# BEHAVIORAL AND NEURONAL EFFECTS OF EEG SYNCHRONIZING STIMULI IN THE CAT

to
my
mother
father
and
sisters

# BEHAVIORAL AND NEURONAL EFFECTS OF EEG SYNCHRONIZING STIMULI IN THE CAT

By

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## Abstract

Electrical stimulation of the medial thalamus (MTH) and lateral preoptic area (LPO), which produces slow, rhythmic "synchronized" waves in the cortical EEG, has been reported also to induce sleep and to inhibit neurons of the brain stem reticular formation that produce fast, irregular "desynchronized" EEG waves. The present findings confirm that MTH and LPO have significant input to brain stem neurons, but that input is not exclusively inhibitory. MTH and LPO stimuli produce discrete cycles of excitation and inhibition in reticular neurons which alternately facilitate and suppress subsequent inputs, from visual (V) and auditory (A) systems for example, which converge with MTH and LPO inputs on single reticular cells. Conversely, it was found that V and A stimuli could facilitate and suppress MTH and LPO responses. These results support the view that synchronogenic and desynchronogenic mechanisms of the cortical EEG are reciprocally organized at a subcortical level, and that the organization involves complex excitatory and inhibitory interactions. However, no convincing evidence was found to support the contention that synchronogenic stimulation of MTH or LPO induces behavioral sleep.

## Preface

Although the cortical electroencephalogram, or EEG, has been intensely studied for many years, its neuronal bases remain largely unknown. One problem for researchers has been the misleading assumption that mechanisms generating the EEG are the same as those generating the behaviors with which the EEG is closely correlated. One aim of this work, therefore, was to determine whether electrical stimulation of the medial thalamus (MTH) and lateral preoptic area (LPO), which clearly produces slow rhythmic EEG waves called synchronization, also necessarily induces the behavioral state of sleep as many authors have claimed (see Experiment I). A second problem in the literature has concerned the mechanisms by which EEG synchronizing structures gain control of the cortical EEG, one hypothesis being inhibition of antagonistic EEG desynchronizing structures located in the reticular formation of the brain stem. Therefore, we examined the effects of MTH and LPO stimulation on spontaneous and evoked activity of single reticular neurons (see Experiments II and III).

Since literature concerning the cortical EEG, its behavioral and physiological correlates, is very extensive, the following introduction is limited to four main topics: 1) cortical electrogenesis of the EEG, 2) desynchronization mechanisms, 3) synchronization mechanisms, and 4) neuronal interactions between synchronizing and desynchronizing systems. The first is intended to acquaint the reader with the general phenomenon of the EEG and its basis in the electrical activity of cortical cells. The second is a description of the anatomy and physiology of reticular neurons involved in cortical desynchronization, since it is from these neurons that recordings are made in Experiments II and III. The third section describes the anatomy and physiology of MTH and LPO synchronization systems, and examines the evidence that these systems are responsible also for the induction of behavioral sleep. The fourth section is primarily an account of descending influences of MTH and LPO on reticular neurons, and the hypothesis that these influences are inhibitory.

There is a discussion of results following each experiment, and a general discussion in which the hypothesis of electrical sleep induction, and the hypothesis of reticular deactivation, are evaluated in light of the present data.

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# Introduction

## I. The cortical EEG

The neural activity of the brain is accompanied by a continuous pattern of moment-to-moment changes in electrical potential which can be recorded from individual neurons and from populations of neurons. Very fine gauge electrodes, small enough to penetrate a single cell, will record from inside the cell a resting membrane potential, and slow hyperpolarizations and depolarizations of the membrane that result from synaptic transmission. Also, intracellular and small extracellular electrodes will record fast, all-or-none action potentials that are propagated along the axon of the cell and cause the release of transmitter substance from axon terminals. Electrodes that are large compared with a single nerve cell will record the sum of the synaptic potentials occurring in millions of cells in the vicinity of the electrode tip. These summated, wave-like macropotentials range in frequency from about .5 Hz to 100 Hz and are called the electroencephalogram, or EEG. The EEG can be recorded through electrodes placed on the scalp or skull, or in the brain tissue itself, of all vertebrates, although there are considerable differences in EEG frequency and waveform among different species. In mammals, in

which the majority of EEG work has been done, the EEG is most commonly recorded from the cerebral cortex, the convoluted outer layer of the brain which is essential for complex behavior.

The origin of the EEG and its relationship to brain function and behavior have intrigued neuroscientists since its discovery in the late 19th century (Caton 1875, Beck 1890, see also Brazier 1959, 1968). Interest in the EEG stems mainly from the fact that it undergoes distinct changes in frequency and amplitude which closely correspond to changes in states of consciousness. The various patterns, or rhythms, of the human EEG were first named by Berger (1929) who observed "alpha" activity, a rhythmic, almost sinusoidal oscillation of about 10 Hz appearing when the subject is relaxed with eyes closed, and "beta" activity, a higher frequency, more irregular pattern of waves appearing during wakefulness with eyes open. Since that time a considerable literature has accumulated describing these and other rhythms of the human EEG, their distribution over the head, ontogeny and modification by disease, drugs and psychological state (see Walter 1950; Brazier et al., 1961, Gibbs and Gibbs 1950, and Rechtschaffen and Kales 1968). Since this thesis deals mainly with experiments done in the cat, the EEG of that species will be described in some detail below, and similarities to the human EEG will be noted.

Figure 1 shows changes in the cortical EEG and electro-oculogram

(EOG) of the cat in different stages of sleep and wakefulness. During wakefulness (Figure 1a) the EEG is composed of low voltage, high frequency potentials between 14 and 100 Hz very similar to the waking beta rhythm of humans. Waking eye movements are reflected in the rapid fluctuations of the EOG. When the cat rests with eyes closed, alpha waves do not appear as they do in humans Instead, as the animal becomes drowsy, 6 - 12 Hz high amplitude oscillations called sleep spindles appear intermittently on a background of low voltage fast activity (Figure 1b). As the spindles increase in frequency of occurrence and in amplitude, the background activity gives way to higher voltage, slower potentials. Humans also exhibit sleep spindles, but they are much lower in amplitude and occur in deeper stages of sleep. Spindles in the cat are most reliably recorded from the frontal cortex (anterior sigmoid, orbital and proreus gyri) but can also be seen in the suprasylvian and lateral gyri (Horvath and Buser 1972). Since the eyes are closed during this period, the EOG is relatively flat, although there are very slow, drifting eye movements. The EEG waves of a fully asleep cat are a continuous series of sleep spindles and other high voltage irregular slow waves called delta waves (Figure 1c), which are also seen in sleeping humans. The eye movements during this stage continue to drift slowly. After several minutes of slow wave sleep (about 30 minutes in the cat (Sterman et al. 1965) and 90 minutes in the human (Dement and Kleitman 1957)), the EEG abruptly reverts to high frequency fast

waves identical to waking EEG, but the subject remains asleep (Figure 1d) Because of the discrepancy between EEG activity and behavior this stage is sometimes called paradoxical sleep, but more often it is called REM (rapid eye movement) sleep because there are quick, sharp fluctuations of the eyes beneath the closed lids. Humans, if awakened from REM sleep, will often report that a dream had been occurring (Dement and Kleitman 1957), but, of course, it is impossible to know if the REM stage of cat sleep is associated with dreaming. Thus, there are four stages to the cat EEG sleep-waking cycle: waking desynchronization, drowsiness or light slow wave sleep with intermittent spindles, deep slow wave sleep with sustained spindles and slow waves, and REM sleep desynchronization. Other normative descriptions of cat EEG may be found in Sterman et al. (1965), Ursin (1968, 1970), Delorme et al. (1964), Jouvet (1967), and Dement (1958).

Cortical mechanisms of the EEG

Cortical EEG is generated by cortical cells themselves. That is, the electrical potentials recorded in the cortex result from currents flowing along cortical cells accompanying differential polarization of parts of the membrane. However, the rhythmic oscillations of the EEG, particularly the sleep spindle, are triggered or patterned by input from subcortical structures. These inputs and their influence on EEG

activity will be discussed in some detail later. The present topic is the nature of the potential generator in the cortex.

The earliest ideas about the neurophysiological basis of the EEG stem from Caton's belief that the slow fluctuations he observed while recording from the surface of the brain in rabbits resulted from the summation of axonal action potentials in deeper cortical layers (Adrian and Yamagiva 1935, and Bishop 1936). Subsequent evidence has shown, however, that while patterns of action potentials are probabilistically related to phases of EEG waves (Fox and O'Brien 1965, Fox and Norman 1968, Li et al. 1952 1956 Whitlock et al. 1953, Fromm and Bond 1967 Buchwald et al. 1966, Creutzfeldt et al. 1966c, Smith 1966, Langsam 1967, Ajmone Marsan 1965, Spencer and Brookhart 1961b), action potentials do not significantly contribute to the EEG because of their fast time course and the very small currents they generate. Additional evidence that action potentials do not produce EEG activity are the findings that spontaneous action potentials are sometimes not detected in areas of the brain clearly displaying spontaneous EEG waves (Renshaw et al. 1940), and that slight hypoxia, anesthesia, or ischemia, which readily suppresses axonal activity, does not also suppress cortical slow waves (Li and Jasper 1953, Mountcastle 1957, Creutzfeldt et al. 1957, Brookhart et al. 1951, Amassian et al. 1964). Also, Babb and Smith (1971) have shown that there is no fixed relationship between the number of spontaneously active neurons in the cortex of brain stem

sectioned cats and the frequency or amplitude of the EEG. Thus there is little support for the supposition that slow cortical waves result from envelopes of spike discharge.

It is now believed instead that the EEG results from summation of potential transients across dendritic and somatic membranes of the cell rather than across axonal membranes (Davis 1936, Gerard 1936, Chang 1951, Eccles 1951, Purpura 1959, Bremer 1958). Dendritic and somatic postsynaptic potentials (PSPs) are generated when neuronal membranes are chemically stimulated at synaptic junctions by the secretions of other nerve cells. They differ from axon potentials in that they are graded in amplitude, longer in duration, and are not propagated along the cell membrane. Depending on the type of synapse, the membrane may be depolarized or hyperpolarized, producing either excitatory postsynaptic potentials (EPSPs), or inhibitory postsynaptic potentials (IPSPs). Action potentials may also be generated in dendrites, and possibly conducted to the soma or axon. Evidence of dendritic spikes has been obtained in the hippocampus (Kandel et al. 1961) and in the cerebellar cortex (Eccles et al. 1966, Llinas and Nicholson 1971), but so far is lacking for the cerebral cortex.

The physiological properties of graded dendritic potentials in the cortex that closely correspond to the waves of the EEG were first described by Chang (1952), Bishop and Clare (1952), Clare and Bishop (1955) and Rall (1962). Simultaneous recordings of slow surface

potentials and intracellular membrane potentials, and of intracellular and close extracellular potentials, confirmed a close relationship among the three phenomena and further supported the hypothesis that EEG waves resulted from the summation of PSPs (Li 1961, 1963, Phillips 1961, Lux and Klee 1962, Stefanis 1963, Purpura et al. 1964, Klee and Offenloch 1964, Jasper and Stefanis 1965, Creutzfeldt et al. 1964a, b, 1966a, b, c). In these experiments PSPs were found to have relatively long durations, 10 - 30 ms for EPSPs and 70 - 150 ms for IPSPs, which correspond to EEG wave frequencies of 6 - 100 Hz. Furthermore, EPSPs were often in phase with the surface negative transients of the EEG, and IPSPs with the surface positive transients. However, these phase relations can vary, and a variety of correlations may be found depending on the depth of recording and the type of preparation used (Pollen 1964, Nacimiento et al. 1964, Creutzfeldt and Meisch 1963, Creutzfeldt et al. 1969, and Sugaya et al. 1964). Also, the relationship between the synaptic activity of single cortical neurons and spontaneous EEG waves may be different for different types of EEG waves.

In order to better relate the activity of single cells to the surface potentials of the EEG the origin of potential differences in different parts of the cell, and the spread and summation of currents along the somadendritic membrane are described more fully below.

Electrical summation of EPSPs and IPSPs

The EEG is a measure of the potential difference generated when current flows along cell membranes from one region of the cortex to another. But Renshaw et al. (1940) realized that "...there is no external field set up and therefore no current flows in the medium about a cell as long as every part of the cell is at the same potential." Thus uniform synaptic activation of a neuron would be ineffective in setting up an external potential field even though the transmembrane potential was changing. External electric fields are set up only when disparate synaptic activity produces potential differences between two or more regions of the cell's surface.

The requirements of differential synaptic contact along the soma-dendritic membrane are met in the cerebral cortex. The cortex is a laminar structure whose major neuronal elements are vertically oriented with dendrites arborizing toward the surface and axons projecting downward. Specific afferents terminate mainly on the soma and basal dendrites, while nonspecific afferents terminate mainly on the apical dendrites (Lorente de No 1943, Globus and Scheibel 1966, Szentágothai 1969, Jones and Powell 1970). Also, inhibitory synapses are localized mainly on or near the soma while excitatory synapses are more uniformly distributed (Szentágothai 1969). Current along the vertical axis of the cell, that is, between the surface and deeper layers of the cortex, is initiated when one type of afferent activity

predominates evoking postsynaptic potentials along limited regions of the cell. The direction and speed of current spread through extracellular space would then depend on several factors including geometry of the cell, the time constant of the neuronal membrane, and timing of successive currents (see Creutzfeldt et al. 1966b). The result is that some membrane transients, particularly the slow, longlasting ones, may be closely in phase with the surface potential while fast potential transients may show shifts of up to 180°. The complexity of the biophysical properties of current spread through a volume conductor make it very difficult to predict exactly the direction and time course of surface EEG potentials from the observation of membrane currents in individual cells.

Nevertheless, in the most simplistic terms, relative surface positivity occurs when the somatic or deep region of the cell is depolarized creating a deep sink into which current flows from a dendritic source. It is also possible to create superficial sinks by direct depolarization of the apical dendrites in which case monophasic negative waves are seen at the surface (Spencer and Brookhart 1961b, Creutzfeldt et al. 1964). IPSPs at the soma produce a current flow toward the apical dendrites which act as passive sinks and the EEG shifts negative (Pollen and Sie 1964). In summary, most surface EEG activity can be traced to vertical transneuronal currents produced by differential activation of excitatory and inhibitory synapses along the

cell membrane.

The potential field which results from this current flow along the cell membrane is similar to that produced by one or more dipoles (Holmes and Houchin 1967), and depth profiles of cortical potentials confirm that the polarity of most potentials is reversed at about layer 4 of the cortex, i. e. the layer of cell bodies (Spencer and Brookhart 1961a, Humphrey 1968). The summation of fields from dipoles oriented parallel to each other would produce large population potentials, while the summation of randomly oriented dipoles would, through cancellation, produce small population potentials. Since in the cerebral cortex there is a vertical stacking of the large bodied cells, there is sufficient anatomical basis for the addition of potentials providing the cells are synchronously activated. Elul (1972) has estimated that a comparatively small proportion of the total neuronal population (about 10%) if synchronously stimulated will generate high amplitude potentials that dominate the EEG record, since the amplitude of the asynchronous activities remains so small. To support his model he cites the finding that gross evoked potentials following discrete afferent stimuli are produced by activation of only 10 - 20% of the population yet they achieve the same amplitude as the large spontaneous EEG waves. So the EEG may reflect the synchronized synaptic activity of relatively small numbers of cells. Verzeano et al. (1970) also suggest that slow EEG patterns result from waves of synchronization circulating in neuronal networks from one

small pool of neurons to another.

More comprehensive reviews of potential generators in the cortex may be found in Creutzfeldt (1974), Adey (1969), Rosenthal (1971), Purpura (1967), Raabe and Lux (1972), and Elul (1972).

Contribution of action potentials

Although PSPs contribute much of the current producing local field potentials and surface polarization, the contribution of action potentials in afferent and efferent fibers of the cortex may be significant. Axon currents are small in amplitude and duration compared to somatic and dendritic currents, yet, if they are synchronized, they may influence surface EEG potentials. For example, following discrete sensory stimulation, or electrical stimulation of sensory or motor pathways, the resulting burst of orthodromic or antidromic action potentials in the cortex is correlated with early surface positive or local deep negative waves indicative of axonal currents (Amassian 1953, Angel and Holmes 1967, Carter et al. 1968, MacKay 1969, Ajmone Marsan 1965, Houchin 1969, Amassian et al. 1964). Humphrey (1968) in particular has demonstrated a close correspondence between theoretical and observed values of potential distribution from the surface to deeper layers of the cortex that would result from synchronized action potential currents associated with the antidromic motor response to pyramidal tract stimulation. Under certain circumstances therefore, components of the EEG may reflect presynaptic as well as postsynaptic voltage fluctuations.

Contribution of glial potentials

Recent studies on the electrophysiological properties of glial cells in simple preparations (in the leech and some Amphibia) suggest fluctuations in glial membrane potentials may contribute to slow EEG potentials recorded from the surrounding tissue. Kuffler et al. (1966) have shown that the resting membrane potentials of glia in these preparations are about -90 mV, or 10 - 15 mV more negative than neuronal membrane resting potentials elsewhere in the same preparation. These and other data suggest that glial cells are permeable only to potassium ions and, therefore, may be used as perfect potassium electrodes to moniter extracellular potassium concentrations in a variety of cellular experiments. In the optic nerve of the mud puppy (Necturus maculosa), Orkand et al. (1966) have shown that neuronal impulse activity causes a depolarization of neighboring glial cells, and that this depolarization is probably generated when potassium ions are released from axons during the falling phase of the action potential, and accumulate in narrow intercellular clefts. Glial cells are also electrically connected to one another by low resistance pathways so that depolarization of one cell draws current from adjacent cells (Kuffler et al. 1966). In experiments where it is possible to alter glial membrane potentials without affecting axonal membrane potentials, it was found that the potential recorded from the surface of the whole nerve was entirely dependent on glial

potentials (Cohen 1970). The above findings seem to describe a glial mechanism for converting axonal spike activity into EEG waves. However, the evidence for such a process in the mammalian brain is not as clear.

Presumed glial cells have been recorded in the mammalian nervous system where they were identified as "silent", "idle" or "unresponsive" cells (Castellucci and Goldring 1970, Karahashi and Goldring 1966, Grossman and Hampton 1968, Kelly et al. 1967, Krnjevic and Schwartz 1967, Sugaya et al. 1964, 1971). Presumed glial cells in the cerebral cortex of the cat may have some of the properties of invertebrate glial cells: resting membrane potentials determined primarily by potassium (Ransom and Goldring 1973a) and slow depolarization shifts associated with adjacent neuronal action potentials (Ransom and Goldring 1973b). However, they show no change in membrane conductance during the slow depolarizations, and no change in slow depolarization amplitude when polarizing currents are applied through the recording microelectrode (Ransom and Goldring 1973b). Therefore, the depolarization is concluded not to be a synaptic effect, but rather results from the transient extracellular accumulation of potassium. Conversely, slow hyperpolarization of presumed glial cells, correlated with surface positive potentials, were shown to result from a transient decrease in extracellular potassium brought about by the operation of the neuronal "sodium pump"

during very high frequency neuronal activity (Ransom and Goldring 1973c).

These data suggest that glial membrane potential shifts, being several seconds in duration and probably related to cortical metabolic processes, contribute to slow electrical phenomena in the cortex, particularly sustained DC potential shifts. However, they are not synaptic in nature, and their role in the genesis of faster EEG potentials has not been clearly demonstrated (Somjen 1975).

