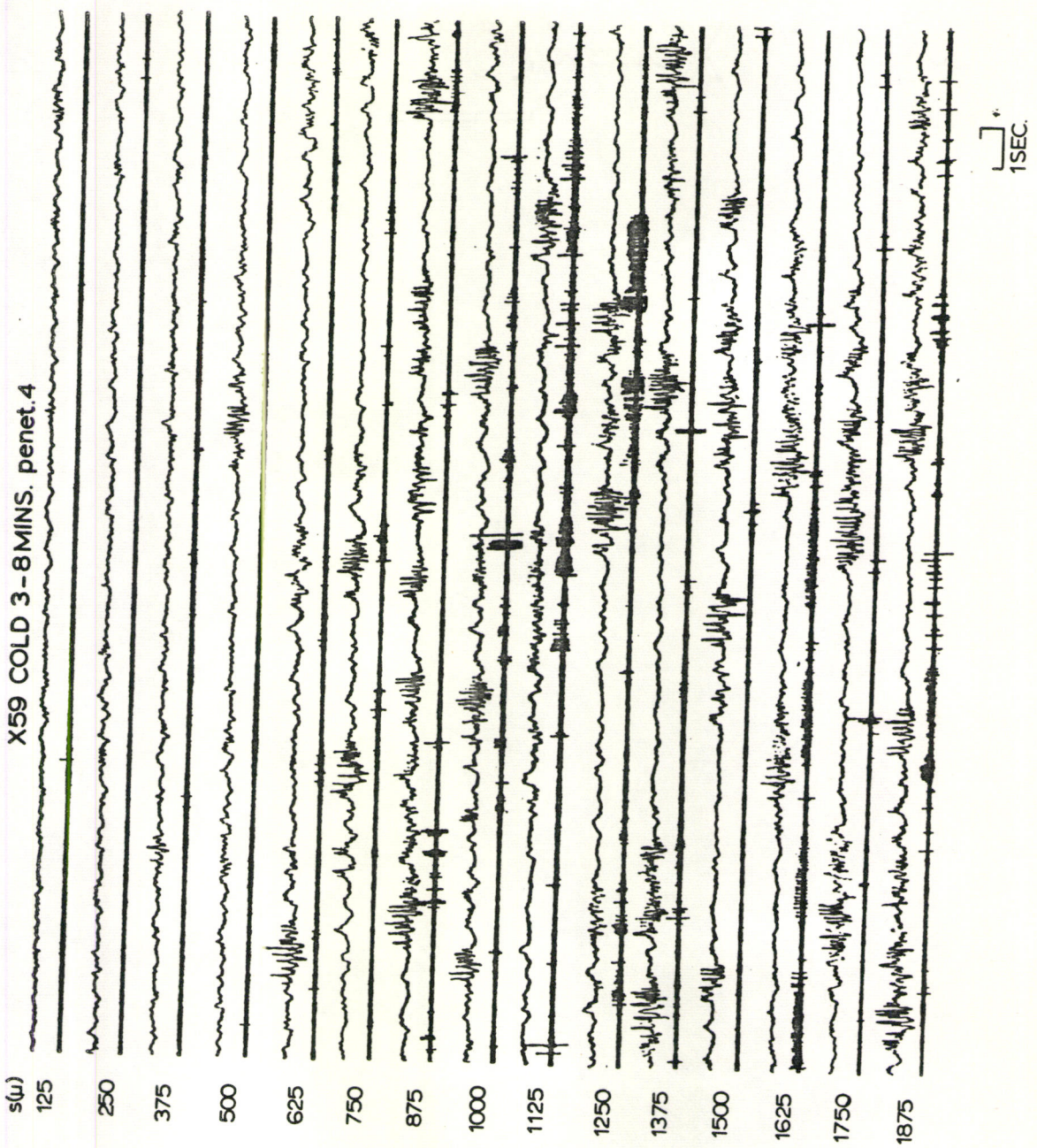

X59 COLD 3-8MINS. penet.4
s(ω)
125
250
375
500
625
750
875
1000
1125
1250
1375
1500
1625
1750
1875
1SEC.

Figure 66.