In summary, the most widely accepted hypothesis of EEG genesis is that it is largely due to the summation of postsynaptic potentials in cortical soma and dendrites. Large field potentials may be generated since the parallel organization of the neuronal elements serves to add the currents around active cells rather than subtract them, especially if they are synchronously generated. The actual form of the surface potential depends on a number of factors including the location of the active synapses (somatic vs. dendritic), whether they are hyperpolarizing or depolarizing, the cytoarchitecture of the neuronal population, and the electrical properties of the conducting medium. These processes describe the EEG potential generator in the cortex, but not the mechanisms of EEG rhythmicity. It is this subject we will consider next.

Subcortical control of the cortical EEG

If a slab of cortical tissue is isolated by knife cuts from extracortical input, so that the only viable synapses are between cortical cells, the spontaneous EEG of the slab is severly modified. Rhythmical EEG activity completely disappears (Creutzfeldt and Struck 1962), and small slabs may show no electrical activity at all, while large slabs may show low amplitude slow waves and some unit activity (Burns 1949, 1950, 1951, 1958, Burns and Grafstein 1952, Grafstein 1959, Sharpless 1969, Frost 1968). Postsynaptic potentials, spikes, and large surface positive EEG bursts may be elicited by electrical stimulation of the cortical surface and repetitive stimulation may lead to self-sustained after discharges (Burns and Grafstein 1952, Creutzfeldt and Struck 1962, Sharpless 1969). While there are reports contradicting these findings (Kristiansen and Courtois 1949, Echlin et al. 1952, Henry and Scoville 1952, Ingvar 1955), the results are generally taken to mean that the regular, rhythmic potentials of the EEG are dependent on afferent input from subcortical structures. Whether these inputs actively pattern the EEG, or rather provide a tonic facilitation necessary for the generation of the rhythms by cortical neurons is not clear.

Experiments using tetrodotoxin, a drug which blocks action potentials but does not affect the spontaneous release of transmitter or

its postsynaptic action.(Narahashi et al. 1964, Furukawa et al. 1959, Elmquist and Feldman 1965), further support the hypothesis of subcortical control of cortical EEG activity. Elul (1972) reported that 10 - 20 minutes after injecting tetrodotoxin into the ventricles, the cortical EEG became flattened intermittently although spike activity could still be recorded in cortical neurons. Since spike firing rate and the amplitude of cortical PSPs fluctuated randomly with respect to the flat periods in the EEG, Elul concluded that a synchronized cortical population was necessary for the production of EEG waves. Furthermore, since tetrodoxin diffuses from the ventricles very slowly and probably did not reach the cortex within 20 minutes, the absence of EEG potentials was probably not due to direct action of tetrodotoxin on cortical neurons. The continuance of cortical unit activity supported that interpretation. Instead, tetrodotoxin must have blocked transmission in structures immediately adjacent to the ventricles, possibly in thalamus, confirming the hypothesis that the integrity of subcortical mechanisms is vital to the production and maintenance of the cortical EEG.

The study of subcortical influences on the EEG began in the 1940's. Morison and Dempsey (1942) found that electrical stimulation of the medial thalamus of the diencephalon evoked rhythmic EEG

potentials similar in many respects to spontaneous sleep spindles. Then, in 1949, Moruzzi and Magoun reported that stimulation of the brain stem reticular formation evoked low amplitude fast EEG potentials like the beta rhythm of the waking EEG. From that time on all EEG rhythms began to be classified as either "synchronization" (any high amplitude low frequency activity) or "desynchronization" (any low amplitude high frequency activity), and the medial thalamus and reticular formation came to be regarded as the synchronization and desynchronization mechanisms of the EEG, respectively.

To a large extent that conceptualization is oversimplified and misleading. First, thalamic synchronogenic mechanisms are involved only in the genesis of sleep spindles; the origin and mechanisms of alpha waves, delta waves, and other slow EEG potentials are essentially unknown. Second, it is not clear that desynchronized EEG is qualitatively different from synchronized EEG since cortical neurons must be synchronized to some degree for any EEG potentials, high or low frequency, to be generated. Third, since 1949 a large number of other subcortical structures have been found to produce cortical EEG potentials when stimulated, implying that EEG rhythms are not a function of particular localized subcortical pacemakers.

Nevertheless, the terms synchronization and desynchronization and implications concerning their physiological substrates persist in the literature, and it is difficult to describe that literature without

adopting the same vocabulary. In the following pages, therefore, we will describe the most extensively studied subcortical mechanisms of the EEG and the reciprocal interactions among them.

## II. Desynchronization mechanisms

### General anatomy and histology

The portion of the brain most closely associated with the function of cortical desynchronization is called the ascending reticular activating system (ARAS). Although neurons of the ARAS are some subset of neurons of the reticular formation (RF) they have no anatomical boundaries but seem to be scattered through the reticular tegmentum and to participate in other reticular functions as well (such as descending motor control or sensory-motor interactions with the cerebellum). An anatomical description of the ARAS, therefore, necessitates a description of the reticular formation in general.

The reticular formation is an extensive territory of intermingled soma, dendrites, and fiber tracts extending through the core of the brain stem from the spinal cord to the medial diencephalon and basal forebrain (Cajal 1909). It is subdivided according to the part of the brain stem it occupies into medullary, pontine, mesencephalic, diencephalic, and hypothalamic regions. The reticular core is surrounded

laterally by long ascending fibers of the classical sensory pathways, and long descending fibers of the pyramidal motor system. Embedded in the reticular substance are cranial nerve nuclei (sensory and motor), which are not generally considered part of the reticular formation, and numerous other cell aggregates which are the intrinsic nuclei of the reticular formation (see Brodal 1957). Some of the intrinsic nuclei give rise to fiber systems projecting to other reticular nuclei, or to the forebrain, cerebral cortex, spinal cord or cerebellum. Some of the rostrally projecting nuclei are thought to be the origin of cortico-petal desynchronizing pathways.

Neurons of the reticular formation have great variability in size and shape, but some general statements can be made regarding their dendritic and axonal morphology. Axons are usually very long and collateralize widely, one main branch ascending to the forebrain and another descending to the spinal cord. Along the way other collaterals of varying length and terminal pattern are directed toward other reticular nuclei, cranial nerve nuclei, and the cerebellum (Scheibel and Scheibel 1958). There is often a recurrent collateral to the region of the cell body. Scheibel and Scheibel (1958) also report a relative scarcity of short-axoned Golgi type II neurons in the reticular formation, but suggest that richly branching collaterals of long ascending and descending axons, which terminate in the vicinity of the parent cell, may constitute the

substrate for close intrareticular and recurrent interactions. Reticular dendrites are also very long processes, over 300 μm according to Ramon-Moliner and Nauta (1966), but they are relatively rectilinear and do not form bushy arborations as seen in some other structures of the brain. Dendrites freely interlace with dendrites and axons of other cells forming a dense network, or "reticulum", which is the main histological feature of the reticular formation. Both histological and physiological studies have stressed the convergence of heterogeneous afferents from sensory systems, the cortex, cerebellum, and spinal cord on small areas or single units of the reticular formation. These inputs terminate densely on both somata and dendrites.

A major cytoarchitectural feature of the reticular formation is the apparent functional distinction between the medial and lateral portions of the reticular core. The medial regions contain the largest concentration of large bodied cells, and these cells give rise to the largest number of very long, vertically oriented ascending and descending reticular fiber systems. Thus the medial region seems to serve an effector function. In the lateral sleeve of the reticular formation are smaller bodied cells, transversely oriented so that their dendrites run laterally to the areas where secondary sensory afferents enter the reticular formation, and axons run medially toward the large bodied cells. Thus these neurons seem

to serve a sensory function, conveying incoming information to the effector regions.

In summary, it appears that the general anatomy and histology of the reticular formation results in maximum convergence of input from numerous sources and divergence of output to rostral and caudal structures, and these features are consistent with its role in the modulation of global cortical EEG rhythms.

EEG and behavioral effects of electrical stimulation of the ARAS

A great advance in understanding mechanisms of the cortical EEG was the discovery that electrical stimulation of the reticular core of the brain stem could produce high frequency, low voltage EEG waves resembling those occurring naturally during wakefulness (Jasper et al. 1948, Moruzzi and Magoun 1949).

To delineate the extent of the desynchronization mechanisms, Moruzzi and Magoun (1949) delivered brief (1 - 2 ms), low voltage (1 - 3 V), high frequency (50 - 300 Hz) pulses to animals that either had brain stem transections at a spinal level or were anesthetized with chloralose. The response was an immediate and sometimes long-lasting electroencephalographic arousal similar to that produced by natural sensory stimulation. The excitable area included the ventral core of the brain stem extending from the bulbar reticular formation forward through the pontine and mesencephalic tegmentum and into

the caudal diencephalon and subthalamus. This region, which later became known as the ascending reticular activating system (ARAS), quickly became the focus of numerous anatomical and electrophysiological studies to determine its function in the control of cortical electrical rhythms and behavior.

It was also observed in other experiments that electrical stimulation of the ARAS produced behavioral arousal in unanesthetized animals (Lindsley et al. 1950, French et al. 1953, Segundo et al. 1955). Almost immediately upon stimulation a sleeping animal will awaken and look about as if having been aroused by a strong sensory stimulus. Continued stimulation may cause the animal to become agitated or pace about the cage.

The only further attempts to study behavioral effects of ARAS stimulation were studies by Fuster (1958) which showed that ARAS stimulation might improve performance by monkeys in a tachistoscopic discrimination task. But since a variety of central processes might have been facilitated by the stimulation, e. g. increased drive level or facilitation of spinal reflexes, the significance of the result is not clear.

Effects of lesions of the ARAS

Before Moruzzi and Magoun's (1949) discovery of the ARAS, it was thought that wakefulness and EEG desynchronization were sustained

by activity in the classical sensory pathways since EEG desynchronization abruptly followed a strong or sudden sensory stimulus and could be maintained by intermittent sensory stimulation. Reciprocally, sleep and synchronization were explained by passive sensory deafferentation, or the spontaneous abatement of impulses in the sensory pathways (see Moruzzi 1964b). For many years, the results of Bremer (1935, 1936) were taken as definitive proof of that hypothesis. Bremer demonstrated that transection of the brain stem at the midbrain level (the cerveau isolé preparation), which destroyed both ascending sensory and ascending reticular fibers, resulted in the cessation of EEG desynchronization and its replacement by slow synchronous potentials. The results of Moruzzi and Magoun's stimulation experiments suggested a new interpretation for that result. It was possible that the RF and not the classical sensory systems was the source of EEG desynchronizing influence.

Lesion experiments testing that hypothesis were done by Lindsley et al. (1949, 1950), French and Magoun (1952) and Sprague et al. (1961, 1963). The results clearly showed that animals with lesions of the classical sensory pathways only, exhibited normal cycles of EEG desynchronization and synchronization, while animals with lesions of the reticular core only, were permanently comatose and exhibited continuous EEG synchronization. EEG desynchronization could be elicited in these animals, but only by strong sensory stimuli, and only

for the duration of the stimulus train.

The major implication of the lesion studies was that, contrary to Bremer's interpretation, cortical desynchronization was a function of ascending reticular fibers, not of ascending sensory fibers. During the 1950's additional lesion and transection experiments, reported below, were performed to further localize the reticular structures controlling the EEG.

As just stated, a brain stem transection placed at the midbrain or precollicular level results in long lasting EEG synchronization. But a brain stem transection lower down at the junction of the spinal cord and medulla (encéphale isolé preparation) results in normal cycles of synchronization and desynchronization in the forebrain and cortex (Bremer 1935). If brain stem transections are made at points progressively rostral to the encéphale isolé, a level is reached in the midpons where cortical desynchronization is eliminated and only synchronization remains. Rossi and Zirondoli (1955) and Roger et al. (1956) had attributed this sudden disappearance of desynchronization to elimination of sensory input which enters the reticular formation through the trigeminal nerve just below that cut. But Batini et al. (1958, 1959a, b, c) showed that a carefully placed midpontine pre-trigeminal cut, even though it excluded trigeminal input, would still allow EEG desynchronization. Slightly more rostral cuts would eliminate the desynchronization. Therefore, specific reticular neurons

rostral to the central pons seemed to be essential for the EEG desynchronization response. Other evidence points to the critical involvement of the nucleus reticularis pontis oralis in the rostral pons (Moruzzi 1964a, Babb and Smith 1971, Carli and Zanchetti 1965, Jouvet 1962a, b, Camacho-Evangelista and Reinoso-Suares 1964). If this region is destroyed or separated from the forebrain, sustained EEG desynchronization by cortical (Mollica 1958) or thalamic (Schlag and Chaillet 1963 ) stimulation is no longer possible.

However, it cannot be concluded that the nucleus reticularis pontis oralis is the sole generator of EEG desynchronization since spontaneous desynchronization may reappear in high cerveau isole preparations after several days of recovery (Bastel 1960, Villablanca 1962, 1965, 1966). So some regions rostral to the mesencephalic reticular formation must also play a role in EEG desynchronization. Both lesion and stimulation data implicate the subthalamus and hypothalamus (Nauta 1946, Bach y Rita et al. 1969, Koella and Gellhorn 1954).

EEG desynchronization during sleep

Until the discovery by Dement and Kleitman (1957) and Dement (1958) of fast cortical rhythms during sleep in man and cats, EEG desynchronization was thought to occur only during behavioral arousal. It is not yet clear whether the mechanisms of waking desynchronization

and sleep desynchronization are the same, but some interesting findings may be mentioned.

Jouvet (1961, 1962, 1965, 1967) has asserted that lesions of the nucleus reticularis pontis oralis and nucleus reticularis pontis caudalis, particularly at their junction, selectively suppress REM sleep in chronic cats without significantly affecting slow wave sleep or wakefulness. More recent investigations, based on biochemical theories of the control of sleep-wakefulness and cortical EEG (see Jouvet 1967, Morgane and Stern 1974), suggest a more specific triggering mechanism in the nucleus of the locus coeruleus (Jouvet and Delorme 1965, Jouvet 1969, 1972, Roussel 1967). These authors claim that after destruction of this nucleus, also located in the pontine reticular formation, EEG desynchronization during sleep is abolished, while EEG desynchronization during wakefulness is unchanged. However, there is not yet sufficient evidence to corroborate this effect or to conclude that there is more than one reticular desynchronization mechanism.

Sensory input to the reticular formation

The above studies have demonstrated that ascending reticular output is the critical factor in the production of cortical desynchronization, since stimulation of the ARAS evokes EEG activation identical to that evoked by natural sensory stimulation, and lesions of the ARAS, but not of classical sensory pathways, abolish the desynchronization response. It is generally believed that the arousal reaction elicited by sensory stimulation represents, therefore, a phasic response of the reticular

formation to sensory input, and, in fact, that the tonic level of reticular output is largely maintained by sensory afferents. The responsiveness of reticular neurons to sensory stimulation is thus an important factor in the desynchronization process, and neurophysiological studies of the reticular formation have been concerned mainly with those sensory responses. A summary of the major findings is given below.

Before beginning that discussion it should be noted that most studies of single unit properties of neurons of the ARAS share a serious limitation, i.e. the inability the know for certain that the unit being recorded belongs to the ARAS, or if neurons belonging exclusively to the ARAS even exist. One anatomical criterion, that of long ascending corticopetal axons, is met by many of the large cells of the mesencephalic and pontine tegmentum, the area from which cortical desynchronization is most easily evoked. However, those same cells almost invariably also have long descending axon collaterals to the spinal cord and lower brain stem, suggesting that they participate in more than one reticular function. Magni and Willis (1963a, b) have tried to identify reticular neurons on the basis of antidromic responses to cortical stimulation. But such cells are few in number and the tedious intracellular recording procedure leaves little time for other physiological tests. More commonly, verification of a unit as reticular has depended on visual positioning of the microelectrode in a "desynchronization" zone, and the accessibility of the unit encountered there to a variety of peripheral sensory inputs. While this is far from a satisfactory

definition, for example, it avoids any specification of axonal projections, it probably includes a substantial proportion of units contributing to the ARAS. Even so, the difficulties inherent in differentiating reticular neurons should not be underestimated, and should be considered as limitations in the interpretation of any results obtained.

Early evoked potential studies of the RF led to the conclusion that the ARAS was diffusely organized in terms of sensory input, since each recording site gave the same response to different types of sensory stimulation (French et al. 1952, 1953, Starzl et al. 1951a, b, Bremer and Terzuolo 1952, 1953). However, later microelectrode studies showed that single reticular neurons could differ greatly in regard to the modality of stimulation to which they responded, and many reticular neurons did not respond to any modality (Scheibel et al. 1955, Baumgarten and Mollica 1954, Moruzzi 1954, Amassian and Waller 1958). It has since been firmly established that the RF, far from being diffusely organized, "should be regarded, rather as being submitted to overlapping spheres of influence" (Scheibel et al. 1955). The following review is of recent data demonstrating the anatomical and functional organization of sensory input to the ARAS.

Organization by sensory modality

Cells responsive to light flashes, movement, and changes in room illumination are generally located in the anterior mesencephalon

below the superior colliculus (Groves et al. 1973, Bach y Rita 1962, Bell et al. 1963, 1964, Horn and Hill 1966, Scheibel et al. 1955). Response latencies ranged from 15 to 110 ms, with a mean of 45.9 ms, the shorter latencies occurring in cells closest to the tectal border (Groves et al. 1973). The loci of visual cells is not surprising in view of the preferential distribution of optic tectum efferents to the dorsal rostral mesencephalon with some terminations in the nuclei of Darkschewitsch and Cajal and the dorsal lateral pontine nuclei (Altman and Carpenter 1961, Kawamura et al. 1974, Pearce and Glees 1957). The superior colliculus is considered the major, if not the only, route for visual input to the reticular formation. Fibers from the visual cortex are unconfirmed.

Auditory neurons are most often encountered in the mesencephalic-pontine region where the nucleus of the lateral lemniscus has greatest exposure to the reticular formation and where the ventral surface of the inferior colliculus merges with the tegmentum. Few or no auditory cells are found in the medulla (Baumgarten and Mollica 1954, Scheibel et al. 1955). Auditory cells differ from visual cells in having shorter response latencies (mean of 12.6 ms in Groves et al. 1973), and in being in the lateral rather than the medial longitudinal plane. Although the auditory region includes the area below the inferior colliculus, there is little anatomical evidence that auditory fibers descend from the tectum. Instead, input is primarily from nearby

second order neurons of the nucleus of the lateral lemniscus, and so the responses tend to be distributed laterally in the reticular core (Brodal 1957, Powell and Hatton 1969, Rasmussen 1961).

A special significance has been attached to the analysis of somatosensory responses in the reticular formation because of the critical role of the trigeminal influx in maintaining the tonic activity of the ascending reticular activating system (Rossi and Zanchetti 1957). Unlike visual and auditory cells, units susceptible to tactile stimulation are continuously distributed through the midbrain, pons and medulla, with concentrations in the nuclei reticularis pontis oralis and caudalis, i.e. the termini of most spino-reticular afferents and the origin of many corticopetal reticular efferents (Bell et al. 1964, Scheibel et al. 1955, Amassian et al. 1961, Amassian and Waller 1958, Lamarche and Langlois 1962, Lamarche et al. 1960, Segundo et al. 1967 a, b). The latencies of stimulus onset to first spike response ranged from 5 to 250 ms with a mean latency of 82.5 ms (Groves et al. 1973).

Until recently there was no evidence of serial representation of peripheral dermatomes in reticular tactile responses. However, Groves et al. (1973) has described an anterior-posterior somatotopic organization in the brain of the rat that corresponds to the anterior-posterior body surface. Cells responding to stimulation in the anterior third of the body were located in the anterior reticular formation or at

the entrance of the fifth nerve. Cells responding to the middle third of the body surface were located in the posterior mesencephalon and anterior pons, and the posterior third of the body was represented in post pontine and medullary reticular neurons. These distributions correspond to the differential anatomical distributions of the trigeminal and spinoreticular afferents (Lamarche and Langlois 1962, Valverde 1962, Rossi and Brodal 1957, Brodal 1957). Cells in the pons have the largest receptive fields and respond mainly to tactile stimulation of the face. The more caudal reticular cells have smaller receptive fields and respond to pain and pressure stimuli as well.

Receptive fields

In general, the receptive fields of reticular cells are larger than the fields of cells in the primary sensory pathways. Somatic receptive fields were extensive, all greater than 1 $cm^2$, according to Amassian and de Vito (1954). Twenty-five percent of all tactile cells sampled responded to one limb only, and another 25% to two or three limbs (Amassian and Waller 1958). About half of the tactile cells in the study by Groves et al. (1973) had receptive fields larger than one-third of the body surface. Similarly, reticular cells were excited by visual stimuli covering large retinal areas (Bell et al. 1964) and by auditory stimuli in a wide range of frequencies (Katsuki 1961). Ipsilateral and contralateral stimuli were equally effective for visual

and auditory responses, but somatic responses were predominantly to stimulation of the contralateral body.

Convergence

The distributions of modality specific cells overlap extensively, especially in the mesencephalon, reflecting the overlapping distribution of visual, auditory, and somatic afferent fibers. In these polysensory areas convergence also occurs at the cellular level, producing neurons that respond to two, three, or more different peripheral and central stimuli. The degree of convergence, however, is limited, some afferent systems making extensive connections and some few, and the patterns of convergence show marked variation among neurons, to the extent that cells within a few micra of each other may have no common response properties (Scheibel et al. 1955, Bell et al. 1964, Amassian et al. 1961). Estimates of the number of cells displaying convergence vary greatly from 4% (Groves et al. 1973) to 80% (Bach y Rita 1964), but these figures are not comparable because of differences in experimental procedure, regions explored, and the criteria defining a response.

Bell et al. (1964) report that about equal proportions of neurons in the mesencephalic reticular formation of the cat respond to one, two, or three modalities, or were not responsive to any stimulus. Groves (1973), however, recording from the rat, included cells from

more posterior sections of the reticular formation, where tactile and nonresponsive cells predominate; and found that 43% responded to just one modality, 4% responded to two modalities, and 5% responded to three modalities of stimulation. Groves has suggested that the type and degree of convergence in a particular cell may be predicted from its anatomical location and the class of afferents in its vicinity. Thus cells polysensory for visual and trigeminal input are most often located in the medial mesencephalon where cells responsive to visual stimuli alone and to tactile stimuli alone overlap (Bach y Rita 1964, Bell et al. 1964, French et al. 1952, Machne et al. 1955, Scheibel et al. 1955). Response combinations including the somatic modality occur lower in the pons or medulla (Scheibel et al. 1955, Amassian and de Vito 1954, Bach y Rita 1962, Groves et al. 1973, Limansky 1963, Segundo et al. 1967). Likewise, in Amassian and Waller (1958), cells responsive to tactile stimulation in combination with auditory stimulation were localized in the lateral reticular zone, while tactile and visual cells clustered in the medial reticular core. All authors agree that the mesencephalon contains the largest concentration of polysensory cells.

When convergence does occur, the two or more responses need not share common properties of receptive field size or laterality. For example, a cell may respond to photic stimuli in the entire visual field, but its somatic response is limited to the left hind limb (Bell et al. 1964).

Cells with restrictive fields in two or more modalities illustrate the striking complexity and uniqueness of afferent terminal patterns on single reticular neurons.

Habituation

A fourth distinguishing feature of reticular neurons, in addition to organization by sensory modality, receptive field characteristics, and convergence, is rapid habituation to repetitive stimuli. The limited capacity to follow repetitive stimuli contrasts with the high following rates reported for neurons in the primary sensory pathways and cortex, and is the probable explanation for the habituation of the cortical EEG arousal response demonstrated by Sharpless and Jasper. (1956) to follow repetitive auditory stimuli. Over 90% of midbrain reticular units studied by Bell et al. (1964) responded to fewer and fewer stimuli as the frequency of stimulation was increased. Most units were maximally driven by somatic stimuli of 1/2, 1, and 2 cycles per second, and in no case was the following frequency pushed beyond 10 Hz. Some cells failed even when test intervals were as large as 10 seconds. The cut off frequency and time course of the attenuation varied with modality, localization, and intensity of stimulation, and where there was convergence a cell might attenuate to each modality at a different rate. In addition, an element habituated to one afferent stimulus remains accessible to other systems converging upon it

(Scheibel et al. 1955). Like the EEG arousal response, habituated reticular sensory responses could be recovered after a rest interval of 20 - 30 seconds or by change to or imposition of a novel stimulus.

In conclusion, the above findings indicate that afferent input to the reticular formation does not result in a homogeneous distribution of sensory responsiveness as had been previously thought. Organization by sensory modality, heterogeneous convergence of two, three, or more inputs on a single neuron, and rapid habituation are newly found properties of reticular neurons that fit well with anatomical data concerning dispersion of sensory afferents to the reticular core, and with behavioral and electrographic data concerning the role of the ARAS in EEG desynchronization in arousal and wakefulness.

Mechanisms of reticular deactivation

Two mechanisms of reticular deactivation--active and passive--have been proposed to explain how the reticulocortical desynchronizing influence is periodically withdrawn, allowing synchronizing influences in the forebrain to dominate EEG rhythms. The passive hypothesis suggests that reticular activity declines because of an endogenous neural or chemical rhythmicity (Scheibel and Scheibel 1965), or because of the natural diurnal fluctuation of sensory input to the reticular formation (Magoun 1954, Moruzzi 1963 1972). While some of these passive mechanisms may be significant, the most recent investigations have followed from the active hypothesis.

The active hypothesis asserts that neural structures antagonistic to the reticular formation functionally inhibit reticular activity, possibly altering its responsiveness to sensory input. This disruption frees forebrain synchronizing structures from reticular dominance, and thus allow them to entrain cortical neurons into synchronized firing patterns.

There is a great deal of evidence suggesting that the antagonistic structures are those generating cortical synchronization, i.e. the medial thalamus and lateral preoptic area, among others. The experiments of this thesis are concerned with possible inhibitory influences descending from the medial thalamus and lateral preoptic area and altering reticular excitability. Before discussing this interaction, the anatomy and physiology, and presumed sleep-inducing capacity of these synchronizing structures is reviewed in section III of this introduction.

Dissociation between reticulocortical desynchronization and mechanisms of wakefulness

A final note on the reticulocortical desynchronization system concerns the separation of its role in EEG genesis from its more ambiguous role in the maintenance of behavioral wakefulness.

Perhaps the most powerful argument for the separation of desynchronization and waking mechanisms is the observation of sustained cortical desynchronization during REM sleep. During this stage the animal is even more deeply asleep than during slow wave sleep in the sense that

a more intense stimulus is required for arousal, yet the EEG is that of the awake animal. Clearly, mechanisms of EEG and behavior can, and do, operate independently under these circumstances.

Dissociation between EEG and behavioral states has also been produced by lesions. Feldman and Waller (1962) showed that destruction of the posterior hypothalamus in cats produced a permanently asleep or comatose animal whose cortical EEG, nevertheless, could be desynchronized by electrical or peripheral stimulation. Conversely, lesions of the midbrain reticular formation that caused EEG synchronization did not cause somnolence.

Most of the remaining relevant data come from experiments using atropine, a drug which produces EEG synchronization in a fully awake and alert animal (Wikler 1952, Bradley 1958, Podvoll and Goodman 1967). Harper (1973) has shown that the pattern of cell discharge in the cortex during atropine induced synchronization is the same as during natural sleep with EEG synchronization. That is, cortical EEG desynchronization mechanisms are definitely being blocked at the same time that wakefulness mechanisms are controlling the animal's behavior.

These studies point out certain difficulties for research based on the assumption of a single mechanism for EEG desynchronization and wakefulness. Jouvet (1967) has suggested, on the basis of data not reviewed here, that the structures responsible for behavioral

arousal are located mainly at the level of the hypothalamus, while those responsible for EEG arousal are located mainly in the reticular formation. In this context, Routtenberg (1968) has also made a case for two arousal systems located in different parts of the brain:

Just as the preceding review has concentrated on EEG desynchronization functions of the reticular formation, the following review will concentrate on EEG synchronization functions of the medial thalamus and lateral preoptic area. However, these two structures have been strongly implicated in the production of sleep as well. One of the major goals of this thesis is to demonstrate that the sleep induction findings are invalid, and that mechanisms of EEG synchronization and sleep should be regarded as independent, just as EEG desynchronization and wakefulness mechanisms are, at least until we better understand the anatomy and physiology of each.

## III. Synchronization mechanisms

### A. Thalamic synchronization mechanisms

#### General anatomy and histology

The thalamus is a large group of nuclei located in the diencephalon just anterior and dorsal to the midbrain. The many nuclei of the thalamus have been differentiated and named on the basis of their histological

appearance and anatomical connections. Although classification systems vary greatly, most authors agree that there are three classes of nuclei: specific nuclei, association nuclei, and nonspecific nuclei.

The nonspecific nuclei of the thalamus, which appear to play the most significant role in EEG synchrony, are situated in the medial two-thirds of the dorsal thalamus and include the unpaired midline bridging nuclei (reuniens, rhomboidalis, centralis medialis and periventricularis), the surrounding paired intralaminar nuclei (centralis lateralis, paracentralis, centre median and parafascicularis), and the remaining paramedian nuclei (ventralis anterior, ventralis medialis and anterior medialis). Another important member, the reticular nucleus, is a sheetlike complex surrounding the lateral and anterior borders of the thalamus.

Neurons of the nonspecific nuclei resemble those of the brain stem reticular formation in that they are multiform with radiating, poorly ramified dendrites and multibranched and collateralized axons, many of which divide into long rostrally and caudally directed components. Such axons communicate with adjacent nonspecific nuclear fields as effectively as with specific and association nuclei, and further project ahead to the basal ganglia, basal forebrain and cortex, and back into the tectum and tegmental reticular core. A wide variety of afferents converge on the nonspecific nuclei from the spinothalamic tract, reticular formation, pretectum, and orbitofrontal cortex. For

these reasons, the neuropil generated in the nonspecific nuclear fields are "among the most complex and enigmatic of the entire central nervous system" (Scheibel and Scheibel 1967, p. 67).

Electrophysiology

The existence of a cortical synchronization mechanism based in the nonspecific nuclei of the thalamus first became known through the work of Dempsey and Morison (1942a, b) and Morison and Dempsey (1943). They applied electrical stimuli of 6 - 12 Hz to the midline nuclei of the thalamus and evoked rhythmical cortical potentials of the same frequency. The induced waves were a stimulus bound sequence of monophasic surface negative potentials whose amplitude progressively increased, reaching a maximum after two to five stimuli, and then decreased. Since the waves grew larger with successive stimulus pulses, as though more and more neurons were being recruited into the process, they have been called the "recruiting response".

By conduction through the massa intermedia, the recruiting response spreads from the point of stimulation to the contralateral hemisphere, and from each hemithalamus through diffuse pathways to the cortex. The recruiting response appears generally over parietal and frontal association cortices with a latency of 20 - 40 ms and may be absent or less well developed in the primary sensory fields (Morison and Dempsey 1942). Recorded from an electrode on the

surface of the cortex, the response appears as a series of surface negative potentials. The negative waves may be preceded by brief positive waves, but the positive wave is usually attributed to inadvertent costimulation of specific sensory fibers. Recorded at a depth of 0.7 - 1.0 mm below the surface of the cortex, the recruiting response appears as a series of positive waves representing depolarization of the more superficial layers. Thus, afferents conveying synchronizing impulses from the thalamus probably synapse on the apical dendrites of cortical cells.

While repetitive thalamic stimulation will produce recruiting, the effect of a single shock is to induce an entire spindle train, which appears first in the frontal cortex and later spreads to the more posterior cortices (Morison and Dempsey 1942, Jasper 1949). The phenomenon, called "spindle tripping", displays both surface negative and surface positive waves and, therefore, is more similar than recruiting waves to naturally occurring spindles (Spencer and Brookhart 1961b).

The significance of the recruiting response and spindle tripping lie in their striking similarity to spontaneous spindling activity that occurs in the sleeping animal. The similarity has led to the hypothesis that the thalamus acts as a pacemaker to induce spontaneous spindling activity in the cerebral cortex. A great deal of experimental evidence has supported this hypothesis. First, the recruiting response has

roughly the same distribution over the cortex as spontaneous spindles, and spontaneous cortical spindling is always accompanied by spontaneous thalamic spindling (Dempsey and Morison 1942b, Andersen and Andersson 1968). Second, simultaneous spindling in the thalamus and cortex occurs even after the entire forebrain is separated from the brain stem by a precollicular transection (Bremer 1935). Also, hemithalamic lesions result in the absence of spindles in ipsilateral cortex (Kristiansen and Courtois 1949, Jasper 1949, Andersen et al. 1967 ), and complete thalamectomy abolishes all cortical spindles (Morison and Dempsey 1943, Angeleri et al. 1969), although other slow cortical waves remain (Villablance 1974). On the other hand, cortical lesions do not interfere with spindles recorded from the thalamus (Villablanca 1972, Arduini and Terzuolo 1951, Verzeano et al. 1953).

Localization of the rhythmic generator within the thalamus is a more difficult problem. While stimulation of the nonspecific nuclei evokes recruiting responses, stimulation of association and specific nuclei evokes a similar rhythmic cortical potential called the augmenting response (Morison and Dempsey 1943). A number of studies have attempted to demonstrate anatomical and physiological independence of recruiting and augmenting mechanisms (Dempsey and Morison 1943, Morison and Dempsey 1943, Hanbery and Jasper 1953, Jasper and Droogleever-Fortuyn 1947), but a larger number have attempted to demonstrate their common origin (Spencer and Brookhart 1961a, b,

Schlag and Villablanca 1967, Bishop et al. 1961, McLardy 1951). We will not elaborate on this literature except to conclude that all portions of the thalamus seem capable of generating rhythmic sequences of cortical potentials, and there may be some topographic specificity between local thalamic synchronizing circuits and their corresponding cortical projection areas (Andersen and Andersson 1968, Horvath and Buser 1972, Hanbery et al. 1954). This is not to say that medial, nonspecific thalamic structures originally implicated by Morison and Dempsey (1942) are not crucial to spontaneous cortical spindling. There is evidence that rhythmic thalamic activity may originate within the midline structures and spread to other thalamic nuclei before being relayed to cortex (Purpura and Cohen 1962, Scheibel and Scheibel 1967, Verzeano and Nigishi 1960, Andersen et al. 1967).

Intrathalamic mechanisms of synchronization

Electrophysiological studies have contributed greatly to understanding how thalamic synchronized waves are generated and propagated. Extracellular recordings show that during spontaneous or evoked cortical spindles neurons in widespread areas of the thalamus, including specific and association nuclei as well as the medial nonspecific nuclei, fire in roughly simultaneous bursts of action potentials. The bursts are separated from one another by 80 - 100 ms "silent periods", during which there is an absence of spikes (Verzeano and Negishi 1960). The

neuronal discharges correlate with the negative waves of the recruiting response or spindles recorded from the surface of the cortex, and the silent periods correspond to the interwave intervals.

Additional information, from intracellular recordings during evoked synchronization, explains how the patterns develop. Prior to stimulation, most thalamic neurons fire spontaneously and randomly. However, when low frequency electrical pulses are applied, the firing patterns become regulated. Following each pulse by 8 - 10 ms is a prominent EPSP which generates the burst of spikes. The number of spikes in the burst increases and then decreases as the surface negative potential waxes and wanes in amplitude. The EPSPs are immediately followed by prolonged IPSPs of approximately 100 ms which effectively suppress both spontaneous and evoked unit discharges. Thus, the extracellular discharge patterns can be seen to be brought about by excitatory and inhibitory postsynaptic potential sequences with an overall duration of 100 - 150 ms. This interval is the same duration as the interwave interval of the spindle and explains why evoked synchronization is optimally obtained with stimulus frequencies of 6 - 12 Hz (Purpura and Shofer 1963, Purpura and Cohen 1962, Purpura et al 1964, Purpura et al. 1966a, Maekawa and Purpura 1967a, b).

If recordings are made from several neurons in different thalamic nuclei, their EPSP - IPSP sequences will be temporally synchronized. Two models have been proposed to account for the development of

synchronous cellular activity among widespread cortical neurons. The one formulated by Andersen and Andersson (1968) involves local recurrent inhibition on the main thalamic cells through short axoned Renshaw-like interneurons. Scheibel and Scheibel (1970), who do not believe the synaptic linkages proposed by Andersen and Andersson (1968) have been adequately demonstrated in the cat, suggest instead a model based on reciprocal connections between the medial thalamic nuclei and the nucleus reticularis thalamis which surrounds the anterior pole of the thalamus. Since neither of these models has been fully corroborated by experimental findings, the internal mechanisms of thalamic synchronization are still in question.

This problem aside, synchronous neuronal discharges of thalamic neurons are ultimately conducted to the cortex (through pathways to be described below) where they in turn induce synchronous firing of cortical neurons. It appears that thalamocortical afferents terminate mainly on the apical dendrites of cortical cells and evoke synchronous depolarizations (EPSPs) seen at the surface as negative waves (Creutzfeldt et al. 1966a, b). As the excitatory activity becomes more intense, the greater the summation of EPSPs and the larger the surface potentials. Since thalamic discharges wax and wane, the surface potential waxes and wanes also, producing the recruiting response or sleep spindle. The synchronous IPSPs that appear in cortical neurons alternately with the EPSPs and generate some components of the EEG rhythm are probably

not due to afferent thalamic activity, but are a product of intracortical physiology, and may include recurrent inhibition following increased cellular discharge. In this way, an excitatory-inhibitory sequence similar to and synchronous with that producing thalamic rhythmicity is triggered in the cortex by thalamic afferents.

Pathways to cortex

The pathways mediating cortical recruitment and spindles from thalamic stimulation are still poorly understood and remain a matter of some controversy. Early attempts to discover a monosynaptic projection system failed because of the conspicuous lack of retrograde degeneration following neocortical lesions (Walker 1938, Combs 1949, Powell 1952, Waller 1934, Lashley 1941, Rose and Woolsey 1943, Murray 1966). It is possible that a fine caliber projection reaches the medial frontal cortex monosynaptically, but a more extensive system, if one exists, is not seen because of sustaining collaterals to the thalamus, brain stem and striatum (Powell and Cowan 1954, Droogleever-Fortuyn 1950, Nashold et al. 1955, Murray 1966). Anterograde degeneration techniques have also failed to show direct, monosynaptic thalamocortical afferents (Nauta and Whitlock 1954).

Nonspecific thalamic projections to cortex, therefore, have been traced electrophysiologically, and have been shown to take a circuitous polysynaptic route through several forebrain structures.

The main pathway passes through the rostral pole of the thalamus and terminates in the orbitofrontal cortex (Eidelberg et al. 1958, Kerr and O'Leary 1957, Starzl and Whitlock 1952, Verzeano et al. 1953).

The rostral pole of the thalamus includes mainly nucleus reticularis and nucleus ventralis anterior. All rostrally coursing fibers from the nonspecific nuclei perforate and collateralize in these nuclei before becoming lost in the inferior thalamic peduncle projections to the basal forebrain and orbital cortex (Scheibel and Scheibel 1967). It is not surprising, therefore, that stimulation of reticularis and ventralis anterior elicits recruiting responses as well as stimulation of the medial nuclei (Jasper 1960) and that lesions of these areas, and of the inferior thalamic peduncle, suppress both spontaneous spindle bursts and recruiting responses (Chow et al. 1959, Hanbery et al. 1954, Skinner and Lindsley 1967, Villablanca and Schlag 1968). However, reticularis and ventralis anterior are probably not the agents of corticopetal transmission, since their axons project caudally into both specific and nonspecific nuclear fields and also onto mesencephalic reticular cells (Scheibel and Scheibel 1966a, b).