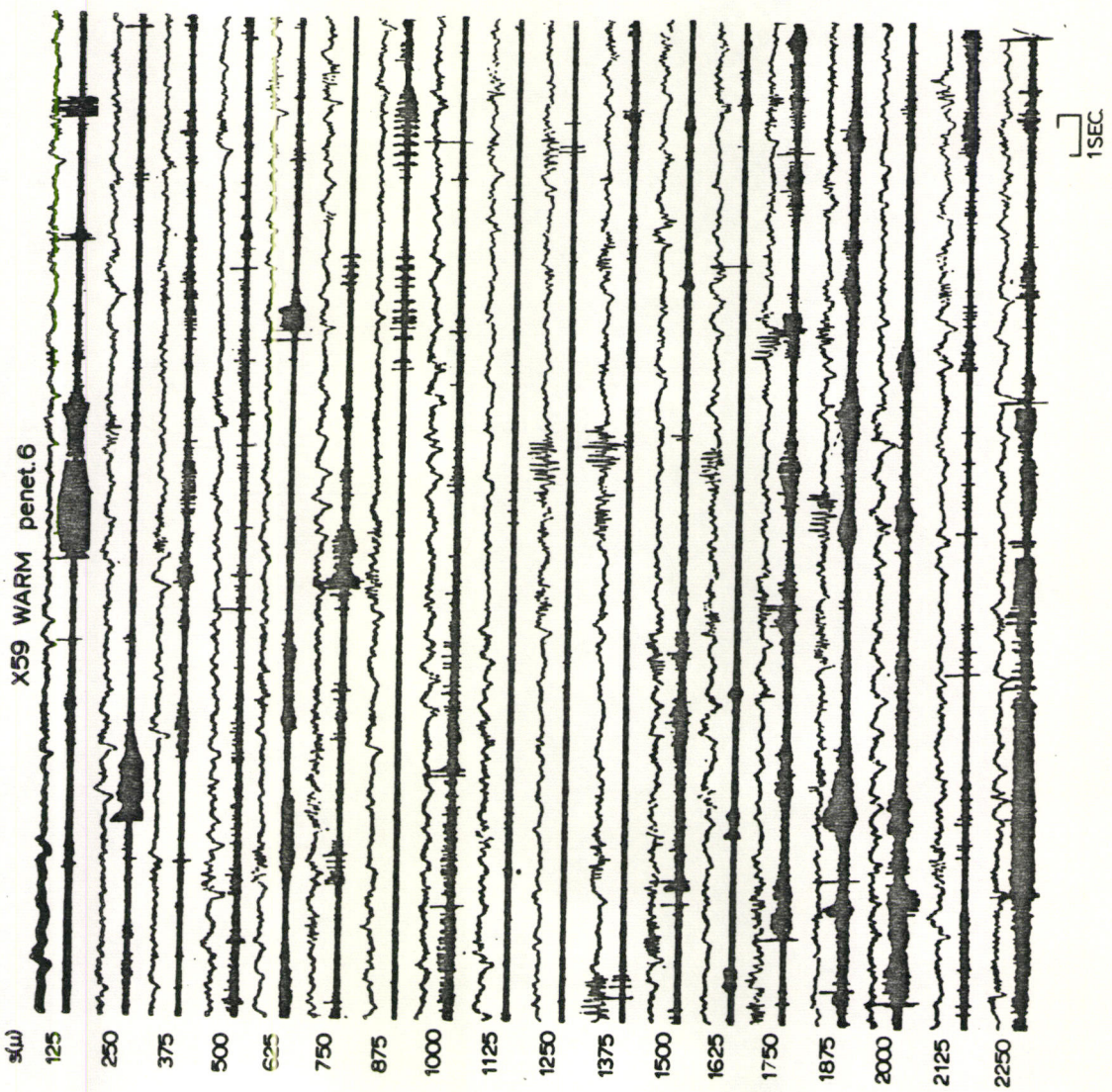

X59 WARM penet.6
s(ω)
125
250
375
500
625
750
875
1000
1125
1250
1375
1500
1625
1750
1875
2000
2125
2250
1SEC

Figure 67.

flow was stopped, either no spikes could be recorded or the amplitudes or firing patterns which were recorded differed from the characteristics of spontaneous discharge which were recorded prior to the cooling. In most cases the amplitudes of spikes recorded after the pons had warmed were lower than the amplitudes recorded prior to cooling. This suggested that the distance between the microelectrode and the membrane may have increased because of movement of the brain or drift of the micropipette over the long silent period (approximately 10 minutes) accompanying the cooling. In light of the fact that midpontine cooling in preparations with an intact brainstem results in a large drop in arterial blood pressure, it is likely that alterations in cerebral circulatory volume causes a shift in the position of cortical neurons which results in a net displacement of a given membrane from a nearby recording microelectrode. The failure of neurons to resume activity after the brainstem had been warm for as long as fifteen minutes is not likely due to cerebral anoxia consequent with the drop in blood pressure, because movement of the microelectrode at that time always detected spontaneous activity in nearby neurons.

In a subsequent experiment (X56) the spontaneous discharges of two different cells were <u>not</u> abolished by pontine cooling but spike frequencies in both cases increased during the cold-induced, large surface-negative spindles.

Figure 68 shows some of the features mentioned so

Figure 68. Some effects of midpontine cooling on single cortical neuron activity in preparations having an intact brainstem. The calibration refers to the transcortical potential (ECoG), surface positive up. Spike amplitudes are not calibrated; however, different spike amplitudes are consistent from one recording strip to the next.