Recent evidence suggests that a true relay may exist instead in the anterior limbic regions of the orbital cortex. Reciprocal anatomical connections between the medial thalamus and frontal cortex have been traced by Minzuno et al. (1969), Rinvik (1968), Waller (1940), DeVito and Smith (1964), and Nauta (1964); and reciprocal evoked

potentials have been recorded by Schlag and Villablanca (1967), Waszak, Schlag and Feeney (1970), Velasco and Lindsley (1965). Velasco et al. (1968) reported that both recruiting responses and spontaneous spindles in the cerebral cortex were totally abolished by bilateral ablation of the orbital cortex in cat. Lesions of other cortical areas not only failed to abolish the spindles, but actually enhanced spindle activity in the thalamus and remaining cortex (Villablanca and Schlag 1968). Reversible cryogenic blockade of the orbitofrontal cortex also abolished spontaneous and induced spindles (Skinner and Lindsley 1967).

Robertson and Lynch (1971) have challenged the conclusion that the orbitofrontal cortex plays a critical role in the synchronization of electrocortical activity. They point out that ablations of the frontal cortex similar to those made by Velasco and Lindsley (1965) have been shown to produce a chronic state of hyperactivity in several species that is incompatible with the appearance of cortical spindles (Brutkowski 1965, Warren and Akert 1964). In Robertson and Lynch's experiments, spontaneous spindling was absent following cortical lesions, but any condition that reduced the arousal level of the animals, such as treatment with barbiturates, extended habituation periods, or lesions of the ARAS, resulted in the reappearance of spindle activity. Similarly, Dahl et al. (1972) found orbital cortex lesions not to be effective in eliminating barbiturate induced spindles.

Therefore, the transmission pathway for thalamocortical synchronization remains unclear. The mechanism appears to have principle output through ventralis anterior, possibly via the inferior thalamic peduncle, but the destination and mode of action of those fibers have not been definitely traced further.

The recruiting response in chronic preparations

Until 1957 there was a widespread assumption that the recruiting response to low frequency medial thalamic stimulation could not be obtained in normally awake and alert animals (see Jasper et al. 1955) Then, Evarts and Magoun (1957) were successful in obtaining recruiting in unanesthetized, freely moving animals and concluded that such electrocortical potentials were not necessarily incompatible with active behavioral states.

Yamaguchi et al. (1964a) subsequently confirmed the production of recruiting responses in chronically recorded cats and further demonstrated their variability during various behavioral states. Responses during waking were either absent or of low amplitude, explaining the difficulty previous researchers had in detecting them. Recruiting responses were more easily obtained in relaxed subjects, and gained full amplitude during the slow wave stage of sleep. No recruiting responses could be obtained during REM sleep. The optimal

frequency of stimulation was 8 10 per second, higher frequencies leading to behavioral and EEG arousal. It was, and still is, believed that the thalamic synchronization mechanisms is functionally opposed to the reticular desynchronization mechanism. Therefore, the increased effectiveness of thalamic stimulation as the animal becomes more and more somnolent was attributed to the concomitant decreased influence of the ARAS over electrocortical rhythms.

'On the other hand, because recruiting responses so readily change with behavioral state, some researchers have asked whether thalamic recruiting stimulation actually induces the change in behavior. Hunter and Jasper (1949) and Evarts and Magoun (1957) reported behavioral arousal similar to that produced by ARAS stimulation as a consequence of thalamic stimulation, but it occurred only during very high intensity stimulation which may have involved a number of other brain structures. Hunter and Jasper (1949) and Yamaguchi et al. (1963) mention sleep as a response to low intensity stimulation, but the references are vague and undocumented. Roitbak and Eristavi (1966) are also noncommital about whether stimuli which produce recruiting also produce sleep. They conclude that recruiting responses vary with behavioral state and the direction of causality, if any, is not known. They also note that after a series of daily stimulations of the nonspecific thalamus, the animal became somnolent whenever it was placed in the testing cage, even though it hadn't yet

been stimulated.

In summary, experiments whose objective is to study thalamocortical synchronization in behaving animals do not generally claim that thalamic stimulation is effective in altering behavior. Other experiments, however, whose objective is to study mechanisms of sleep induction, do conclude that stimulation which produces cortical synchronization puts the animal to sleep. Some of these are described below.

Stimulation induced sleep

Several years before Morison and Dempsey (1942) discovered the capacity of the medial thalamus to control cortical EEG synchronization, the same thalamic nuclei were being studied by Hess (1929, 1931, 1932) for their capacity to evoke a variety of autonomic behaviors. For this discussion we are interested in the general patterns of sleep responses obtained by Hess by applying trains of electrical brain stimulation through fine wire electrodes to different regions of the diencephalon, a technique which Hess pioneered. A complete list of reports elaborating on the electrical sleep induction phenomenon of Hess may be found in Hess (1969). The present review is taken mainly from the following reports, symposia and monographs: Hess 1944, 1954 a, 1954 b, 1957.

The region from which sleep was said to be elicited, the so-called

"hypnogenous zone", according to Hess lies in the thalamus beginning about 2 mm lateral to the midline and extending somewhat laterally. It is located at the level of the massa intermedia and corresponds closely to the intralaminar and diffuse nonspecific nuclei. Drowsiness was said to result from the mere mechanical stimulation of implanting electrodes which occurred under nitrous oxide anesthesia. The gas was discontinued after the surgery, and the experiment performed on freely moving unanesthetized animals a few hours later.

To produce sleep only low voltage, low frequency pulses (1 - 2 V, 4 - 10 per second) of a specific rise time and duration (12.5 ms) were adequate. The low intensity and frequency were necessary to avoid costimulation of neighboring arousal structures, and the long duration pulses were thought to differentially excite parasympathetic fibers over sympathetic and motor fibers which also traverse the area. Such pulses were applied in approximately 30 second trains each minute to monopolar electrodes located in only one hemisphere. It has been reported that when such stimulations were repeated two or three times the animal showed a progressive decrease of activity, with clear signs of behavioral drowsiness followed by true physiological sleep.

The sleep was characterized by the natural curled up posture, closure of eyelids and nictitating membrane, pupillary constriction, slowing of respiration, appropriate EEG stages, etc. It was clearly

not a state such as is produced by anesthesia, coma or stupor. After the stimulation was terminated at sleep onset, the animal continued to sleep for several hours if left undisturbed, but could be easily aroused by loud noises or the smell of meat. After such an arousal the animal usually resumed its sleep without further stimulation, displaying what Moruzzi (1972, p. 93) has called "an extraordinary ingertia of the response".

Hess' results have been repeated and confirmed several times in a number of species by investigators who acknowledge the necessity for using Hess' precise methods to achieve the effect (Hess Jr. et al. 1950, 1952, 1953, Monnier 1950 a, b, Hölsi and Monnier 1962 Tissot and Monnier 1958, Monnier et al. 1960, 1962, Akimoto et al. 1956, Parmegianni 1962, Caspers and Winkel 1954). The total reversibility of the response and the absence of lesions in postmortem histological sections refute the criticisms of Ranson and Magoun (1939) and Harrison (1938, 1940) directed at Hess' early work (1929, 1931, 1932) that the lethargy resulted not from activation of a thalamic "sleep center" but from electrolytic damage to hypothalamic regions regulating wakefulness.

Yet many researchers continue to find the data and arguments for electrical sleep induction unconvincing. The long latency and persistence of the response, the lack of precise criteria defining sleep onset, and the vague descriptions of sleep in comparison to sleep-like

states of depressed activity have supported speculation that the sleep observed in Hess' and other experiments was actually a spontaneous event unrelated to the stimulation per se. Jouvet (1967) states:

> The interpretation of the results yielded by this (i. e. Hess') method indeed meets numerous difficulties . . . it is difficult to know whether sleep is induced by the stimulations or has been spontaneously produced, for most investigators agree that the occurrence of sleep is favored by stimulating a 'relaxed' animal and that it is extremely difficult to induce sleep during a state of intense alertness. . . Our personal experience, during which we have stimulated hundreds of chronic cats over a period of several years, has not convinced us that a cat, asleep after any stimulation (other than painful ones), would not have gone to sleep spontaneously.
>
> (p. 135.)

Similar opinions have been expressed by Bremer (1954), Kleitman (1963), Moruzzi (1972) and Berlucchi (1970).

In conclusion, the interpretation of stimulation related sleep is in dispute. On the one hand, a number of reports state that drowsiness and/or true sleep follows with some delay low rate stimulation of the medial thalamus. The behavioral evidence relies for support mainly on electrophysiological evidence that the same stimulation induces sleep-like synchronization in the cortical EEG. On the other hand, the sleep experiments are poorly controlled, lack objective measurements, and have not dealt with the possibility of the occurrence of spontaneous sleep. When the behavioral effects of thalamic stimulation were measured under more rigorous conditions, no differences were found

between the latency of sleep following stimulation and the latency of sleep under identical conditions without stimulation (Leisinger-Trigona and Hunsperger 1972, Hunsperger 1972).

It has already been shown in the case of the reticular activating system that a structure may function in the genesis of electrocortical rhythms and not be the driving force for the behavior usually associated with that rhythm. The same may be true concerning functions of the medial thalamus. Electrocortical and behavioral effects of medial thalamus stimulation may be dissociated, as several authors have suggested and begun to demonstrate. Experiment I of this thesis, to be described later, deals with this question.

B. Basal forebrain synchronization mechanisms

General anatomy and histology

The basal forebrain is a loosely defined region of cell bodies and ascending and descending fiber tracts at the base of the brain anterior to the hypothalamus. It includes the lateral preoptic region of the hypothalamus and portions of the anterior commissure, diagonal band of Broca, and orbitofrontal cortex.

The anatomical connections of the basal forebrain area have been traced using various histological, electrophysiological and chemical mapping techniques. They indicate that this area contributes to the

medial forebrain bundle, a major fiber system connecting forebrain and brain stem, and participates in reciprocal circuits involving limbic structures (amygdala, hippocampus and septum), the medial thalamus, the brain stem reticular formation, and the cerebral cortex. For example, Golgi studies by Scheibel and Scheibel (1967) have described a fiber system arising from the basal forebrain area, coursing caudally into the intralaminar fields of the thalamus, and terminating in the dorsomedial nucleus. Another fiber system descends from the orbito-frontal cortex and basal forebrain to terminate diffusely in specific and nonspecific thalamic nuclei. A different cortico-limbic-pontine system originates in the orbital cortex and extends through the dorsal preoptic area down into the midbrain and pontine tegmentum via structures belonging to the limbic-midbrain circuit of Nauta (Nauta 1958, 1964, Hernandez-Peón et al. 1963).

Afferent and efferent connections of the basal forebrain have been difficult to trace because they overlap with reticular, limbic, and thalamic pathways. Many of these circuits also have been found through histochemical approaches to contain catecholamines (dopamine and norepinephrine), serotonin or acetylcholine (Dahlstrom and Fuxe 1965, Shute and Lewis 1967, Jouvet 1972, Olson and Fuxe 1971, Ungerstedt 1971, Krnjevic 1969, Morgane and Stern 1974). This confluence of fiber tracts, and the lack of distinctive nuclear regions, has generally delayed investigations of basal forebrain functions.

Electrophysiology

In a series of related papers, Sterman and Clemente (1961, 1962 a, b, 1968) and Clemente and Sterman (1963, 1967 a, b) present evidence that the basal forebrain-lateral preoptic area described above is, like the medial thalamus, an area from which cortical synchronization may be evoked by electrical stimulation. In the acutely prepared cat immobilized with gallamine (Sterman and Clemente 1962 a) low frequency (5 - 7 Hz) stimulation of the lateral preoptic area and diagonal band of Broca produced an abrupt onset of widespread bilateral EEG synchronization in the frontal cortex, though frequently the response was also prominent in the occipital and parietal cortices. Synchronization was closely time locked to the stimulus train and did not continue after the stimulation had ended. Optimal results were obtained with .75 ms pulses 2 - 4 mV in amplitude. If the reticular formation was stimulated at high frequency during a train of lateral preoptic stimuli, the synchronization response was replaced by EEG desynchronization: If the reticular stimulation preceded the lateral preoptic stimulation, the findings were reversed--desynchronization was replaced by synchronization. That is, the cortical response was dependent on the order of stimulation, neither stimulus being dominant. Also, both reticular desynchronization and preoptic synchronization responses were abolished by intravenous injection of nonanesthtizing doses of Nembutal.

The forebrain synchronization response was similar in some respects, but different in others, to previous reports of thalamically induced synchronization. First, the thalamic recruiting response is characterized by a waxing and waning of surface negative potentials, while the forebrain synchronization response, while it may recruit, often attains full amplitude upon the first stimulus, and it has both surface negative and surface positive components. Second, whereas the recruiting response tends to be restricted to nonspecific association cortices, the forebrain synchronization response always appears bilaterally in both association and primary sensory fields and is most prominent over the occipital cortex. Third, intravenous injection of Nembutal abolishes the forebrain synchronization response, and the reticular desynchronization response, but such barbiturates have no effect on, or may even enhance, synchronization produced by medial thalamic stimulation. This finding suggests that the physiological basis for forebrain synchronization may be intimately tied to reticular mechanisms of desynchronization, while the thalamic synchronization mechanism is relatively autonomous. Finally, single pulses to the medial thalamus may produce entire spindle trains resembling spontaneous sleep spindles. But the effects of single lateral preoptic pulses have not been investigated.

The EEG synchronization response to low rate stimulation was also reported to occur in freely moving, unanesthetized animals

(Sterman and Clemente 1962 b) However, examination of the published records reveals some discrepancies. For example, the synchronization "response" to 6 Hz lateral preoptic stimulation (p. 106, Figure 2) begins several seconds after the onset of stimulation, not immediately as in the acute experiments, and continues beyond the end of stimulation. Also, synchronization is clearly evident in both stimulus and interstimulus intervals. Therefore, it appears that the synchronization may be spontaneous and not related to the stimulation since it is also prevented by excessive sensory stimulation, food deprivation, or emotional agitation of the subjects.

Sterman and Clemente (1962 b) claim that high frequency stimulation of the lateral preoptic area (up to 250 Hz) is also effective in producing low frequency synchronization of the EEG. Such an effect (low frequency response to high frequency stimulation), if reliable, is very rare in the literature (see Doty 1969). However, again, the published record (p. 110, Figure 6) does not fully corroborate the report. It can be seen that the EEG synchronization begins over 20 seconds after the onset of stimulation, and looks as if it occurred spontaneously as the cat was falling asleep.

In conclusion, there is clear evidence that low frequency lateral preoptic stimulation will evoke sustained and recruited synchronization in the cortical EEG in acute and possibly in chronic preparations. However, responses to high frequency stimulation are ambiguous.

Also, reports that lateral preoptic induced synchronization is conditionable to an auditory signal (Clemente et al. 1963) are open to criticism. (Attempts by Seigel and Yamaguchi to condition thalamocortical recruiting responses apparently have failed--see Clemente et al. 1963.) Nevertheless, there is sufficient reason to believe, on the basis of the low frequency effects, that the basal forebrain-lateral preoptic area is involved in mechanisms of the EEG, and that it is reciprocally organized with respect to the brain stem reticular arousal system, since their responses interact. Whether the interactions occur in the cortex, or between the forebrain and reticular formation, will be discussed later.

Stimulation induced sleep

Following the report of induced EEG synchronization (Sterman and Clemente 1962 a), the same authors reported that bilateral stimulation of the same basal forebrain area, in unanesthetized, freely moving cats, was followed by the appearance of fully integrated behavioral sleep. The effective stimuli were identical to those producing synchronization, i.e. trains of brief (.75 ms), low intensity (1 - 3 V), low or high frequency (5 - 250 Hz) electrical pulses. Following the onset of stimulation the subject was reported to cease ongoing behavior, retreat to a comfortable corner of the cage, and assume a reclining

posture. Only if left undisturbed would this behavior progress to true sleep. One prolonged or several short periods of stimulation were sufficient to achieve the effect which was accompanied by the appropriate appearance of sleep spindles and slow waves in the cortical EEG.

The latency of the sleep effect was frequently, though not consistently, lower than that observed in Hess' experiments following medial thalamic stimulation. Latencies as short as 5 - 30 seconds are mentioned, but the authors state that the observations were necessarily qualitative, and no statistics of latencies are supplied. Also, sleep durations were not measured or used as criteria for the response, though it was observed that occasionally the subject awoke after less than one minute of sleep and stimulation had to be reapplied.

Like the synchronization response, the sleep response is reported to be sensitive to several extraneous conditions. Satiation, fatigue, or a general lack of disturbing influences enhanced the induction of sleep. Also, time of day, time of last feeding, and prior experience with the stimulation could affect the latency. Apparently, individual differences play a role, since the authors state that some animals became increasingly indifferent to stimulation while others became model subjects. Some subjects also developed a conditioned response to the experimental chamber, falling asleep in it even before stimulation was applied.

Sleep following stimulation was accompanied by a suppression of a number of somatic, autonomic, and endocrine functions, which are suppressed naturally with sleep, and many of which had previously been observed upon stimulation of basal forebrain sites (Hess 1954, 1957, Kaada 1951, Grastyan et al. 1953). This finding supported the hypothesis that basal forebrain synchronization and sleep zones were part of a general forebrain mechanism for inhibition and suppression of behavior. It was proposed that the basal forebrain area may function antagonistically to the brain stem reticular activating system described by Moruzzi and Magoun (1949) in producing cycles of synchronization and desynchronization, and cycles of sleep and wakefulness. The relationship between the basal forebrain synchronizing zone and other synchronogenic mechanisms, in the thalamus for example, required further investigation. The above data and interpretations may also be found in Clemente and Sterman (1963, 1967 a, b) and Sterman and Clemente (1968, 1974).

Other data from lesion, thermal, and chemical stimulation experiments have been used to corroborate the evidence for stimulation induced sleep. They are summarized briefly below.

Lesion studies

McGinty and Sterman (1968) confirmed in cats Nauta's (1946) observation in rats that anterior hypothalamic transections, i.e.

separation of the basal forebrain from more caudal brain stem structures by destructive knife cuts, severely altered the sleep-waking cycle. Various degrees of chronic sleep loss were observed, which in some cases were so extreme as to lead to death. These findings are in accord with previous reports by von Economo (1929) that clinical cases of insomnia in humans were related to pathological lesions of the basal forebrain.

In McGinty and Sterman (1968) experimental hyposomnia, produced in 10 cats by preoptic-basal forebrain lesions, began about three days after surgery and reached a peak after about two weeks. At that time, sleep was totally abolished in some subjects. Continuous observations revealed that these animals were constantly standing or walking in their cages, and even after collapsing, apparently from exhaustion, continued to display EEG and EMG patterns of wakefulness. In addition, responses to all modes of sensory stimulation were markedly depressed. Sleeplessness persisted after transient temperature and feeding irregularities had passed, and in three subjects resulted in death four to eight weeks after the surgery.

The animals with less extreme sleep loss survived the critical period at about two weeks and began showing increasing amounts of sleep by four weeks. REM sleep, which was all but abolished in all subjects when slow wave sleep fell to 15% - 5% of the recording time,

also recovered in these subjects when the amount of slow wave sleep exceeded that minimum. The gains in sleep time occurred only after spontaneous feeding had reappeared and body temperature returned to the normal range.

Histological analyses confirmed that the deaths occurred in subjects with the largest bilateral lesions of the medial forebrain bundle, supraoptic nucleus, lateral preoptic nuclei, and inferior thalamic peduncle. Surviving subjects sustained less extensive damage. Eight subjects in which control lesions were placed outside the basal forebrain area did not exhibit severe changes in sleeping behavior. Similar results have been reported by Sterman et al. (1964) and Madoz and Reinoso-Suárez (1968).

McGinty and Sterman (1968) conclude that lesions of the rostral preoptic region and of the basal portions of the diagonal band of Broca caused a significant increase in wakefulness and decrease in sleep possibly because the lesions released the brain stem reticular activating system from antagonistic control by descending basal forebrain influences. These conclusions assume that destruction of sleep related structures, and not lesion-induced irritation of surrounding tissue was responsible for the results. The assumption is consistent with other reports that damage to basal forebrain regions produces a general hyperactivity marked by increased waking time (Warren and Akert 1964, Butter 1969, Koranyi 1965, Hernandez-Peón and Sterman 1966). The phenomenon

of recovery, on the other hand, suggests that the lesions did not completely inactivate sleep inducing mechanisms, which seem to have a wide distribution. The deaths in the most extensively lesioned animals may have resulted from disruption of hypothalamic homeostatic functions rather than from acute sleep loss. Therefore, the effects of basal forebrain lesions support but do not fully confirm the hypothesis that the basal forebrain area has a critical sleep inducing function.

Thermal stimulation studies

Roberts and Robertson (1969) showed that warming of the preoptic region and of the anterior hypothalamus with radio-frequency stimulation produced sleep-like behavior in the freely moving cat. When radio-frequency currents were adjusted to 25 - 30% below threshold for tissue damage, the animals would lower their heads, lie on the floor in a posture similar to that described by Sterman and Clemente (1962 b) following electrical stimulation of the same region, and close their eyes. The nictitating membranes relaxed, the pupils constricted, and slow waves appeared in the cortical EEG. The latency of the effects were in the order of 20 - 150 seconds, and the behavior outlasted the duration of stimulation by about the same amount. Application of 60 Hz electrical stimulation through the same electrodes never produced sleep, but rather elicited sniffing, locomotion, pilo

erection, hissing, jumping, and circling. The anatomical location for the diathermic sleep response was restricted to the basomedial anterior hypothalamus, stimulation of surrounding loci being ineffective.

The preoptic region studied in the above experiment had already been established as a thermoreceptor zone. Electrical stimulation or warming of the area typically produces panting, grooming, and other heat dissipating behaviors (Hess 1954, Andersson et al. 1956), and specific heat sensitive neurons have been identified there (Nakayama et al. 1963, Wit and Wang 1968). Apparently, thermoregulatory and sleep related structures overlap in this area, because several other authors have reported the thermal sleep induction phenomenon, in cats (Freeman and Davis 1959), the oppossum (Roberts et al. 1969), and in the dog (DeArmond and Fusco 1969).

The literature on the interrelationships between sleep and thermoregulation has been reviewed by Parmeggiani and Rabini (1970). There is some evidence that peripheral and humoral stimuli activating thermal receptors in the brain are also important for the facilitation of sleep produced by a warm environment, e. g. a close relationship exists between ambient temperature and total sleep time (Parmeggiani et al. 1969). On the other hand, sleep and thermoregulation are probably produced by separate physiological oscillators, since the circadian sleep-waking cycle and the circadian temperature cycle, though normally phase locked, can be decoupled under conditions of

sensory isolation (Aschoff 1969).

In summary, the sleep and EEG synchronization induced by thermal stimulation in the preoptic forebrain area indicate that temperature regulation and sleep regulation may involve overlapping neuronal systems, and that one of the normal physiological inputs to the basal forebrain sleep mechanism may be thermoreceptor discharge.

Chemical stimulation studies

Hernandez-Peón (1962) independently reported the induction of behavioral sleep by electrical stimulation of the lateral preoptic area of the forebrain in the same year of Sterman and Clemente's report (1962 b). In addition, he demonstrated that application through cannulae of minute amounts of crystalline acetylcholine (ACh) or carbacol to the same lateral preoptic area was followed by the same behavioral and EEG signs of sleep within 2 - 5 minutes. The chemically-induced sleep lasted up to one hour, but the animal could be aroused during that time by a strong auditory stimulus. The chemical specificity of the effect was demonstrated by the fact that adrenaline, noradrenaline, gamma-amino-butyric acid, and strychnine sulphate similarly placed did not elicit sleep (see also Hernandez-Peón and Chavez-Ibarra 1963, and Hernandez-Peón et al. 1963, 1967).

The cholinergic pathway for sleep was shown by Hernandez-Peón et al. (1963) to be descending, since small electrolytic lesions, or

atropine, placed caudal to the point of ACh stimulation abolished the effect (Velluti and Hernandez-Peón 1963). The pathway closely corresponded to the limbic-midbrain circuit described by Nauta (1958), i. e. it followed the trajectory of the medial forebrain bundle from the preoptic region through the lateral hypothalamus and into the midbrain area surrounding the ventral tegmental area of Tsai. Based on these findings, Hernandez-Peón (1965) proposed a neurochemical theory of sleep-waking regulation involving descending inhibition of the ARAS very similar to the proposition of Sterman and Clemente (1962 b). In fact, Hernandez-Peon suggested the descending ACh pathway was the substrate for the reported sleep inducing effects of electrical basal forebrain stimulation.

Although Cordeau et al. (1963), Morgane (1969), Yamaguchi et al. (1964b), and Sterman and Clemente (1968) have replicated the sleep induction effects of ACh, cholinergic stimulation of many loci in the preoptic region has failed to induce sleep in experiments by Myers (1964) and MacPhail and Miller (1968). General arousal and defensive behavior was elicited instead, as it was when Hernandez-Peón (1962) applied ACh to points in the midbrain. MacPhail and Miller (1968) reject the possibility that the discrepant results were due to differences in dosage, technique, or site of stimulation, and suggest instead that the sleep Hernandez-Peón observed with preoptic ACh stimulation was spontaneous. Spontaneous sleep also may have followed carbacol

stimulation in Hernandez-Peón s experiments, for Sterman and Clemente (1968) and Yamaguchi et al. (1964b) cout not produce sleep with this drug.

Another problem for Hernandez-Peón's theory is that the cholinergic pathway he describes is descending, but Shute (1970) maintains that ACh fibers only ascend in the medial forebrain bundle (Shute and Lewis 1966, 1967, Lewis and Shute 1967). Shute (1970) suggests that a more probable explanation for Hernandez-Peon's results is that there is a noncholinergic descending pathway containing relays in which transmission can be modified by ascending cholinergic input. Since these speculations are vague, and the possibility remains that the observed sleep was spontaneous, we must conclude that chemical stimulation studies do not contribute greatly to the position that the basal forebrain region is hypnogenic.

In summary, data from lesion, thermal, and chemical stimulation experiments usually offered as corroboration of the basal forebrain sleep induction phenomenon all give rise to problems of interpretation. Yet sleep induction through forebrain electrical stimulation continues to be generally accepted as a valid effect. Therefore, Experiment I of this thesis will test the hypothesis of basal forebrain sleep induction, as well as the hypothesis of medial thalamic sleep induction, using controls for spontaneous sleep.

IV. Interactions between synchronizing and desynchronizing systems

The preceding information regarding EEG synchronization and desynchronization mechanisms provides the context in which interactions between synchronogenic and desynchronogenic systems may be discussed. At the outset, it should be noted that relatively little is known about these interactions even though many reviews of cortical electrogenesis refer to them (Moruzzi 1972, Elul 1972, Parmeggiani 1968, Desiraju 1971, Bremer 1972, Sterman and Clemente 1968). To date, most work has been done on the influence of desynchronizing neurons over synchronizing neurons, i. e. the influence of ascending reticular formation efferents on neuronal activity in the medial thalamus and basal forebrain. These influences will be discussed below.

More relevant to the experiments of this thesis, and less frequently studied, is the reciprocal influence of medial thalamic and basal forebrain efferents over neuronal activity in the reticular formation. So far, a number of anatomical and electrophysiological studies have demonstrated descending input from thalamus and forebrain to brain stem, and some of this input has been reported to suppress reticular electrical activity. These studies will also be discussed below.

Ascending reticular influences on thalamic and basal forebrain neurons

The most comprehensive analysis of ascending reticular projections was made by Scheibel and Scheibel (1958, 1966, 1967, 1970). Golgi stained material indicated that reticular projection fibers, mainly from the medial magnocellular regions, ascend in the midbrain to a point just behind the centre median-parafascicular complex of the medial thalamus. There the system bifurcates into a shorter dorsal leaf which ascends to the intralaminar and medial nuclei of the thalamus, and a longer ventral leaf which takes a ventral route through the subthalamus and continues on to the basal forebrain (see Figure 2). A number of other anatomical and evoked potential studies are in close agreement with these anatomical data (Starzl et al. 1951, Rossi and Zanchetti 1957, Nauta and Kuypers 1958, Minderhoud 1967, Fuller 1975, 1976, Chi 1970, Dila 1971, Lynch et al. 1973, and Robertson et al. 1973). Both the dorsal and ventral systems, described more fully below, probably function in reticulocortical desynchronization, but possibly through different mechanisms.

Ascending reticular fibers in the dorsal system can be followed into the intralaminar thalamic fields where they collateralize widely and terminate along the dendrites of thalamic neurons. Their function has been described by Purpura and his associates (see Purpura 1969, 1970), whose experiments, in part, reproduce the classical experiments

of Moruzzi and Magoun (1949) in which high frequency brain stem stimulation resulted in EEG desynchronization and the suppression of thalamically evoked cortical recruiting responses. Purpura and Shofer (1963) and Purpura et al. (1966b) found that during the transition from cortical synchronization to cortical desynchronization, the sustained IPSPs in thalamic neurons that determine the frequency of cortical slow waves are blocked, and there is also a marked enhancement of the excitatory synaptic drive on thalamic neurons resulting in sustained EPSPs as well as prolonged postexcitatory facilitation. These and other experiments indicated that IPSPs were blocked because reticular afferents inhibited the interneurons involved in the production of IPSPs in thalamic neurons. Thus, the change from cortical synchronization to desynchronization may be described as disinhibition imposed by reticular fibers on thalamic processes. As yet, however, the exact organization of this influence is not fully known. The reticular disinhibitory effect could be exerted through small local interneurons (Mancia et al. 1974, Fukada and Iwama 1971), or through neurons of the thalamic reticular nucleus (Scheibel and Scheibel 1970, Schlag and Waszak 1971, Filion et al. 1969, Massion 1968, Yingling and Skinner 1975). It should be noted, however, that the silencing of inhibitory neurons does not necessarily require post-synaptic inhibition, but may result from the temporal dispersion of excitatory PSPs so that

the inhibitory cells reach firing threshold less frequently.

The ventral projections of the reticular formation to the basal forebrain region, which also play a role in the desynchronization process, are far more diverse and complex than the dorsal projections to the thalamus. They ascend through the zona incerta and hypothalamus and continue forward into the fields of the preoptic and basal forebrain regions. About 10% of these fibers may reach cortical neurons directly while the majority reach these levels through one or more synapses (Scheibel and Scheibel 1958). The same authors (Scheibel and Scheibel 1967) believe that the ventral route is the most effective pathway for cortical desynchronization. Nauta (1946) had previously associated this pathway, leading to the basal and medial parts of the telencephalon as well, with the ARAS and mechanisms of wakefulness, and Magoun (1954) has noted that EEG desynchronization is most effectively evoked by stimulation of this pathway. Lynch et al. (1973) have traced a similar pathway with anterograde degeneration techniques. After unilateral lesions of the medullary-pontine reticular formation, degenerating axons were found through the subthalamus into the striatal complex, with a few fibers reaching deep layers of the frontal pole. They interpret their results as evidence of a large monosynaptic reticular-telencephalic pathway, slightly dorsal and lateral to the trajectory of the medial forebrain bundle, which, it is reasonable to assume, is the pathway of the diffuse ARAS.

Unfortunately, there is little electrophysiological data relating to these fibers (see Fuller 1975, 1976). The chemical anatomy of ascending reticular projections to the basal forebrain has also been described by Morgane and Stern (1974), Shute and Lewis (1966), Dalstrom and Fuxe (1964) and Jouvet (1972).

In summary, the neurophysiological substrate for the EEG desynchronization response to high frequency brain stem stimulation is not yet clearly understood. One reticular output pathway through medial thalamic synchronogenic neurons inhibits their capacity for generating long duration IPSPs and, therefore, functionally inhibits cortical synchrony. The resulting cortical desynchronization may simply be a passive result of suppression of the thalamocortical synchronization mechanism. A second output pathway through the basal forebrain region may exert a more direct and active control of cortical neurons that results in their desynchronized firing patterns. However, both the anatomy and physiology of this pathway are poorly understood, and the effect of desynchronizing impulses in this pathway on neurons of the synchronogenic regions through which it passes are equally unclear.

Descending thalamic influences on reticular neurons

It will be recalled that low frequency stimulation of the medial thalamus evokes cortical synchronization in the form of recruiting

responses and spindles (Morison and Dempsey 1942). Yet cortical desynchronization may also be produced by stimulation of the same neurons with high frequency pulses, much as high frequency reticular stimulation produces cortical desynchronization (Moruzzi and Magoun 1949). Apparently, the output pathways of the medial thalamus are frequency specific. Scheibel and Scheibel (1967) suggest that the reticular nucleus of the thalamus acts as a "band pass filter", allowing only the low frequency impulses to pass forward from the thalamus into the inferior thalamic peduncle and ultimately to the cortex. The question then remains of where the high frequency desynchronizing impulses are shunted.

It was determined by Schlag and Chaillet (1963) and Weinberger et al. (1965) that the pathways for thalamically induced desynchronization are separate from the synchronization pathways, and in fact pass back into the mesencephalic tegmentum since tegmental lesions caudal to the point of stimulation abolish the desynchronization response. Scheibel and Scheibel (1967) suggest that high frequency medial thalamic stimulation either 1) antidromically activates the ascending dorsal projections of the ARAS back to the point of bifurcation with the ventral leaf, and from there activity is carried forward through the ventral leaf to the basal forebrain, or 2) orthodromically activates axons descending from the medial thalamus to the reticular tegmentum. While there are other

interpretations as well, we are concerned here with the evidence for thalamic descending fibers, since we will, in Experiment II of this thesis, investigate the effect of their stimulation on reticular responses.

Actually, few anatomical data are available regarding descending thalamic efferents to the brain stem (see Figure 2). Early researchers described a variety of descending fibers impinging on tegmental structures, only some of which may have been from the thalamus (Clark 1932, Clark and Boggon 1933, Nauta 1958, Hugelin and Bonvallet 1957). Scheibel and Scheibel's (1967) field analysis of nonspecific thalamic nuclei shows that thalamic efferents, like reticular efferents, branch extensively making connections with both more rostral structures (basal ganglia, basal forebrain and cortex, among others), and with more caudal structures, one of which was very likely the mesencephalic reticular tegmentum.

Despite the sparse anatomical data, a variety of electrophysiological responses, including recruiting responses and spindles, may be evoked in the reticular formation by thalamic stimulation (Schlag and Chaillet 1963, Schlag and Faidherbe 1961), and recent extracellular and intracellular recordings have demonstrated actual synaptic contact between thalamus and brain stem.

For example, Purpura (1970) reports that low-frequency medial thalamic stimulation evokes predominantly depolarizing EPSPs in

mesencephalic reticular neurons. A unique feature of the EPSPs was that they were not the high amplitude, smooth, long duration transients that would typically be evoked in thalamic, caudate, or cortical neurons, but instead were composed of small, irregular summating responses. This variability was most apparent when displayed during the injection of hyperpolarizing currents. Another feature of the response was its variable and rather long latency (10 - 20 ms). The temporal dispersion of the responses in relation to the stimulus, their variable amplitude and duration, and their irregular components all reflect an extraordinary complexity of interneuronal pathways from the medial thalamus to the reticular formation.

Excitation was also the major feature of intracellular responses elicited from 50 - 75% of impaled reticular neurons in studies by Grantyn et al. (1971) and Mancia et al. (1974). EPSPs with spikes appeared with latencies of 2 - 10 ms but were quite variable, and earlier and later (20 - 25 ms) EPSPs were also seen. No inhibition was ever observed in mesencephalic units during low frequency thalamic stimulation. A small number of neurons responding with spikes at .7 - 1.3 ms were considered to have been fired antidromically. The short latencies of some reticular responses suggested mono- or paucisynaptic thalamo-reticular projections although longer pathways (possibly through the cortex) could not be excluded.

It is interesting to compare the effects on reticular neurons of low rate thalamic stimulation which synchronizes cortical and reticular EEG and high rate thalamic stimulation which desynchronizes it. Mancia et al. (1974) show that with low rate stimulation EPSPs can be seen increasing in amplitude with each pulse as the amplitude of the recruiting waves builds up. The EPSPs synchronous with the recruiting responses are, consequently, also acting in phase with thalamic neurons, and, therefore, presumably less accessible to their other desynchronizing inputs. The thalamus thus exerts some suppression over the reticular activation process. On the other hand, high rate thalamic stimulation produces shorter latency EPSPs and a general increase of firing rate. In this case the thalamus is facilitating the desynchronization process by itself activating reticular neurons.

In summary, electrophysiological and anatomical data establish the existence of reciprocal thalamic-reticular relations. In many ways the relationship appears to be functionally antagonistic. In the rostral direction, reticular input to the thalamus disrupts the thalamic synchronization process and so expedites the reticular desynchronization process. Caudally, thalamic fibers have excitatory influences on reticular neurons which may be used to synchronize reticular firing patterns and so prevent the desynchronization process.

Descending basal forebrain influences on reticular neurons

Localized lesions of the basal forebrain area from which EEG synchronization can be elicited yield fiber degeneration extending caudally to the hypothalamus and ventral tegmental regions, and to some reticular nuclei (Scheibel and Scheibel 1967, Mizuno et al. 1968 a, 1969a, b). Electrical stimulation of the same area evokes short latency evoked potentials in the nucleus of the posterior commissure and in the mesencephalic reticular formation, as well as in limbic nuclei and in the medial thalamus (Clemente and Sterman 1967). These results suggest the involvement of the basal forebrain in a "limbic loop" with septum, amygdala, and hippocampus, and in a descending pathway to the mesencephalic brain stem (see Figure 2). Clemente and Sterman (1967) proposed that the descending pathway actively suppressed reticular desynchronization mechanisms during evoked cortical synchronization. Essentially, they were suggesting a substrate for the long-sought mechanism of active reticular deactivation (see Dell et al. 1961, Dell 1963, Moruzzi 1963).

Bremer (1970) was the first to test that hypothesis. He found that stimulation of basal preoptic regions in the encéphale isolé preparation evoked short latency, positive-going field potentials in the brain stem tegmentum correlated with a cessation of spontaneous activity. The positive deflection, lasting from 20 - 60 ms, was generally

preceded by a small, sharp negative potential and burst of spikes. Another slow negative wave followed the positive wave like a post-inhibitory rebound. The positive response was elicited along the whole vertical extension of the mesencephalic reticular formation, and had the same latency and threshold as Clemente and Sterman's (1967) evoked potential (see also Bremer 1972, 1973, 1975).

Several properties of the positive wave led Bremer to characterize it as the extracellular counterpart of postsynaptic inhibitory processes which, by depressing the arousal system, would facilitate thalamocortical synchronization circuits. First, it was correlated with a suppression of multiunit spike discharge while the subsequent negative wave was associated with increased unit activity. Second, a small negative-going reticular response to auditory stimuli was generally suppressed when preceded by a conditioning pulse to the preoptic region, the response reduction being maximal at test intervals of 20 - 40 ms. Interestingly, the auditory suppression effect could be evoked at test intervals exceeding the decay of the positive wave of the preoptic response. Third, physiological or amphetamine arousal tended to reduce the positive wave, while light barbiturate anesthesia prolonged it, as would be expected if the positive wave represented an antagonistic input to the reticular formation.