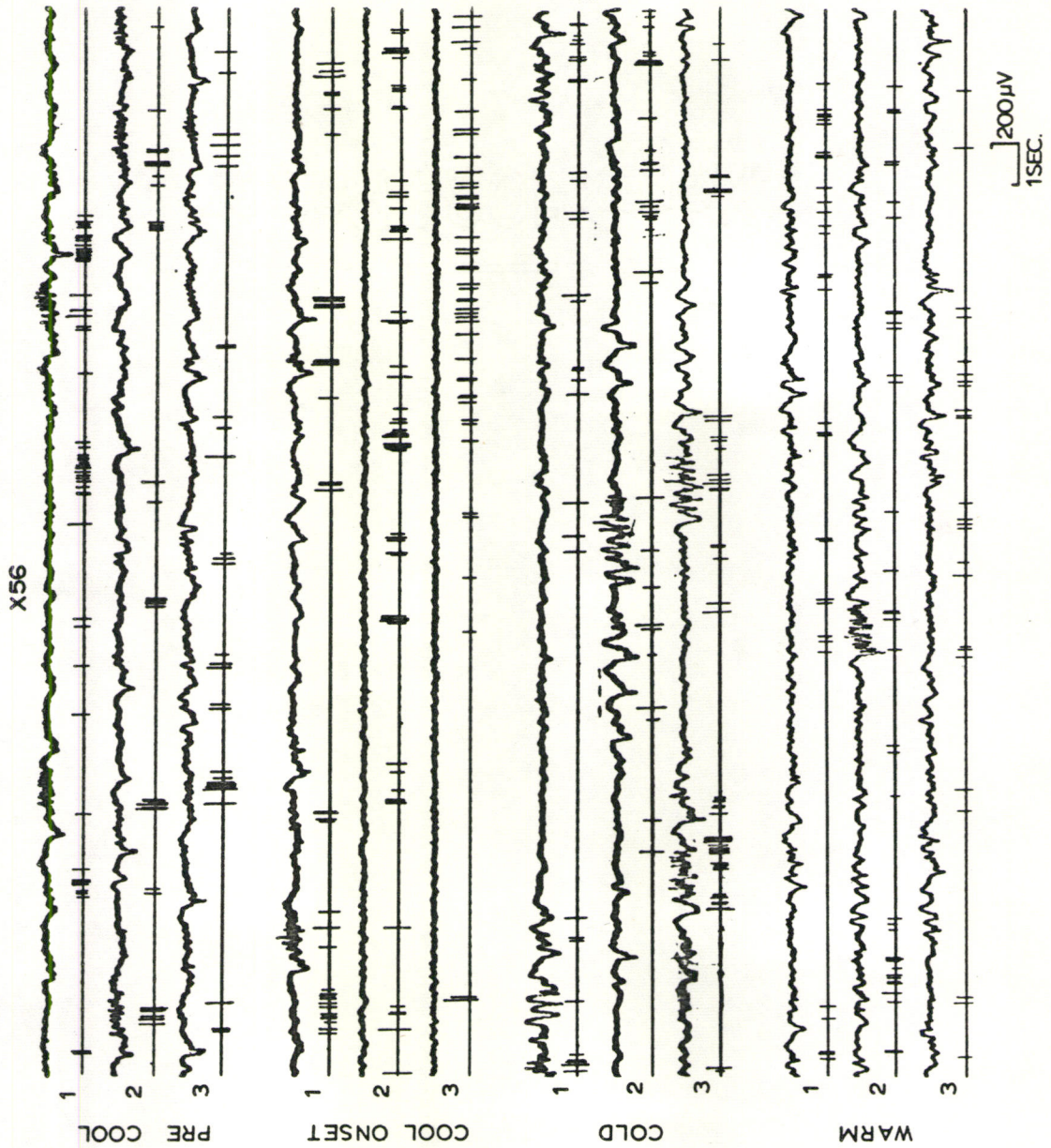


Figure 68.

far. Prior to cooling, two cells of different amplitudes can be seen (Figure 68, PRE-COOL). Initially, cooling activates the ECoG (Figure 68, COOL ONSET) and accelerates unit firing (COOL ONSET, strip 3). When pontine blockade induces large spindles, cells of both amplitudes fire during the spindle complex (COLD, strip 3). After warming, the larger cell appears reduced in size, suggesting again that the position of the micropipette tip relative to the membrane changed concurrent with a change in brainstem temperature.

The results of these last experiments involving manipulation of spontaneous unit discharge by pontine inactivation were inconclusive, and further efforts were discontinued. Although a majority of cells recorded (8 out of 10) stopped firing during pontine blockade, the fact that the pre-cooling firing patterns were not re-established after brainstem warming does not provide an unequivocal demonstration that pontine inactivation inhibited spontaneous unit discharge.

CHAPTER FIVE

DISCUSSION

The preceding experiments were designed to study pontine reticular influences on gross field and unit spike potentials of the cat association cortex. The pattern of slow surface potentials was altered by complete transections at different levels of the pons or by reversible mid-pontine cooling. Samples of extracellular action potentials in the underlying cortex under these conditions revealed that the number of spontaneously active units encountered following mid-pontine pretrigeminal lesion or cooling was reduced as compared to that following post-trigeminal section. In addition, discharges of the membrane due to microelectrode contact were less prevalent following pretrigeminal lesion. Bilateral "gasserectomies" did not reduce the unit activity in post-trigeminals; although they did change the EEG pattern from desynchronized to synchronized.

These results led to the conclusion that a <u>non-specific</u> neuronal system located between these levels of transection determines the number of suprasylvian neurons which remain spontaneously active or excitable due to microelectrode contact. This influence may be mediated through the withdrawal of excitatory input to cortical neurons (either directly or indirectly) or through some inhibitory

process which changes the excitability of cortical neurons to incoming excitation, (e.g., Elul and Adey, 1966).

One possible mechanism of injury discharge which may have been operative in the present experiments is that penetration of the microelectrode tip through the cortical tissue caused distortions of dendritic membranes with electrotonic depolarizations of the cell soma. Intracellular penetration by the microelectrode tip (as evidenced by large positive spikes or negative potential shifts) was rarely observed and most injury discharge amplitudes were smaller than would be recorded if the tip were in direct contact with the soma. If this explanation actually represents the physical relationship between the recording tip and the parts of the neuron, it is perhaps fair to regard injury discharges as <u>evoked</u> unit activity in that dendritic depolarizing currents eventually "trigger" action potentials, whether this dendritic activity is initiated synaptically or mechanically. In order to account for the fact that injury discharges as well as spontaneous activity is reduced after mid-pontine cooling, it is assumed that the somata of a large percentage of suprasylvian neurons become hyperpolarized, thereby elevating the threshold for spike activity. It would then follow that structures in the lower pontine reticular formation are instrumental in the control of the transmembrane potentials of suprasylvian neurons. Mechanisms for this proposed pontine

action will be discussed later.

An important observation in these experiments was that slow potentials recorded from the cortical surface were not correlated with the density of spontaneously active neurons in the underlying cortex. In cortex with a relatively dense distribution of spontaneous activity, both desynchronized (post-trigeminal) and synchronized (gasserectomized post-trigeminal) surface potentials were recorded. In cortex displaying little spontaneous activity, synchronized (rostropontine) or desynchronized (mid-pontine pretrigeminal) EEG patterns were found. The potentials recorded from the cortical surface apparently do not reflect the summated spike discharge of the many neurons within the vicinity of the large electrode tip. This finding may not seem unexpected when considering the results obtained by intracellular recordings which have demonstrated that, regardless of the incidence of cell discharge, PSPs may show a good cross-correlation with (Creutzfeldt et al., 1966) or a frequency spectrum (Adey, 1967) similar to the surface EEG. However, considering that action potentials are preceded by unusually large PSPs, it might be expected that a high incidence of unit discharge would be an indicator of large PSPs in the neuron population which might then generate a characteristic EEG pattern at the surface. On the contrary, it was found that almost any EEG pattern could be recorded from cortical

surfaces under which either a high or low density of unit activity was found.