However interesting these results may be, Bremer's conclusions

far exceed the strength of the data, since the data lack intracellular or even close extracellular recordings. Also, the encephale isole preparation is not suitable for studying complex neural interactions which very likely depend on an intact brain.

Lineberry and Seigel (1971), who had previously studied the effects of caudate stimulation on mesencephalic unit activity (Siegel and Lineberry 1968), extended their investigations to the effects of caudate, basal forebrain, and orbital cortex stimulation on the same neurons in chronic preparations. They found that caudate stimulation was by far the most effective of the three, producing responses in 92% of cells sampled. Fifty-eight percent of those responses consisted of a clearly definable and immediate suppression of spontaneous unit activity lasting 100 - 250 ms, replicating the results obtained in acute preparations. In contrast, orbital cortex stimulation affected 62% of reticular cells tested, and basal forebrain (lateral preoptic) stimulation affected 54%. Those responses were evenly distributed among five response categories: stimulus locked facilitation, stimulus locked inhibition, facilitation followed by inhibition, inhibition followed by facilitation, and multiple cycles of facilitation and inhibition. Cells influenced by one or more stimulation sites were uniformly distributed in the mesencephalic tegmentum. The results were thought to be, in general, consistent with Clemente's (1968) proposition that the basal

forebrain system acts reciprocally with the reticular activating system.

Also of interest was the finding that the mesencephalic unit effects of forebrain stimulation occurred at the same time as induced cortical EEG synchronization and inhibition of behavior (cessation of eating). The close association of the three responses, and the fact that they occurred at the same threshold values, suggested that modulation of midbrain units is functionally related to the behavioral and EEG responses. Also, basal forebrain sites which did not produce either EEG synchronization or behavioral arrest had little effect on reticular unit activity.

However, the lack of a predominant response type in the reticular unit responses makes it difficult to assess the nature of the forebrain influence. The simplest conclusion, stated by Lineberry and Siegel, is that the modulation is in the form of a nonspecific disruption of ongoing unit firing patterns rather than a purely inhibitory drive, as suggested by Bremer (1970), although a number of units did seem to be strongly inhibited. The response types also were not distributed differentially in the reticular formation, and in fact could be found in nonreticular tegmental structures as well. This is not surprising, since it is known that the basal forebrain projects to brain stem centers for autonomic and somatic reflexes as well as to the reticular arousal system (Clemente et al. 1966, Sauerland et al. 1967, Nakamura et al. 1967).

Taken together, the results of Lineberry and Seigel (1971) demonstrate a diffuse influence of forebrain synchronizing structures over single unit activity of the brain stem. If an antagonistic relationship exists, it was manifest in their study only by a generalized disruption of unit firing patterns. The close correspondence among stimulus thresholds for single unit, EEG, and behavioral effects lent support to the hypothesis that the unit disruption is of functional significance in the generation of cortical slow waves and behavioral inhibition.

Because in the foregoing experiments forebrain influences were extended not only within the reticular formation but in the surrounding brain stem nuclei, Siegel and Wang (1974) explored the possibility of an even wider distribution of forebrain efferents. In their experiments, up to 87% of cells contacted in the visual cortex, intrinsic, extrinsic and association thalamic nuclei, subthalamus and hypothalamus showed either an initially facilitatory or initially inhibitory response to stimulation of the caudate nucleus, basal forebrain or orbital cortex. Sixty-three percent of those responses had latencies less than 5 ms. A typical response pattern consisted of a short latency unit discharge followed by a variable period of inhibition which tended to lengthen with higher stimulus intensities. Positive going field potentials correlated with the silent periods, and rebound facilitation was often observed.

There were no obvious differences in response type as a function of the cells' location. Convergence was a common occurrence since 59% of cells responded to basal forebrain as well as orbital cortex stimulation, and 40% responded to those as well as caudate stimulation. As a control procedure, forebrain sites not producing EEG synchronization were also stimulated, and they produced responses in only 6% of the cells. This analysis implies that all three structures participate in a widespread though rather specific forebrain synchronizing system. In one respect it contradicts the notion that forebrain synchronization is achieved by reticular deactivation, since many other neurons in other brain structures are similarly affected, yet it is consistent with the underlying concept that cortical synchronization requires an active alteration of subcortical neuronal processes.

In conclusion, recent investigations into the cellular basis of forebrain induced synchronization have generally failed to demonstrate a true synaptic inhibition of reticular neurons as predicted by the theories of Sterman and Clemente (1968), Bremer (1971), and Moruzzi (1972). There is, however, indisputable evidence of forebrain projections to the reticular formation which alter the firing patterns of large numbers of reticular cells. Therefore, Experiments II and III of this thesis further investigate basal forebrain influences in the reticular formation, particularly in regard to possible postsynaptic

effects on reticular neuronal excitability.

The present experiments

From the above literature review, it is clear that many questions remain concerning neural mechanisms of the cortical EEG and its alternation between synchronized and desynchronized states. A serious obstacle to answering those questions has been the uncritical identification by many authors of mechanisms of synchronization and desynchronization with mechanisms of sleep and wakefulness. For example, it is common to find in the literature reports in which inferences about neural bases of the EEG are drawn primarily from behavioral data concerning sleep or wakefulness, and vice versa. In order to facilitate research on the EEG, the degree to which EEG and behavioral mechanisms are related at the neuronal level should be clarified.

The purpose of Experiment I of this thesis is to determine whether neurons known to trigger cortical EEG rhythmic synchronization also play a crucial role in the induction of sleep. Specifically, an attempt is made to replicate studies by Hess (1944, 1954, 1957) and by Sterman and Clemente (1962 b, 1967) which claim that electrical stimulation of the medial thalamus (MTH) and lateral preoptic area (LPO) of cats induces behavioral sleep. A critical inadequacy in those experiments was the failure to control for spontaneous sleep. The present findings

are that while low frequency MTH and LPO stimulation is effective in evoking rhythmic cortical synchronization, it is not followed by sleep having a significantly shorter delay or longer duration than naturally occurring sleep. This result strongly suggests that thalamic and preoptic synchronogenic areas should no longer be considered as sleep centers, and, more importantly, that EEG synchronization and behavioral sleep be recognized as having separate, if not independent, physiological bases.

Experiments II and III also deal with MTH and LPO synchronogenic mechanisms and examine the hypothesis that they act reciprocally and antagonistically with respect to desynchronization mechanisms in the brain stem. The work of Sterman and Clemente (1967), Bremer (1970, 1972, 1973, 1975) and Lineberry and Seigel (1971) suggests that thalamic and preoptic input to the reticular formation of the brain stem results in inhibition of the spontaneous activity of reticular neurons. The present experiments attempt a more direct test of the effects of MTH and LPO stimuli on reticular sensory responses. We found in Experiment II that the number of spikes generated by reticular neurons in response to visual or auditory stimuli could be both increased and decreased by prior conditioning pulses to MTH, or LPO, depending on the relative timing of the two stimuli. Since the facilitatory and inhibitory effects may have occurred within the recorded neurons, or

at one or more other levels in the multisynaptic pathway between forebrain and brain stem, intracellular recordings were made from reticular neurons in Experiment III to directly measure reticular excitability changes. Some evidence was found that LPO and MTH influences act directly on reticular cells. These results are interpreted in the context of theories of reticular deactivation.

## Experiment I

### Background

Several authors have reported that using certain techniques of electrical brain stimulation it is possible to induce behavioral sleep in unrestrained animals (Hess 1929, 1944, 1954, Akert et al. 1952, Sterman and Clemente 1962 b, Parmeggiani 1962, Holsi and Monnier 1952, Hernandez-Peon 1962). The techniques require that low intensity currents (and long duration pulses in the case of MTH stimulation) be used, since high intensities produce behavioral arousal, and that the subject be sedate and undisturbed during the stimulation period. Under these circumstances low frequency MTH and low and high frequency LPO stimulation was said to be followed by sleep within minutes, although repeated measures were not obtained and sleep latencies were not quantified.

Other authors have criticized the conclusions of these experiments because alternative interpretations were not considered. For example, Harrison (1940) demonstrated that sleep effects of thalamic stimulation could be related to tissue damage caused by the stimulating electrodes, and Hunsperger (1974) demonstrated that sleep which followed MTH

stimulation did not differ in latency from spontaneous sleep. Moruzzi (1972), Jouvet (1972), and Berlucchi (1970) have summarized the general opinion of many researchers that sleep induction results are obtained in poorly designed experiments that lack statistical evaluation and controls for spontaneous sleep, a frequent behavior of laboratory cats.

The possibility that sleep in cats following MTH and LPO stimulation, according to the techniques of Hess (1944) and Sterman and Clemente (1962 b), might be spontaneous was investigated in the following experiment by comparing the latency and duration of sleep between trials where stimulation was applied and matched trials where no stimulation was applied. It will be shown that quantitative differences are small, and there is no convincing evidence that sleep on stimulation trials had a significantly shorter latency. Nor did the stimulation produce overt changes in the animal's behavior unless the intensity was increased to the threshold for arousal. Cortical EEG responses to MTH and LPO stimulation, on the other hand, were consistent with a large number of other findings. If it is not true that synchronogenic electrical stimulation produces sleep, current neurophysiological concepts of sleep and EEG mechanisms must be reconsidered.

## Methods

### Subjects

Experiments were performed on 11 mature, healthy, nonpregnant female cats, weighing approximately 4 kilograms (kg), obtained from the McMaster University Medical Center. Four of the 11 cats served in pilot experiments to establish stimulation parameters, trial procedures, and running schedules, and their data are not reported.

When not being tested, the animals were housed, uncaged, in a 20' x 9' sound attenuating room with a 12-hour light-dark cycle (light from 7 a. m. to 7 p. m.). Standard laboratory chow and water were offered once a day at 7 p. m., after the day's experiment, and removed the next day at 7 a. m.

### Surgical preparation

Surgical anesthesia was induced with Nembutal (40 mg/kg) injected intraperitoneally (I. P.). When the subject became unresponsive to tactile stimulation of the cornea and pinna, and peripheral stretch reflexes were absent, it was placed in a Kopf stereotaxic head holder, after Xylocaine ointment had been applied to the ear bars. A midsagittal section was then made and the temporal muscles retracted in order to expose the cranium from the frontal sinus to the lambdoidal

ridge. The frontal sinus was opened unilaterally, and two stainless steel jeweler's screws soldered to connecting wires were inserted into the bone overlying the orbit in order to record the electro-oculogram (EOG). A bipolar electrode for recording the electromyogram (EMG) was made from another pair of jeweler's screws fixed to a small strip of plexiglass (8 mm x 4 mm x 1 mm). The plexiglass was then sewn into the superficial cervical musculature.

Four bipolar electrodes for recording the cortical electroencephalogram (EEG) were placed directly on the cortical surface through small holes drilled in the skull overlying the frontal and occipital cortices bilaterally. The electrodes were constructed of two lengths of insulated nichrome wire .01" in diameter shaped into a "ski pole". Insulation was removed from the point of the pole, which penetrated the cortex to a depth of about 1 mm, and from the bottom surface of the loop of the pole, which rested on the dura.

Stimulating electrodes were also made of two lengths of nichrome wire .01" in diameter, insulated except at the tips, twisted together and separated at their tips by 1 - 2 mm. They were stereotaxically lowered through small holes drilled in the skull to bilateral points in the MTH (anterior 10.0 mm, lateral 2.0 mm, depth 1.3 mm) and LPO (anterior 14.0 mm, lateral 1.0 mm, depth - 3.6 mm) according to the stereotaxic brain atlas of Snider and Niemer (1961).

All stimulating and recording electrodes were soldered through wires to a 19 pin Amphenol connector which was then fixed to the skull with dental acrylic. The scalp incision was treated with veterinary wound powder containing procaine penicillin G and then fixed to the head cap with more acrylic cement. Postoperatively, 100,000 I. U. of benzathine penicillin G were injected intramuscularly (I. M.), and the animal's health was carefully monitored throughout its one to two week recovery period.

Apparatus

For testing, subjects were placed in a lighted, ventilated, thick-walled chamber, the inside dimensions of which were 24" x 24" x 48", containing a closed-circuit television camera for observing the animal's behavior. The subject's implanted electrode assembly was connected to a flexible cable made of low noise Microdot wires suspended from the ceiling of the chamber. That cable and other electrical connections between the chamber and the peripheral apparatus were led out through small holes in the top of the chamber. A TV monitor and all other peripheral stimulating and recording apparatus to be described below were in the same room as the chamber, and were controlled by the experimenter. The room was kept quiet and the door was always closed.

Stimulation apparatus

Electrical stimuli were generated by Grass S4 stimulators and delivered through Grass stimulus isolation units to either the MTH electrodes connected in series, or the LPO electrodes, also connected in series. Before each trial, the pulse width, pulse frequency, and pulse intensity were monitored on a Tektronix 502A oscilloscope and adjusted to the desired values. While the cat was connected in series with the circuit, the current was calculated by measuring the voltage drop across a 1 KΩ resistor also in series with the stimulation circuit. The 1 KΩ resistor and measuring device were disconnected before the stimulation was applied.

MTH stimulation was always presented at 6 Hz, but the pulse shape and intensity varied. In subjects M14 and M15 pulses were .2 ms capacity coupled square waves; in subjects M16, M17, and M36 they were .4 ms biphasic square waves; and in subjects M18 and M19 they were 12.5 ms capacity coupled D. C. pulses. A Grass stimulator triggered a photon decoupled active filter circuit which delivered a 12.5 ms pulse conforming to the specifications of Wyss (1965) and Hess (1957) (Figure 3). The slowly rising onset of the pulse is considered essential for selectively stimulating "hypnogenic neurons" of MTH (Hess 1954). The 12.5 ms pulses were applied unilaterally in MTH in order to replicate the Hess procedure.

LPO stimuli were presented at 6 Hz in some trials and at 150 Hz in other trials. They consisted of .2 ms capacity coupled square waves in subjects M14 and M15, and .4 ms biphasic square waves in subjects M16, M17, M18, M19, and M36.

Stimuli were applied bilaterally to LPO in all subjects, and bilaterally to MTH in subjects M14, M15, M16, M17, and M36. MTH stimulation was applied unilaterally in subjects M18 and M19, first to the right thalamus for a number of trials and then to the left thalamus in another series of trials. The intensity of all MTH and LPO stimuli ranged from .05 mA to .4 mA, which corresponded to a voltage range of .7 V to 16 V.

Stimulus parameters described above included those reported in the literature as effective in producing sleep, and slightly higher and lower values were added to assure some overlap with the critical parameters. Information concerning the actual stimulus conditions used for each subject, and the number and order of those stimulations, are given in Table I.

Recording apparatus

The animal's electrical activity was filtered, amplified, and recorded on an 8 channel inkwriting Beckman type R Dynograph. EEG potentials were recorded with filters set for 3 dB attenuation at 1.6 Hz and 32 Hz, EMG filters were set for 3 dB attenuation at 5.3 Hz and

150 Hz, and the EOG was recorded DC. Stimulus markers and time markers were also registered on the paper which ran continuously throughout each stimulation and control trial.

Procedure

At the beginning of each test day (approximately 10 a. m.) the subject was placed in the experimental chamber, where it remained until the end of testing approximately 8 hours later. A test consisted of a series of trials, half of which included brain stimulation (stimulation trials) and half of which did not (control trials). Stimulation trials were run consecutively and separated from each other by 10 minutes. An equal number of control trials were also separated from each other by 10 minutes. The stimulation block was separated from the control block by one hour. The procedures for stimulation and control trials did not differ in any way except for the presence or absence of brain stimulation. No environmental cues were available to the subject signalling the beginning or the end of any trial.

Trial protocol

The beginning of the first trial of the day, and the beginning of each subsequent trial, was determined by the animal's behavior. That is, a trial would begin only on a baseline of two minutes of active wakefulness (AW). AW and other behavioral states defined in this study

will be more fully described below. Following two minutes of AW, on stimulation trials, brain stimulation was applied for 30 seconds each minute (i. e. 30 seconds on, 30 seconds off) until the animal fell asleep. Criteria defining sleep onset will also be described below. Once the animal was asleep, stimulation was terminated and the animal was allowed to remain asleep until it spontaneously awoke. That awakening marked the end of the trial. If sleep did not occur within 40 minutes of the beginning of the trial, the trial was terminated and a sleep latency of 40 minutes was recorded in order to provide data for statistical analyses to be done later. Control trials followed the same procedures, except, of course, the subjects were not stimulated.

Experimental design

The general design for the presentation of stimulation and control trials is shown in Figure 4. As can be seen, several trials of one type, either stimulation or control, were run consecutively beginning in the morning. Some time in the afternoon, after a lapse of one hour, an equal number of trials of the other type were run. The order for stimulation and control blocks was randomly determined on the first day of testing and reversed on the next day. All stimulation trials for those two days would employ the same set of stimulus values, i. e. the same site of stimulation, frequency, current, and waveform. On the following two days a different set of stimulus values was tested in the

same way. One exception to this procedure occurred in subject M14. During the 8 days of tests for MTH stimulation, stimulation trials were run on one day and control trials on the next.

Data collection

Throughout each stimulation and control trial, the experimenter continuously monitored the subject's behavior on closed circuit television and through the electrographic recordings of EEG, eye movements, and muscle tension. For each 30-second interval, the overall state of the animal was determined and recorded. Five separate states were defined:

1) active wakefulness (AW)--the animal is obviously awake and displaying some limb movement, e. g., walking, climbing, grooming, or exploring the chamber.

2) quiet wakefulness (QW)--wakefulness with eyes open, but no limb movement.

3) transition to sleep (TS)--the animal is in a posture usually preliminary to sleep, i. e. reclining or curled up, with eyes closed, but there are no sleep spindles or slow waves in the EEG.

4) slow wave sleep (SWS)--characteristic sleep posture, eyes closed, spindles and slow waves in the cortical EEG, reduced amplitude of EOG and EMG.

5) deep sleep (DS)--sleep with rapid eye movements (REMs) and

desynchronized EEG.

These data regarding the fluctuations of behavior in each trial were then used to determine four quantitative measures: SWS latency, SWS duration, DS latency, and DS duration.

SWS latency was the time from the beginning of the trial to sleep onset. Sleep onset in turn was defined as the beginning of two consecutive minutes of the SWS state. That is, SWS must have exceeded two minutes before a sleep episode was counted. These criteria were developed after observing in pilot experiments that transient states of drowsiness up to two minutes in duration often occurred during spontaneous behavior and did not ultimately lead to sleep. SWS duration was the time from SWS onset to awakening, or to the beginning of a DS period, whichever came first.

DS onset was usually abrupt and easily recognized by inspection of the EEG and EMG recordings. DS latency, therefore, was the time from the beginning of the trial to DS onset, and DS duration was the time from DS onset to awakening, or to the reoccurrence of SWS. In the great majority of cases, however, DS was followed by awakening.

Data analyses

A number of statistical tests were done comparing SWS latencies and SWS durations between stimulation and control trials, and determining the degree of relationship between latency and duration of sleep on the

same trial, and the duration of sleep with latency on the next trial.

Histology

After receiving a lethal dose of Nembutal (5 cc injected I. P.), animals were perfused through the right carotid artery with approximately 50 cc of .9% saline solution followed by approximately 40 cc of 10% formalin. The brains were removed and stored in formalin until they were frozen, and cut into 40 μm sections. Every tenth section was mounted on a slide and stained with cresyl violet (Drury and Wellington 1967).

To determine the positions of subcortical electrode placements, the basal forebrain sections were projected with an enlarger onto a drawing of the appropriate histological section taken from Jasper and Ajmone Marsan (1954), and the positions of the electrode tips were plotted. The thalamic sections were compared to the atlas of Snider and Niemer (1961), and the coordinates of electrode tips were plotted onto a drawing taken from Akert et al. (1952).