The absence of a relationship between the number of active cortical neurons and the waveforms of gross cortical potentials indicates either that these potentials are not determined by discharges in cortical neurons or that neurons which do influence gross potentials were not sampled by either of the present techniques (i.e., by manual exploration or continuous penetration of the microelectrode tip). Clearly, the relatively large microelectrode tip (1 - 3 u inside diameter) would not record spikes from small cells as often as from large; however, the relative potential field of small cells would be expected to be small and thereby less likely to contribute to a gross field recording. Of course, if it is assumed that the population of small cells is influenced by different pontine sections so as to determine the characteristic EEG patterns observed after such sections, it is possible that samples of larger cell activity would not detect such a correlation. In fact, when the spike activities of such larger cells are influenced by different brainstem structures than those that influence gross cortical potentials (as demonstrated in the present experiments), the conclusion that cortical waves are <u>not</u> determined by the density of unit activity is dependent on having sampled mostly large cells.

If it is assumed that cortical gross potentials are generated solely by PSPs, it would be predicted that PSPs evoked by intracortical connections would be more numerous and EEG patterns different in cortex with dense unit activity as compared to relatively inactive cortex. However, it is not known to what extent intracortical connections influence cellular potentials in normal cortex; however, in neurologically isolated cortex large surface potentials may be recorded distant to stimulating points, indicating that intracortical synaptic activities could be sufficient for generating normal EEG potentials.

Burns (1958) has argued that such intracortical connections form complex self re-exciting networks which maintain bursts of spike discharge. If such a re-excitation process were operative, it would be predicted that, in cortex with dense unit activity, synaptic bombardment would occur more frequently than in cortex of reduced unit activity and average firing rates would be higher. However, there was no observed difference in patterns or mean average firing rates for cells recorded from relatively active or inactive cortex. This may suggest that bursts are not dependent on intracortical self re-exciting networks. On the other hand, the reduction in activity observed in our experiments may have been insufficient to disrupt such networks. Alternatively, the networks may involve only clusters of a few neurons, all of which are active if any one of them is active. Mid-pontine inactivation may inactivate many of these clusters

with the remaining clusters exhibiting spontaneous activity patterns and rates not different from those with an intact pons and more clusters active in the cortex.

Recordings of the discharge pattern of individual neurons from different preparations were not apparently related to the pattern of the surface EEG. All neurons sampled exhibited the basic bursting pattern and mean discharge rates were often similar despite large differences in EEG patterns. The dissociation between spike pattern and surface EEG is a common finding (e.g., Evarts, 1961; Murata and Kameta, 1963). However, by measuring slow potentials in the vicinity of the discharging neuron, it was possible to detect a correlation between bursts of spikes and local negativity. That a relationship between rate of single neuron discharge and extracellular slow potentials should be detectable while the spontaneous surface potentials seemed so unrelated to neuron discharges may be due to the fact that slow potentials recorded from the microelectrode tip are more likely to be generated by slow membrane potentials (EPSPs) of the discharging neuron. Since EPSPs and action potentials are highly correlated, the extracellular transient would correlate with any neuron discharge and better with repetitive discharge. This correlation might be further influenced by simultaneous membrane potential fluctuations in nearby neurons, such that EPSPs might summate at the micro-

electrode tip. Smith (1969) has demonstrated that bursts of spike discharge are correlated among adjacent neurons.

It was also found in these experiments that the amplitude ratio of slow waves to fast waves was higher for a transcortical derivation (ECoG) than for a monopolar surface EEG recording (see Experiment I, Figure 12). The simplest explanation for the presence of large slow transients in the transcortical record is that relatively large (5 - 15 mV) PSPs are less attenuated at the nearby microelectrode tip than they are at the surface. In addition, the gross surface electrode would not be selective to one or a few potential generators and as a result probably represents the average of summated dendritic and cellular potentials near the surface.

Another possible interpretation is that the potential generators for fast and slow waves are differentially spaced in the cortex. For example, fast potentials may be generated at a point remote to the cortical leads (e.g., in the afferent radiations). The potential differences detected between the differential transcortical leads (ECoG) may be insignificant compared to a monopolar derivation (EEG). On the other hand, slow wave generators may lie in deeper cortical layers and would hance be less attenuated at the microelectrode than at the surface lead.

Using the assumption that surface or extracellular

slow potentials are generated by slow membrane potentials (PSPs), it may seem plausible that following pretrigeminal transection the remaining specific, non-specific and intracortical afferents to suprasylvian neurons evoke PSPs and characteristic EEG patterns but are insufficient for threshold membrane depolarization, presumably due to critical withdrawal of phasic non-specific brainstem afferents. However, this interpretation cannot explain the lowered excitability of neurons upon microelectrode contact, (i.e., the fewer injury discharges following pretrigeminal as compared with post-trigeminal section). That finding requires postulation of a tonic decrease in membrane excitability.

In accounting for the dissociation of slow potentials and action potentials, it may be argued that the density of neuron discharges can be easily reduced without influencing extracellular or surface slow potentials if the thresholds for regenerative depolarization of neuron membranes are increased.

Relative membrane hyperpolarization (i.e., a net increase in the steady transmembrane potential) would not influence subthreshold slow membrane depolarization (PSPs) due to synaptic inputs; whereas the probability of reaching threshold for the action potential with these inputs would be decreased. This interpretation would account for the

decreased incidence of injury discharge in pretrigeminals, if it is hypothesized that a tonically-active lower pontine fiber system (perhaps originating in n. reticularis pontis caudalis) is instrumental in the control of the average transmembrane steady potential of large numbers of suprasylvian neurons. Withdrawal of this control by mid-pontine lesion or cooling would result in a net transmembrane hyperpolarization and decreased likelihood for spontaneous or evoked (injury) discharge. Fiber systems supplying input to neurons to produce phasic PSPs, which perhaps generate the various EEG patterns observed, are presumably located in rostral or mid-pons and are not critically dependent upon the activity of lower pontine nuclei, since rostropontine lesion results in synchronized EEG patterns often absent in MPP preparations.

In the present experiments the development of synchronized EEG activity in MPP cats is, to some extent, not in agreement with the classical report of Batini et al. (1959a) which emphasized the intense activation of the EEG following MPP section. Other workers have reported EEG synchrony following mid-pontine sections by electrolytic lesion (Rossi and Zirondoli, 1955; Roger, Rossi and Zirondoli, 1956) or by spatula (Rossi, and Zirondoli, 1955; Zernicki, 1964). However, since a desynchronized pattern was usually found for at least one hour or more post-operatively, the development of synchrony in the present experiments may have been due in part to hyperventilation, which

has been shown to be sufficient for inducing EEG synchrony in encéphale isolé cats (Abeles, Magnes and Samueloff, 1964). In the present experiments, expired CO_2 content was regulated to 2.5%.

The influence of trigeminal afferents on pontine nuclei, as indicated by EEG synchrony in gasserectomized post-trigeminal preparations, has been reported before (Roger, Rossi and Zirondoli, 1956) and remains unexplained, in light of the fact that complete pretrigeminal section does not invariably lead to synchrony. This implies that lower pontine nuclei promote synchronized EEG patterns when "released" from trigeminal influences. For example, n. reticularis pontis caudalis may, after gasserectomy, inhibit rostral pontine activating structures and thereby precipitate EEG synchrony. However, it is not known how such a brainstem mechanism would account for the fact that high levels of unit activity recorded in post-trigeminal preparations are not substantially reduced after gasserectomies. Lacking knowledge of the exact excitatory or inhibitory mechanisms involved in either the control of EEG patterns or the maintenance of unit activity, it is difficult to speculate on whether this result is actually problematic or only apparently so.

The foregoing interpretation of the results of these experiments has indicated the importance of lower pontine nuclei

for the maintenance of excitability of suprasylvian neuron membranes. Calma and Arduini (1954) reported that only a few anterior sigmoid neurons increased rates of firing and that most stopped, during mesencephalic reticular stimulation. On the other hand, Creutzfeldt (1961) has reported the opposite result in marginal (lateral) gyrus units during intercollicular reticular stimulation: 90% of the units were activated, 10% inhibited. In posterior suprasylvian cortex, Imbert (1960) observed a temporary increase in spontaneous discharge during reticular stimulation (exact location not reported), as well as improved responsiveness to visual stimuli. These experiments, while superficially discordant, reaffirm the existence of non-specific brainstem influences on cortical neurons. The finding of improved responsiveness to a specific stimulus (Imbert, 1960) suggests that this influence, induced with electrical stimulation, is a tonic one which normally controls the threshold for neuron discharge. This was also suggested in Li's (Li, 1956) experiment in which prior centromedian stimulation increased the number of spikes evoked from a posterior sigmoid gyrus neuron by a VPL shock.

A direct example of relative hyperpolarization of neuron membranes due to withdrawal of a tonic reticular influence has never been demonstrated in cortical neurons. However, Barnes, Joynt and Schottelius (1962) reported hyperpolarization of spinal motoneurons at the 5th lumbar segment

during cooling at the 10th thoracic segment. The cold block presumably interrupted descending influences on the motoneuron. This result fits in with the classical finding of depressed reflexivity following high cord transections and may be analogous to the present experiments demonstrating reduced neuron excitability after pretrigeminal lesion or cooling. The exact synaptic mechanism by which brainstem influences could operate on cortical neurons so as to tonically control the average or steady transmembrane potential fall under two general hypotheses. Relative hyperpolarization of cortical neuron membranes may occur by 1) reduction of excitatory synaptic inputs (disfacilitation hypothesis) or 2) increase in inhibitory influences (inhibitory hypothesis).

One synaptic mechanism which could account for relative hyperpolarization following pontine lesion or cooling would be a simple disfacilitation hypothesis. Removal of tonic non-specific afferents which may excite thalamic neurons synapsing on suprasylvian neurons would result in a reduction in depolarizating currents and a net increase in "steady" transmembrane potential. That cortical membrane potential may be determined by the relative number of active excitatory inputs is suggested by Creutzfeldt (1961) who demonstrated cessation of spontaneous unit discharge during retinal ischemia in cerveau isolé cats. Retinal ischemia in encéphale isolé (intact non-specific inputs) did not alter

the firing pattern of spontaneous visual cortex neurons. In the present experiments, it might be argued that lower pontine inactivation by mid-pontine lesion or cooling simply eliminated a significant number of excitatory inputs to suprasylvian neurons and thereby hyperpolarized the cells.

A second mechanism would be a disinhibitory effect of brainstem inactivation. If the function of lower pontine nuclei is viewed as basically inhibitory, so as to inhibit tonic inhibition by other subcortical structures on cortical neurons, pontine inactivation would result in withdrawal of inhibition (pontine source) of inhibition (other source) on cortical neurons, thereby releasing the activation of tonic inhibitory synapses on suprasylvian membranes and resulting in membrane hyperpolarization.

There is little evidence bearing on this interpretation; however, Purpura, McMurty and Maekawa (1966) have demonstrated attenuation of thalamic IPSPs during brainstem reticular stimulation. Seven-per-second medial thalamic stimulation always resulted in a ventrolateral cell EPSP followed by a long-lasting IPSP, which inhibited discharge due to brachium conjunctivum (BC) stimulation. Simultaneous reticular stimulation reduced this phasic IPSP, and BC stimulation became effective in evoking discharge, suggesting that brainstem reticular neurons functioned to <u>inhibit</u> inhibitory synapses from medial thalamic to ventrolateral neurons. However,

this model experiment may not be analogous to the pontine inactivation experiments. First of all, the occurrence of phasic IPSPs does not imply a tonic action. Secondly, intrathalamic relations may be quite different from thalamocortical ones. Finally, in records during reticular stimulation evoked EPSPs were attenuated as well as the IPSPs, suggesting that the reticular activation did not selectively inhibit inhibitory synapses. However, Angel, Magni and Strata (1965) have demonstrated long-lasting pre-synaptic inhibition in the lateral geniculate nucleus during midbrain reticular stimulation. Nevertheless, since their cats had mid-pontine sections, the role of lower pontine reticular structures is not clarified.