## Results

### Location of electrodes

MTH electrodes were found to end in the massa intermedia, 2 - 4 mm from the midline, between AP 11.0 and AP 7.0 (stereotaxic atlas of Snider and Niemer 1961). Within this region are the midline and intralaminar nuclei of the nonspecific thalamocortical projection system (Jasper 1949). The stimulation sites have been plotted onto a saggittal section through the thalamus 2.5 mm from the midline (Figure 5 a). Below that is a reproduction of a figure from Akert et al. (1952) showing thalamic loci from which sleep was induced in their experiment (black circles) as well as thalamic loci where the same stimulation did not induce sleep (open circles) (Figure 5 b). A comparison between the two diagrams shows that points stimulated in the present experiment closely correspond to the "hypnogenic" area described by Hess and coworkers except for the two most dorsal points, representing the position of electrode tips in subject M16.

The lateral preoptic electrodes were found to lie in the basal forebrain in the general region of the rostral and lateral preoptic region of the hypothalamus. In one animal, the electrodes end in the diagonal band of Broca. These placements are illustrated in Figure 6 a,

and compared to points where stimulation resulted in cortical synchronization in experiments by Sterman and Clemente (1962 a) (Figure 6 b). It may be concluded that similar neuronal systems were stimulated in both experiments.

Cortical EEG responses

Cortical synchronization consisting of spindles, recruiting responses and following responses were recorded from the occipital and frontal cortex in five of seven subjects when relatively high intensity MTH and LPO stimuli were applied at the end of the experiment. In some of these subjects the responses also occurred during the experiment at the lower stimulus intensities used in the stimulation trials. The other two subjects did not receive the more intense stimuli after the experiment, and did not exhibit responses at the lower intensities during the experiment. Cortical responses were found to vary with frequency and intensity of stimulation, and with the behavioral state of the subject as described below.

Responses to MTH stimulation

EEG spindles having a duration of about 1 second and intraspindle frequency of about 8 Hz were elicited by single pulses to MTH (Figure 7 a). The responses were often facilitated by synchronized background activity in that their threshold was lowered, and amplitude and duration

increased. Such evoked spindles have been fully documented by previous authors (Morison and Dempsey 1942, Jasper 1949, Horvath and Buser 1964), and so were not analyzed further in the present experiment.

Continuous MTH stimulation at 6 Hz, the frequency used in the stimulation trials, evoked recruiting responses and smaller following responses like those in Figure 7 b. Below 1.5 mA no response is discernable, but at 1.5 mA biphasic responses appear and increase in amplitude, reaching a maximum after about 4 pulses, and then become smaller again. Another series of recruiting waves follow after about 2 seconds. For the remainder of the stimulation period only following responses are evoked. Usually, as seen here, the recruiting responses appeared only at the beginning of a long train of pulses, but occasionally continued to wax and wane throughout. Thalamocortical recruiting responses have also been thoroughly documented by previous authors (Morison and Dempsey 1943, Hanbery et al. 1954, Schlag and Villablanca 1967).

Responses to LPO stimulation

Single pulse LPO stimulation evoked cortical spindles very similar in appearance to thalamically evoked spindles (Figure 7 c). The LPO spindle had a frequency also of about 8 Hz, but it was preceded by a large evoked potential having a duration of about 100 ms.

LPO spindles have not been reported previously, and some of their characteristics are further described with the results of Experiment II of this thesis.

Low frequency 6 Hz LPO stimulation elicited EEG synchronization shown in Figure 7 d. The response, having a threshold of 1.5 mA, may be described as a following response or sustained synchronization, and is very similar to responses reported by Sterman and Clemente (1962 a). The evoked responses also periodically recruit, but do not achieve the same amplitude as the thalamic recruiting responses.

High frequency 150 Hz LPO stimulation did not elicit low frequency responses in any of the subjects, contrary to the reports of Sterman and Clemente (1962 a), and Clemente et al. (1963). For example, Figure 8 shows the continuous EEG and EOG of one subject during alternate 30 second periods of stimulation and no stimulation. The record begins 2 minutes after a stimulation trial has begun and continues to the onset of SWS. While the animal is awake (Figure 8 a), the EEG is desynchronized and does not seem to change between stimulated and unstimulated periods. Toward the end of the last stimulation period (Figure 8 b), some slow wave activity seems to be developing. Following this period, however, spontaneous high amplitude slow waves are clearly evident as the animal is falling asleep. It is more likely that the slow waves in the preceding stimulus period were spontaneous

and related to the cat's behavior than that they were evoked by the stimulus since they did not appear during any of the earlier stimulation periods. The coincidence of spontaneous EEG synchronization with a long period of stimulation is, therefore, not an unusual observation.

Effects of behavioral state on thalamocortical recruiting responses

The recruiting response to MTH stimulation was found to vary strikingly with states of sleep and wakefulness. This effect has been known since Evarts and Magoun (1952) first reported the possibility of obtaining recruiting responses in unanesthetized, freely moving animals. The responses are poorly developed during wakefulness, but they increase in amplitude and in frequency of occurrence as the animal becomes drowsy and falls asleep. They achieve maximum amplitude during SWS, and they are totally absent during DS.

This effect can be seen in Figure 9, a continuous record of EEG taken from the left occipital cortex while ipsilateral MTH stimulation at 6 Hz and .2 mA is applied for approximately 30 seconds each minute. Arrow up indicates the onset of stimulation and arrow down the offset.

In trace A, the cat is awake and sitting quietly as indicated by the desynchronized EEG prior to stimulation. When stimulation is applied, low amplitude recruiting responses appear at a latency of about 1 second. As signs of drowsiness appear (the animal is in a sphinx-like posture, purring, and the eyes are closed), the stimulation evokes

recruiting responses at a shorter latency and with greater frequency of occurrence (B-F). It should be noted that the EEG does not show slow waves during the interstimulus intervals although the animal did show behavioral signs of falling asleep correlated with changes in the recruiting responses.

Behavioral observations

The most striking result of this experiment is that while MTH and LPO stimulation was effective in eliciting EEG synchronization identical to responses reported by other workers, those same stimuli were not generally effective in decreasing the latency or lengthening the duration of sleep when compared to control, unstimulated conditions. All subjects, after being placed in the experimental chamber, explored the surroundings, groomed, paced about, and eventually fell asleep. There were considerable individual differences in these behaviors, for example, some animals seemed to be in a constant state of agitation and fell asleep only after long periods of wakefulness, while others were placid and lethargic and consistently fell asleep within minutes of each awakening, but these differences were not related to the presence or absence of stimulation. Statistical analyses of sleep latency and duration reported below confirm these observations.

SWS latencies and durations

The SWS latency for each trial for each subject (except subject

M36, who was run in a comparatively small number of trials), are plotted in Figures 10 - 15. Control trials on a particular day are indicated by closed circles and stimulation trials on that day by open circles. The crossed data point indicates which session (stimulation or control) occurred first on that day. Stimulus parameters for stimulation trials are listed above each day's data. In Figures 16 - 21, depicting corresponding SWS durations, blank spaces mean that no SWS duration was measured because the animal failed to fall asleep within 40 minutes.

On visual inspection there appear to be no consistent differences between stimulation and control data either within or between days. Nor does there appear to be a trend toward longer or shorter latencies or durations, or greater or less variance among the points, from the beginning of the experiment to the end. In other words, the graphs support the general observation that the cat's behavior was relatively constant from trial to trial and from day to day, and did not vary with stimulation.

A summary of these results is given in Figure 22. For each animal, latencies on all MTH stimulation trials were averaged and plotted next to the average latencies for associated control trials (Figure 22 a). The same is done for latencies on LPO stimulation and control trials (Figure 22 b), durations on MTH stimulation and control

trials (Figure 22 c), and durations on LPO stimulation and control trials (Figure 22 d). Two clear relationships can be seen in these graphs. First, while there are substantial individual differences in sleep latency and duration between animals, there is little difference in one animal between stimulation and control conditions. Second, stimulation and control latencies within animals are highly correlated using the Pearson product moment coefficient of correlation (Mendenhall and Ramey 1973):

r = .80 for latencies on MTH stimulation and control trials

r = .92 for latencies on LPO stimulation and control trials

r = .90 for durations on MTH stimulation and control trials.

r = .53 for durations on LPO stimulation and control trials.

This means that behavior observed during unstimulated control trials is a good predictor of behavior occurring during stimulation trials, and implies that stimulation was not effective in altering behavior.

Other correlations were obtained indicating the strength of the relationship between the latency on any particular trial and the subsequent duration of sleep on that trial; and the duration of sleep on any trial and the latency of sleep on the subsequent trial. A small number of tests achieved significance at a criterion level of $\alpha = .05$ ( 8 out of 84 tests). The 8 significant results were evenly distributed with regard to positive and negative correlations and so cannot be used to

draw any general conclusions about the direction of relationship between sleep latency and sleep duration. The 76 nonsignificant results imply the length of time an animal slept was not related to the latency of sleep on that trial, and that how long an animal slept did not significantly determine how quickly it would fall asleep on the next trial, which began approximately 10 minutes later. These results support the assumption that independent samples were obtained from trial to trial, but since it is not possible to prove this assumption, statistics which assume independent samples were not used and the following nonparametric statistical tests for related samples were applied to the data.

The mean SWS latencies for each subject were compared across 6 stimulation conditions, across 6 control conditions and across all 12 stimulation and control conditions in a Friedman two-way analysis of variance by ranks (Siegel 1956). The same tests were applied to measures of SWS duration. The 6 stimulation conditions were low ($\leq$ .1 mA) and high ($>$ .1 mA) intensity 6 Hz MTH stimulation, low and high intensity 6 Hz LPO stimulation, and low and high intensity 150 Hz LPO stimulation. The results, presented in Table 2, indicate that there is insufficient evidence to reject the null hypothesis that sleep latency or sleep duration differed significantly among stimulation conditions, among control conditions, or between stimulation and

control conditions. However, since the earlier authors claim that the effects are highly dependent on electrode placement and that hypnogenic and arousal effects are in competition, it is quite possible that hypnogenic effects might be seen in only one or a small number of trials when the placement and stimulation conditions were just right. In particular, a single combination of stimulus strength and duration might be effective in a given subject while parameters below it are subthreshold and above it the net effect is arousal. Hence it is imperative for a rigorous test of the hypothesis that individual cases be examined to discover effects that may be overwhelmed in the averages used in the analysis of variance.

Therefore, a Wilcoxon matched pairs signed ranks test (Siegel 1956) was done for each set of stimulation and control trials for each animal. The data were matched by pairing latencies on stimulation trials with latencies on control trials, during the two days of testing, on the basis of the order and time of day in which they were run. For example, the first stimulation trial run on the morning of day 1 was paired with the first control trial run on the morning of day 2, the second stimulation trial of day 1 with the second control trial of day 2, etc. The same matching procedure was used to compare SWS durations between stimulation and control trials.

The results are presented in Table 3. In the far left columns

are the subjects, and the stimulus parameters for stimulation trials in terms of stimulation site, frequency, current intensity, and voltage. The Wilcoxon "T" statistic is then listed for the comparison between stimulation and control latencies on "N" trials, and between stimulation and control durations on "N" trials. The Ns sometimes differ because on some trials animals did not fall asleep within 40 minutes, so no sleep duration measure was obtained. Also, the number of comparisons was sometimes reduced because of tied scores. Positive signs of T indicate the average control measure was greater, and negative signs that the average stimulation measure was greater.

None of the duration comparisons is significant, confirming the results of the analyses of variance done on averaged data. However, 4 of the 36 comparisons of SWS latency achieve significance using a criterion of $\alpha = .05$ (two-tailed). In 3 of the 4 cases (M15, 6 Hz LPO stimulation, .05 mA; M16, 6 Hz LPO stimulation, .20 mA; and M19, 150 Hz LPO stimulation, .09 mA), the latencies of sleep on stimulation trials were found to be greater than the latencies on control trials. That is, stimulation may have had an arousal effect since the animals remained awake an average of 7 minutes (M15), 6 minutes (M16) and 4 minutes (M19) longer on stimulation trials compared to control trials. But since they eventually did fall asleep in all of these cases, despite continued stimulation, any arousal effect could not have been

very powerful. The differences may also be accounted for by considering the nature of the Wilcoxon signed ranks test. Since the relative magnitude of the data points are utilized rather than their absolute values, it is possible to obtain highly significant differences simply because of a constant direction of difference. In other words, if control latencies were around 20 minutes, and stimulation latencies were consistently greater by only a few minutes, a statistically significant effect would be found even though the difference of a few minutes is not very meaningful in such a situation. In any case, the finding that some stimulus conditions may have delayed the onset of sleep contradicts the hypothesis that stimulation induces sleep.

The only evidence in this experiment for stimulation induced sleep appears in the data of subject M14 where sleep latencies, on trials in which 6 Hz MTH stimulation at .2 mA was applied, were significantly shorter than latencies on associated control trials. While the same interpretive limitations apply here as described above, the effect cannot be easily dismissed. It is possible, however, that a significant difference in this direction occurred in this animal and not in the others because a different testing procedure was used. As mentioned in the Methods section, MTH stimulation and control trials were run for this subject on alternate days, while in other subjects they were run on the same day and their order was counterbalanced on

a second day. As can be seen in Figure 10, there was in subject M14 a general decline in sleep latency, on both stimulation and control trials, across the first 5 days of the experiment. For the Wilcoxon test reported here, day 1 control data were paired with day 2 stimulation data, and day 3 control data with day 4 stimulation data. Because of the relatively long control latencies on day 1, a significant difference was found. If, however, day 5 control latencies are substituted for day 1 control latencies, i. e. day 2 measures paired with day 3, and day 4 with day 5, no significant difference results. ($T = 13$). This phenomenon illustrates the extent to which experimental conditions must be controlled before it is possible to conclude that any sleep behavior following stimulation is related to the stimulus and not a spontaneous event.

DS latency and duration

When episodes of REM sleep occurred in stimulation and control trials their latency and duration were also measured. Although these measures could not be statistically evaluated because the data were too few, the general impression of the experimenter was that DS was not affected by prior MTH or LPO stimulation.

Some confirmation of this impression is given in Figure 23, which shows DS latencies and DS durations for one subject (M16) who happened to display a particularly large number of DS episodes. The

top graph shows the latency of DS episodes occurring on each day of the experiment. Closed circles represent DS latencies on control trials and open circles DS latencies on stimulation trials. Both stimulation and control latencies vary between 10 and 50 minutes, and there are no systematic differences between them. Similarly, the duration of REM sleep on those same trials, shown below in Figure 23 b, does not seem to be affected by stimulation.

Summary of results

Sleep following MTH and LPO stimulation was compared to spontaneous sleep in order to determine the validity of claims that such stimulation is a direct cause of sleep. We found that:

1) MTH and LPO stimulation evoked slow, rhythmic EEG potentials like those occurring naturally during sleep, but the same stimulation was not followed by behavioral sleep that occurred significantly sooner, or lasted significantly longer than spontaneous sleep.

2) Neither were there obvious effects of stimulation on the latency or duration of DS.

3) The spontaneous sleep behavior we observed did not seem to differ qualitatively or quantitatively from behavior previous authors have interpreted as induced sleep.

4) Therefore, we conclude there is no more reason now to believe that sleep can be induced by electrical brain stimulation than there was prior to the findings of Hess (1944) or Sterman and Clemente (1962b).

## Discussion

Before discussing the behavioral results, it is important to establish that stimulating electrodes were properly placed and that animals were adequately stimulated. LPO electrodes were found in histological sections to end in the same basal forebrain region stimulated in experiments by Sterman and Clemente (1962 a). Since effective stimulation sites in their experiment ranged through four anterior-posterior planes, and included loci in the optic chiasm, it is not clear that highly localized stimulation is critical. Similarly, MTH stimulation sites reported by Akert et al. (1952) to induce sleep are scattered over an indistinct region of the medial thalamus, and are interspersed with ineffective stimulation sites. In the present experiment, all but one subject's electrodes were within the effective region.

Electrocortical responses to MTH and LPO stimuli may also serve as criteria for proper electrode placements since the literature claiming sleep induction often relies on such neuronal effects for corroboration. In the present experiment, single pulses to MTH and LPO elicited spindle trains in the frontal and occipital cortex, and low frequency (6 Hz) pulses elicited recruiting waves and stimulus bound synchronization. The responses were most easily obtained when the

animal was drowsy or asleep, but they also occurred with lower amplitude during wakefulness. These results are in agreement with a large body of literature, including some sleep induction reports, that have demonstrated the involvement of MTH and LPO in the genesis of rhythmic EEG potentials (Morison and Dempsey 1942, Evarts and Magoun 1966, Andersen and Andersson 1968, Purpura 1970, Sterman and Clemente 1962 a).

One discrepancy in the present experiment, however, was the failure to elicit low frequency EEG responses to high frequency LPO stimulation as described by Sterman and Clemente (1962 a, p. 110). Because the "response" to which they refer begins almost 30 seconds after the onset of stimulation, continues beyond the end of stimulation, and occurs while the animal is falling asleep, we suggest it may have been spontaneous synchronization unrelated to the stimulus. It was shown in the present experiment that a loose temporal association between stimulation and commonly occurring EEG potentials is not sufficient to conclude a causal relationship. Since there are no other published illustrations in which to evaluate the validity of the claim, we conclude that high frequency LPO stimulation does not produce EEG synchronization.

Many of the present EEG responses to low frequency stimulation described above were obtained with stimulus intensities higher than

those used in the stimulation trials. The question then arises whether the lower intensity stimuli were above threshold for a possible sleep induction effect. The question is not easily answered since the original experiments report stimulation intensity in terms of voltage, a variable that cannot be equated between experiments because of differences in electrode resistance. In the present experiment, an attempt was made to approximate reported voltages while keeping currents within a reasonable range. The result was current pulses that were just below threshold for behavioral arousal and other overt responses, yet were occasionally high enough to produce neural responses.

In summary, histological, electrocortical, and behavioral criteria confirm that subjects in the present experiment received stimulation equivalent to that used by previous investigators when sleep induction was reported. Under these conditions it was true that sleep followed MTH and LPO stimulation with relatively short latencies, at least as short on some trials as latencies reported by other investigators. However, it was also true that spontaneous sleep, occurring when the animal was not stimulated, had similar latencies. The problem then becomes one of statistical evaluation--does sleep following stimulation occur signigicantly sooner than spontaneous sleep? In the present investigation it did not. This conclusion was reached after finding that only one of 38 tests indicated shorter sleep latencies on

stimulation trials, but this difference could be attributed to a method of data analysis which was different for that animal, and not to the effects of stimulation.

On the other hand, stimulation was followed by significantly longer sleep latencies in 3 other tests, implying the stimulation was arousing. The arousal hypothesis is rejected, however, since the animals did eventually fall asleep during stimulation. An alternative hypothesis is that these differences, occurring in a total of 4 out of 76 observations, might be expected to result from random sampling. The very fact that so many trials are required to produce few differences of small magnitude suggests that behavioral effects of low intensity MTH and LPO stimulation are extremely subtle, and, if they exist at all, would not have been evident in the casual observations of Hess (1957), Akert et al. (1952), and Sterman and Clemente (1962 b).

In regard to this point it is important to recognize that the present results are qualitatively and quantitatively very similar to the observations of those authors, in spite of the opposite conclusions. Those authors emphasize that sleep following stimulation in their experiments is identical to natural sleep, in that it is preceded by grooming and postural adjustments until a comfortable position is found, and it is accompanied by slowing of the cortical EEG, progressive muscular atonia, pupillary constriction, and a lowered respiratory rate. These

behaviors are said to progress at a normal rate and can be interrupted by arousing environmental stimuli. In short, their descriptions of cats falling asleep during stimulation exactly parallel the events observed in this experiment--on stimulation and control trials.