Indications for Further Research

A direct demonstration of reticular control of steady membrane potential is planned for future experiments in which intracellular recordings from suprasylvian neurons in post-trigeminal cats will be compared before, during and after midpontine cooling. Secondly, it is important to distinguish further between the relative contributions of non-specific and specific input, if such a distinction exists experimentally. To this end, extracellular records of spontaneous suprasylvian discharge in a PoT preparation will be compared before and after removal of all remaining cranial afferents, i.e., bilateral sections of N.I, N.II and N.V. If spontaneous

discharge is not significantly reduced by these deafferentations in a PoT preparation, then the role of lower pontine non-specific fiber systems would be proved essential for the maintenance of suprasylvian neuron excitability, i.e., maintaining membrane threshold at a level which results in a high probability of spontaneous discharge (due to some phasic synaptic input) as well as of "evoked" discharge due to microelectrode contact. It is entirely possible, however, that following total sensory isolation the efficacy of reticular neurons may be so reduced that their tonic ascending influence would be insufficient to maintain suprasylvian excitability. This result would not be easily interpreted: it might indicate that specific afferents do contribute directly to the maintenance of cortical excitability or more likely it would suggest that a functional distinction between specific and non-specific influences would require evidence of reduced excitability following a restricted pontine reticular lesion. Here again, however, coma due to reticular damage may reduce the efficacy of sensory stimulation, especially projections to association cortex.

CHAPTER SIX

SUMMARY

In experiments designed to study pontine reticular influences on gross field and action potentials in the cat association cortex, pontine structures were inactivated by different pontine transections or reversible mid-pontine cooling.

1. The amplitude ratio between slow and fast wave components was found to be higher for a transcortical recording derivation (ECoG) than for a monopolar surface recording (EEG). It is assumed that the presence of large slow waves in the ECoG is due to large cellular PSPs detected by the nearby microelectrode tip.

2. Post-operatively, EEG patterns were synchronized following rostropontine lesion, desynchronized following mid-pontine pretrigeminal (with one exception) and post-trigeminal transections. Synchrony was found to develop after a few hours in most MPP and PoT preparations. Bilateral gasserectomies in PoT preparations always precipitated EEG synchrony. The desynchronized EEG pattern of PoT preparations was synchronized by mid-pontine cooling, eventually resulting in large surface negative spindles (10 - 11 Hz).

3. Samples of extracellular action potentials were taken

under these various experimental conditions and compared with the different EEG patterns. The number of spontaneous and injury discharges recorded was reduced after pretrigeminal lesion (MPP or RP) or during mid-pontine cooling as compared to that recorded from post-trigeminal preparations. Bilateral gasserectomies did not substantially reduce unit activity.

4. Slow potentials recorded from the cortical surface were not correlated with the density of spontaneously active neurons in the underlying cortex. It was concluded that a pontine reticular influence controls the excitability of cortical neurons (as evidenced by a decreased incidence of injury discharges following mid-pontine inactivation) and that a rostral pontine influence may affect gross cortical potentials (as evidenced by EEG synchrony following RP section, often not seen after MPP section).

5. Patterns of spontaneous discharge and the mean and median average firing rates were not demonstrably different for individual cells after high (MPP or RP) or low (PoT or PoT + V) pontine section.

6. A correlation between large, tip-negative slow potentials of the ECoG and bursts of spike discharge was found regardless of the level of brainstem section, perhaps because the microelectrode preferentially detected the EPSP preceding the spike.

7. Brainstem mechanisms which may alter the number of

active suprasylvian neurons without influencing either potential generators of gross cortical field potentials or the activity patterns of individual neurons are discussed.

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APPENDICES

CRYOPROBE SYSTEM [1]

Construction of Cryoprobe [2]

In order to cool brain tissue surrounding the cryoprobe, a coolant, 95% alcohol passed through copper tubing immersed in a dry ice and alcohol bath, was circulated through the cryoprobe. The cryoprobe was constructed of two stainless steel hypodermic tubes (Superior Tube Company, Norristown, Pa.) arranged as a concentric double-cannula with the input tube (26 gauge) inserted into the larger output cannula (18 gauge) by penetration of its wall. This junction and the end of the output cannula were sealed with solder (see Figure). This arrangement formed a probe which provided increasing heat exchange with the brain as the rate of coolant flow increased, (see Figure for drawing of complete system). Construction of these probes so as to withstand the high pressures at solder joints required for a high coolant flow rate requires attention to certain details. First, to facilitate insertion, the tubing must be annealed (Hypoflex 304 stainless steel) and for

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1. The construction and operation of the cryogenic probes are based upon a system commercially available from the Neuropsych Company (Altadena, California), first reported in detail by Skinner and Lindsley (1968).

2. The cryoprobes were manufactured by Klaus Fabich, Hamilton Research Instruments, Hamilton, Ontario.

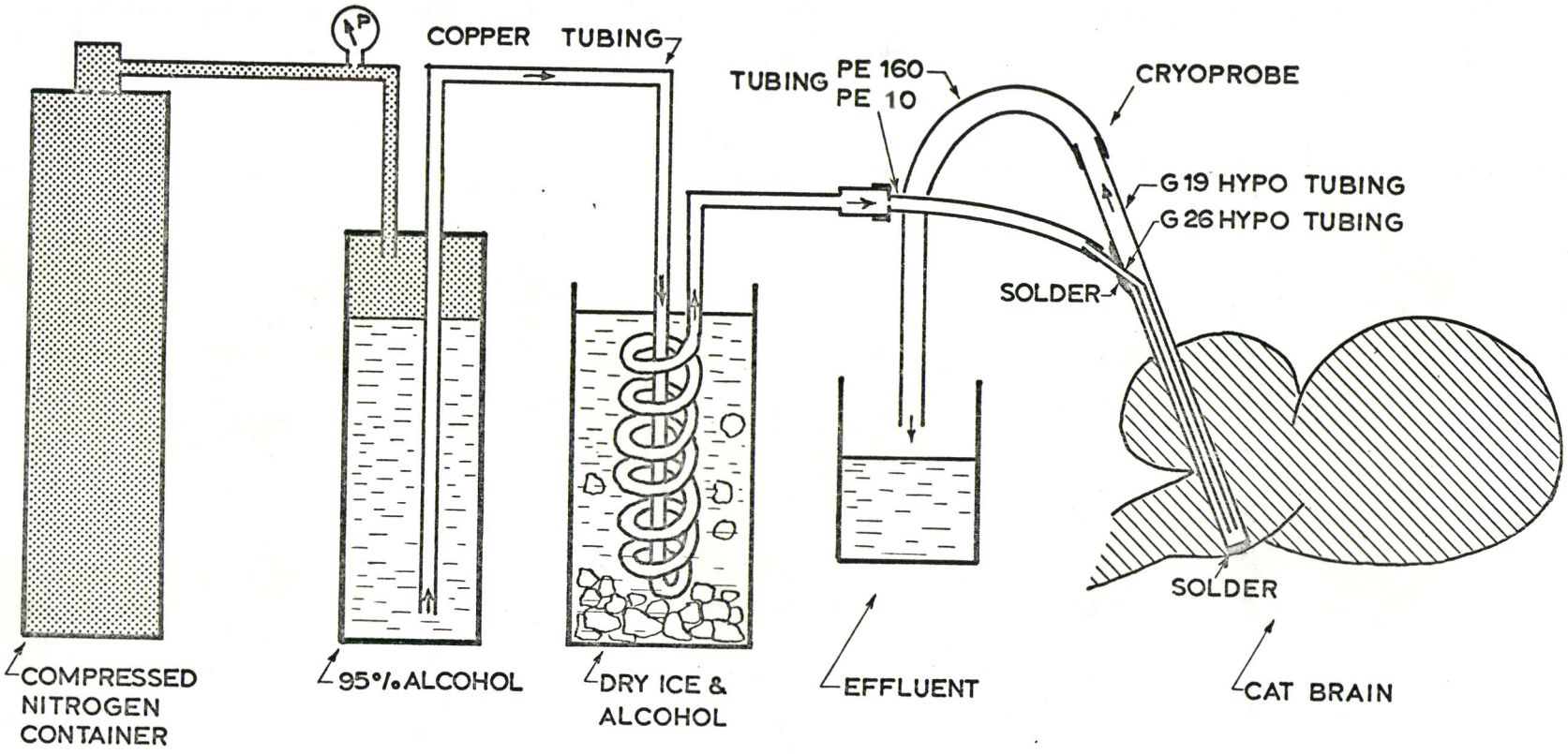

Figure 69. Composite drawing of the cryoprobe system.

soldering a special stainless steel flux (Autochemic Compound 157) and solder (Eutectic Canada, Ltd., Pointe Claire, Quebec) is recommended.

Delivery of Coolant

In order to block conduction of action potentials in a region of brain tissue exceeding a 4 mm radius of each cryoprobe, extremely high coolant flow rates (1 ml/sec) were required for rapid heat exchange. To achieve these rates through the small tubing (26 gauge) the alcohol was put under high pressures (400 - 450 p.s.i.) by connecting the stainless steel alcohol cylinder to a pressurized nitrogen cylinder through an adjustable gas pressure gauge (0 - 1000 p.s.i.). Adjustment of this gauge controlled the coolant flow rate, which could be measured as milliliters per second in the effluent.

Further Information

Cryoprobes may be designed for a variety of different local cooling tasks. The extent of the cooling may be restricted to the tips of the cryoprobe while a D.C. current passed through a wire wrapped around the shaft of the probe maintains body temperature in that region.

Details for specially-constructed cryoprobes may be obtained from (1) Klaus Fabich, Hamilton Research Instruments, Hamilton, Ontario, (2) Neuropsych Company, Altadena California, or (3) by reference to Skinner and Lindsley (1968).

Figure 70. Horizontal or parasagittal sections of the brainstems of cats with complete post-trigeminal lesions. Bilateral gasserectomies in X19 and X22 were performed extracranially. Destruction of trigeminal rootlets in X26 by electrolytic lesion was verified by a Prussian blue reaction but cannot be distinguished in the section shown here due to poor contrast in the photography of the pale thionin stain.

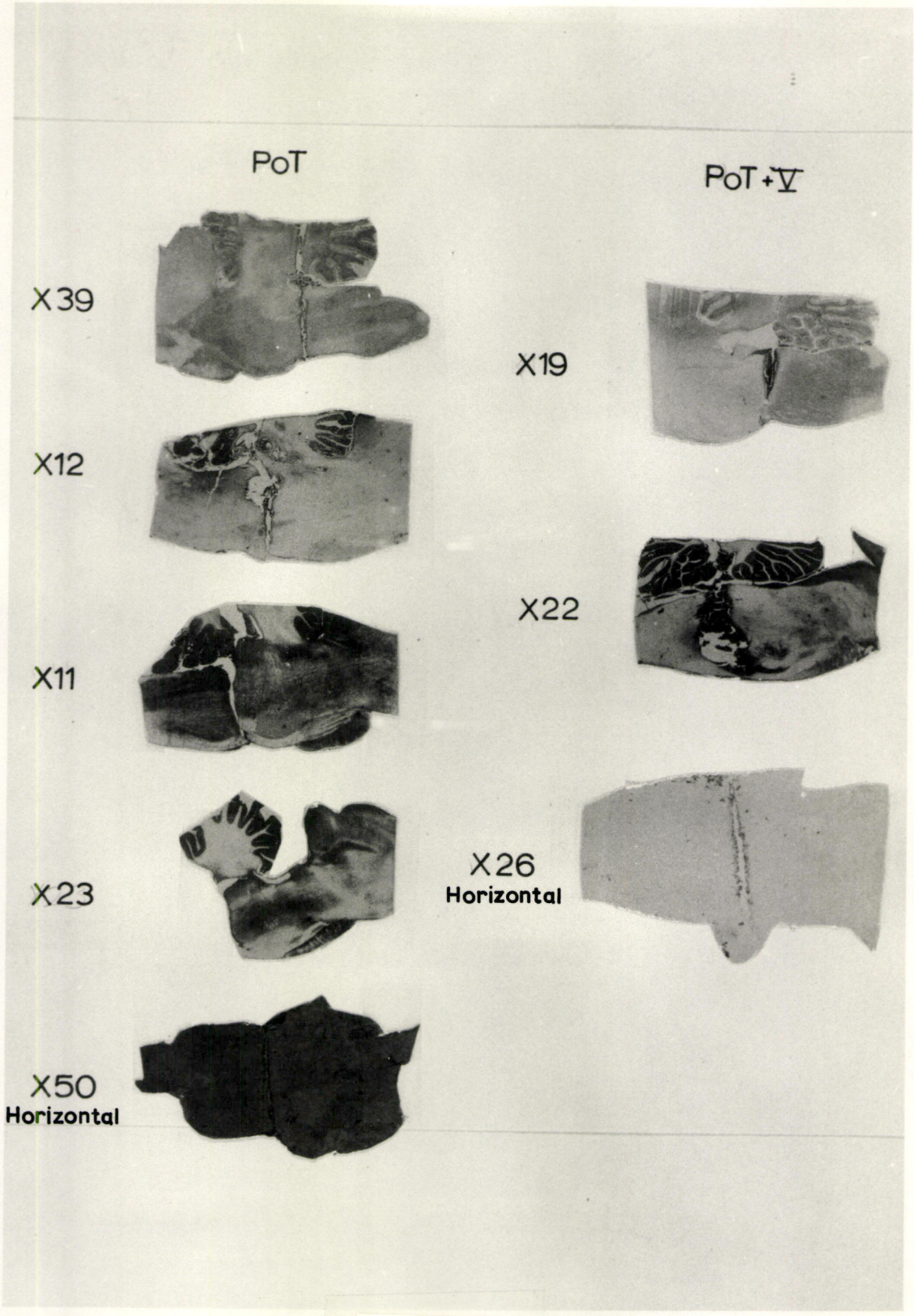
PoT
PoT + V
X39
X19
X12
X22
X11
X23
X26
Horizontal
X50
Horizontal

Figure 70.

Figure 71. Parasagittal sections of cat brainstems with midpontine pretrigeminal (MPP) or rostropontine (RP) sections. Note the integrity of the brachia pontis in some MPP sections (e.g., X38, X31 and X9). Strands of the pyramidal tract may be intact in X30. Rostropontine preparations reported were always complete.

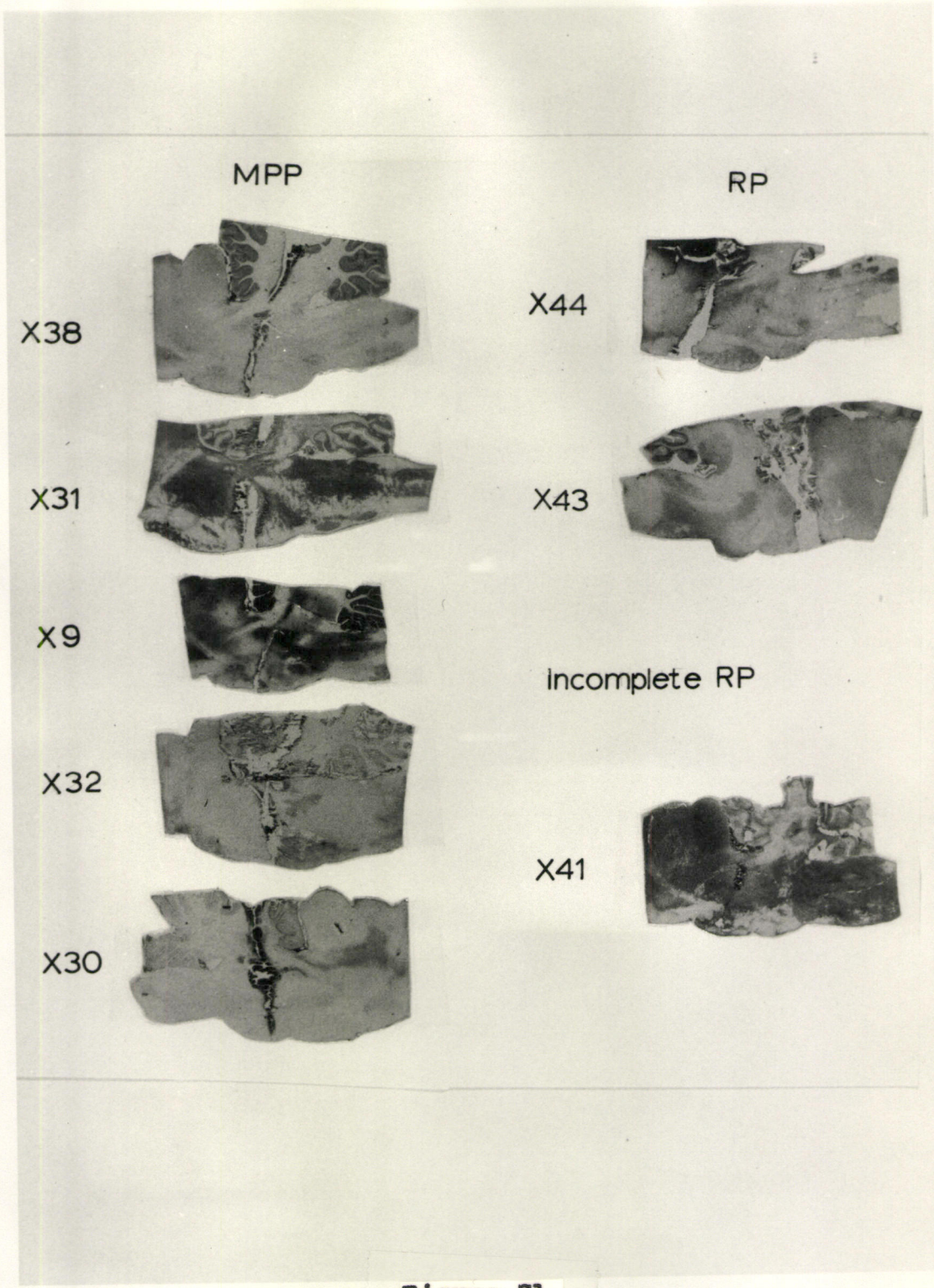

Figure 71.