The actual latencies measured in this and previous experiments may also be comparable. In Hess' studies sleep is said to occur after 2 or 3 stimulation periods. But the length of the stimulation periods and the interstimulus intervals vary. Latencies as great as 10 - 15 minutes, and even in one case over an hour, may be extrapolated from the published reports. The average time reported for sleep following LPO stimulation is only 30 seconds, and occasionally sleep was said to appear after delays as short as 5 seconds (Sterman and Clemente 1962 b). But, these figures are not statistically documented and not entirely supported by other data in the study.

The relatively longer latencies in the present experiment (2 - 40 minutes) can be explained by the more stringent criteria used here to determine the time of sleep onset. These criteria were developed after observing in pilot experiments that transient states of drowsiness up to 2 minutes in duration often occurred during spontaneous behavior and did not ultimately lead to sleep. So a 2 minute minimum duration of SWS was required before sleep onset was counted. Similar criteria were used by Sterman et al. (1972) in their study of periodicity in the

spontaneous sleep habits of laboratory cats, but not by Sterman and Clemente (1962 b), who report, for example, that sleep was induced and then spontaneously terminated 3 times during a 130 second stimulation period (p. 106).

Therefore, we suggest that sleep reported in other experiments to result from stimulation was actually spontaneous, as it seemed to be in the present experiment. This interpretation is supported by the frequent observation that "induced" sleep occurs most readily on a background of spontaneous drowsiness, and that it is facilitated by satiation, fatigue, or a general lack of disturbing influences, while excessive sensory stimulation, food deprivation, or emotional agitation favors the maintenance of active, waking behavior. Also, the "response" could be interrupted at any time by a novel stimulus in the environment.

The notion that the classic sleep response to MTH and LPO stimulation is in fact spontaneous behavior is also supported by the fact that laboratory cats spontaneously sleep or exhibit quiescent behavior 89% of the time (Sterman et al, 1965), and that the latency and duration of sleep per episode under free running conditions are similar to the latency and duration observed in this experiment (Delorme et al. 1964, Ursin 1968). Jouvet (1967) among others shares the view that a cat asleep after any stimulation would probably have gone to sleep spontaneously. The only published report that attempts a quantitative

evaluation of sleep latencies following stimulated and unstimulated control periods similar to the present investigation concurs that spontaneous sleep, ranging in latency from 5 - 90 minutes, is indistinguishable from sleep following stimulation (Hunsperger 1972, see also Leisinger-Trigona and Hunsperger 1972).

In spite of this evidence and line of reasoning, the idea that sleep can be induced by direct cerebral stimulation perhaps should not be so quickly dismissed. The effect has been called "striking" and "convincing" even though it lacks adequate statistical evaluation. It is possible, therefore, that a true sleep response to brain stimulation can occur but only under very specific and not easily replicated conditions. Akert et al. (1952) acknowledged this possibility when noting the difficulty other researchers have had in confirming the sleep phenomenon. Slight differences in electrode placement, current intensity, or pulse waveform or frequency will produce arousal or no response at all. But if the phenomenon is so elusive, a great number of observations would be required to demonstrate it. Such large scale parametric procedures were not, however, employed by these authors, although they imply the effect is obtained reliably.

Another interpretation of their results is that sleep may have been a conditioned response to experimental conditions not related to the brain stimulation. For example, sleep is often reported as a

conditioned response to repetitious or monotonous environmental stimulation (see Pavlov 1929). Roitbak and Eristavi (1957), who experimented with MTH recruiting stimuli in cats, report that "after a series of day per day NTS (non-specific thalamic system) stimulations the animal became somnolent whenever it was placed in the testing cage." And Sterman and Clemente (1962 b) note that "the reaction of the cat to the experiment changed markedly after several stimulations. The apparent apprehension characteristic of earlier trials was replaced by docility, and the animals would purr in many instances."

Therefore, whether sleep following stimulation in the present and most previous experiments was spontaneous, as suggested by control data, or resulted from extraneous environmental factors or sporadic stimulus conditions, it is clearly not a robust and reliable physiological response to brain stimulation. On the other hand, neural responses in the form of cortical EEG synchronization are easily and reliably obtained with such stimulation. We thus conclude that neural processes and substrates for the production of EEG potentials and those for the induction of behavioral sleep are not identical as is commonly assumed, and that they may even be independent at the level explored in this experiment.

## Experiment II

### Background

Experiment I confirmed that low frequency, but not high frequency, stimulation of MTH and LPO induces spindles and other slow waves in the cortical EEG. Several authors have described neuronal events within the thalamus and within the cortex during these periods of evoked synchronization (Creutzfeldt et al. 1966 a, b, c, Purpura 1969, 1970, Andersen and Andersson 1968), but the pathway for the response is not clear since there are no widespread projections from MTH and LPO to cortex. It has been suggested that instead of direct action on the cortex, MTH and LPO exert their effect through descending pathways which inhibit desynchronization mechanisms of the reticular formation. This hypothesis is consistent with the theories of Dell et al. (1961) and Dell (1963) which ascribe the abatement of cortical desynchronization, and onset of synchronization, to "active reticular deactivation". That is, they propose the existence of central synchronizing structures whose output to the retucular formation functionally inhibits the capacity of reticular neurons to generate corticopetal desynchronizing impulses.

Several lines of evidence suggest that MTH and LPO may be such structures. Descending fibers from MTH and LPO, some of which may contact reticular neurons monosynaptically, have been identified in Golgi stained and Nissl stained material (Scheibel and Scheibel 1967, Minzuno et al. 1969, Troiano and Siegel 1975). Electrophysiological data also show influences of MTH and LPO neurons over spontaneous reticular unit activity (Purpura 1970, Mancia et al. 1974, 1976, Mancia 1975, Grantyn et al. 1971, Bremer 1970, Lineberry and Siegel 1971).

Based on these reports, the present experiments explore further the nature of MTH and LPO input to the reticular formation. Since it has been suggested that such input is inhibitory in nature, and suppresses the responsiveness of reticular neurons to normally desynchronizing inputs, particularly sensory inputs, we have recorded single unit and field potential responses of the reticular formation to MTH, LPO, visual (V) and auditory (A) stimuli applied separately, and applied in pairs so their response interactions could be observed. It was concluded that each of the four stimuli produced reticular responses having both excitatory and inhibitory components since each stimulus, when acting as a conditioning stimulus, could both facilitate and suppress subsequent responses to a test stimulus. The results indicate there is no qualitative difference between EEG synchronizing inputs to the reticular formation (MTH and LPO) and EEG desynchronizing inputs (V and A), and that the

net output of the reticular ascending system is determined by complex interactions between excitatory and inhibitory influences from both sources. There may, however, be quantitative differences in the time course and site of action of various stimuli which determine whether the synchronizing or desynchronizing influences predominate.

## Methods

### Subjects

Experiments were performed on seven healthy, mature, non-pregnant female cats, each weighing three to four kg., obtained from the McMaster University Medical Center.

Two different preparations were used. In one, animals were surgically prepared under Nembutal anesthesia with indwelling stimulating and recording devices, allowed to recover for one to two weeks, and then paralyzed with curare during one, or two, day long recording sessions. In the other preparation, the surgery and recording were done on the same day under continuous chloralose anesthesia.

### Curarized preparation

Four cats were surgically prepared under Nembutal as in Experiment I with bipolar depth electrodes for stimulating MTH and LPO bilaterally, and monopolar skull screw electrodes for recording cortical EEG. In addition, after removing a disk of bone approximately 1.2 cm in diameter, a stainless steel chamber, about 1 cm in diameter, was implanted in the skull overlying the brain stem region from which single unit recordings were to be made.

The chamber was stereotaxically placed in the skull above the

cortex so that its midpoint was located 1 mm anterior to the interaural line, and 2 mm lateral to the sagittal midline, and its surface was aligned parallel to the horizontal plane so that a reference point could be established for calculating the depth of recording. Once cemented in place, the chamber was filled with sterile mineral oil and sealed with a threaded nylon stopper.

Acrylic cement was added surrounding the stimulating and recording electrodes and the recording chamber until a smooth and secure cap was formed. Penicillin G powder was applied to the scalp wound, and the scalp too was cemented to the head cap. After removing the animal from the head holder, 100, 000 I. U. of benzathine penicillin G were injected I. M. The animal was returned to a heated chamber for the first 24 hours, and then to its home cage for a one to two week recovery period.

On the day of the experiment the animal was brought into the recording room and anesthetized with nitrous oxide and fluothane while an endotracheal tube was inserted and 2 cc of Flaxedil (curare) were injected I. P. (total time approximately five minutes). The anesthesia was then discontinued and, when its breathing became shallow, the animal was connected through the endotracheal tube to a Large Animal Respirator (Model 607, Harvard Apparatus Co., Inc., Dover, Mass.) and artificially ventilated. Additional Flaxedil was injected approximately

every two hours or at the first sign of movement.

The subject, unanesthetized but paralyzed, was then positioned so that it lay on its stomach with its head supported upright on foam padding. A lightweight cable with leads for the stimulating and cortical recording electrodes was plugged into the animal's head cap and this plug was clamped to a table support to further stabilize the animal's head. The animal's eyes were covered with transparent contact lenses and irrigated periodically with physiological saline. Small plastic earphones delivering auditory stimuli were inserted in each ear and held in place with adhesive tape. Subjects were kept warm by a heating pad placed under the abdomen which regulated rectal temperature to 37°. C.

An hydraulic microdrive (Trent Wells, Inc., South Gate, Calif.) containing a tungsten microelectrode was then attached through a micropositioner connector with vernier scales graduated to .1 mm to the chamber above the brain stem. The tungsten microelectrode was housed inside a stainless steel guide tube, .6 mm in diameter, premeasured to extend to the surface of the brain stem when the microdrive was in place. The electrode was then driven an additional 15 mm beyond the end of the guide tube. After one such penetration of the brain stem, the microdrive was removed from the chamber, positioned at a different set of stereotaxic coordinates within the chamber with a

different electrode if necessary, and lowered again to the brain stem.

After the 10 - 12 hour recording session, two of the four subjects were allowed to recover from the paralysis and removed from the respirator. The microelectrode chamber was washed out with sterile saline, refilled with sterile mineral oil, and resealed. Subjects were held in a heated chamber for 24 hours and then returned to their home cages. Both recovered well, and were used in the same experiment about a week later. The other two subjects were used just once.

Chloralose preparation

Three subjects were anesthetized with nitrous oxide and fluothane while the femoral vein was cannulated. The gas anesthesia was then discontinued and 60 mg/kg of chloralose dissolved in physiological saline was immediately injected through the cannulated vein. When reflex responses had diminished, and EKG and respiration stabilized, the subject was placed in a Kopf stereotaxic head holder.

MTH and LPO stimulating and cortical recording electrodes were placed in the same manner described for the curarized preparation. But instead of implanting a chamber for microelectrode recordings from the brain stem, the posterior cranium overlying the tectum was removed unilaterally, leaving the underlying dura and cortex intact. To keep the cortex warm and moist, 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. The same Trent Wells microdrive containing a tungsten microelectrode housed in a guide tube was used as in the curarized preparation except that instead of being locked into an implanted chamber, the device was held in a stereotaxically mounted micromanipulator. For each penetration the guide tube was lowered through the cortex to predetermined points in the brain stem.

The only additional preparation was the insertion of contact lenses over the eyes. No auditory stimuli were used in the chloralose preparations.

Stimulation apparatus

Four different types of stimuli were applied: visual (V), auditory (A), medial thalamic (MTH) and lateral preoptic (LPO).

Visual stimuli were diffuse light flashes 10 microseconds in duration produced by a Grass Model PS 22 photostimulator set at maximum intensity. The flash bulb was placed at the midline approximately 18" in front of the animal's eyes.

Auditory stimuli were .2 ms trains of 2.5 KHz rectangular pulses (pulse width 100 $\mu$sec) delivering approximately 12 milliwatts of power to each of two hearing aid speakers wired in series and inserted in the animal's ears. The intensity of the tone was above threshold necessary to produce evoked responses in the reticular formation.

Brain stimulation, both MTH and LPO, consisted of .4 ms

monophasic square pulses originating in a Tektronix pulse generator (type 161) and relayed through a constant current stimulus isolation unit (W. P. Instruments, model PC-1) with which current intensity could be adjusted between .05 mA and 5 mA. The output of the isolation unit was connected in series to the two MTH or two LPO stimulating electrodes. Stimuli were usually presented at a rate of one every 2.5 seconds.

All four stimulation circuits were synchronized through several Tektronix pulse and waveform generators (type 161 and 162) so that any one stimulus could be triggered independently, or any combination of two could be triggered in pairs with variable interstimulus intervals. The master circuit also generated prestimulus pulses and stimulus markers.

Recording apparatus

Extracellular recordings were obtained with insulated tungsten microelectrodes etched to a tip diameter of approximately 5 $\mu$m. The potentials recorded from the microelectrode were referred to a screw electrode in the frontal sinus and led to two Grass P-15 AC preamplifiers in parallel. One preamp acted as a low pass filter (3 - 100 Hz) and the other as a high pass filter (300Hz - 50 kHz) so that both field potentials and action potentials could be derived from the same microelectrode. EEG potentials from the occipital cortex were also filtered

through a Grass P-15 preamplifier (3 - 100 Hz) and all three outputs were recorded at a gain of 1000 on an Ampex PR500 seven channel frequency modulated magnetic tape recorder. Also recorded were pre-stimulus markers, stimulus pulses, and a voice monitor. All bio-electrical potentials and event markers were simultaneously displayed on a Tektronix RM 565 dual beam oscilloscope while the high frequency unit activity was monitored on a loudspeaker.

Recording procedure

After positioning the microelectrode recording device, several penetrations of the brain stem were made to search for spontaneously active units. The exploration was limited to a region of the mesencephalic and anterior pontine reticular formation extending approximately 6 mm caudal to AP + 3 (atlas of Snider and Niemer 1961), 0 - 4 mm lateral to the sagittal midline, and in the dorsal-ventral plane from the superior colliculus to the ventral surface of the brain.

Units in this area were selected for recording after meeting the following criteria: a spontaneous firing rate, absence of injury discharges, constant amplitude of extracellular action potentials after approximately one minute of observation, and a spike height sufficiently above the background noise to be easily and consistently discriminated by visual inspection.

Isolated cells were tested for their responses to each of the four

stimuli applied separately, and then to various pairs of stimuli at a number of interstimulus intervals. The first stimulus of the pair is defined as the conditioning stimulus, and the second as the test stimulus. The protocol for stimulus presentations is rather long and involved, as described below, so some steps were abridged or eliminated in order to maximize the number of data that could be obtained per cell. In general, the experiment proceeded as follows:

1) starting with V stimulation, then proceeding to LPO, MTH and A usually in that order, each stimulus was presented alone, once every 2.5 seconds for approximately 50 trials, to establish the general characteristics of each response type.

2) V and LPO stimuli were presented simultaneously, and another 50 responses to that combination of stimuli were recorded.

3) The V stimulus acting as the test stimulus was delayed about 2 - 3 ms relative to the LPO or conditioning stimulus. A large number of responses were recorded if the response to the test stimulus seemed modified and a small number of responses if no noticeable changes had occurred.

4) The V stimulus was successively delayed in progressively larger steps up to about 1000 ms. At each delay the experimenter decided whether to record a large number of responses (if a clearly observable change had occurred in the response compared to the

previous response) or go on to the next delay (if no change had occurred). These subjective judgements were necessary considering the relatively short time available for recording. At random times, the test stimulus was presented alone to ensure its basic response had not changed over time.

5) After completing the sequence described above, the same two stimuli were again paired, this time the LPO stimulus being delayed relative to the V stimulus.

6) Having recorded the responses to paired V and LPO stimuli, the same procedures (steps 2 - 5) were followed with paired LPO and A stimuli, MTH and V stimuli, MTH and A stimuli, and V and A stimuli. MTH and LPO stimuli were not paired.

7) The order of those subsequent tests was determined by the experimenter based on a few sample observations and considering the time available for recording.

When a cell was no longer useable because of injury discharges, drifting of the electrode or changes in the cell's basic response characteristics, the electrode was lowered until another cell was encountered. As far as possible, the same tests (steps 1 - 7) were applied to it. Generally 1 - 4 cells were recorded per penetration, and there were 8 - 10 penetrations per subject.

Data analysis

The method of pairing conditioning and test stimuli and observing their response interactions at various interstimulus intervals has been used commonly to examine features of convergent inputs to a single neuron or neuronal pool (e.g. Dubner and Rutledge 1965, Hernandez-Peon and Hagbarth 1955, Lindsley et al. 1972, Adey et al. 1957). While the method does not provide a direct measure of the changes in the cell's excitability produced by a given stimulus, it does indicate the degree of sharing of neurons by various inputs and possible sites and mechanisms of interactions. The term "blocking interaction" will be used to describe the situation where conditioning stimuli reduce the number of action potentials or amplitude of the field potential produced by a test stimulus, compared to the response evoked when the test stimulus is applied alone, and the term "facilitatory interaction" will be used to describe increases in the test response produced by the conditioning stimulus (see Amassian 1952, and Hernandez-Peón and Hagbarth 1955). The latency of blocking or facilitation is defined as the time following the conditioning stimulus that the test response is first noticeably altered, and the duration of blocking or facilitation as the subsequent interval during which the test response remains altered. For the most part, only blocking interactions were quantified, since they were the most conspicuous and easily measured.

Reticular responses and response interactions were analyzed by two methods: 1) visual inspection of the activity played back from magnetic tapes and displayed on a Tektronix 565 oscilloscope, and 2) poststimulus histograms drawn from 35 mm films of the oscilloscope tracings.

In the first method, each sweep of the oscilloscope was synchronized with a stimulus or prestimulus pulse so that each response could be visualized. For several reasons it was necessary to measure parameters of the response by visual inspection rather than by computer methods or electronic counters. First, the signal to noise ratio in some recordings was not always high enough for the action potentials to be reliably discriminated by an electronic gate. In other recordings, two or more cells were recorded simultaneously, and their responses could not always be separated by selective gating. Third, because of the necessity for rapidly sampling a large number of stimulus conditions, it was not possible to collect a large sample of responses for each condition. Fourth, even when long trains of response were recorded, they were periodically interrupted by tests of the cell's viability or the interposition of other stimuli to compare responses.

Therefore, criteria were developed for measuring responses which depended on certain characteristics that were easily discriminable by eye. Only activity which was highly consistent from trial to trial

in its temporal relationship to the stimulus was counted. Responses to single stimuli were defined as an action potential or burst of action potentials of approximately the same amplitude which reliably followed the stimulus on at least 75% of the trials with approximately the same latency ($\pm$ 3 ms), latency being defined as the time from stimulus onset to the first spike of the burst. Any data considered ambiguous were discarded.

Responses to single and paired stimuli were photographed on Polaroid film. Also, in three cells, poststimulus histograms were drawn by projecting 35 mm film of consecutive oscilloscope sweeps onto graph paper and adding, and then averaging, the number of action potentials per 4 ms interval.

Histology

Histological procedures were the same as for Experiment I. The brains of five of the seven subjects were examined.

## Results

### Location of electrodes

Stimulating electrodes were located in the ventromedial thalamus (Figure 24a) and in the basolateral forebrain (Figure 24b), loci corresponding to MTH and LPO stimulation sites in Experiment I. Therefore, similar synchronogenic regions were stimulated in both experiments.

### Cortical EEG responses

As in Experiment I, and confirming the appropriate electrode placements, single pulse and low frequency stimulation of MTH and LPO elicited cortical synchronization in the form of recruiting waves (Figure 25a) and spindles (Figure 25b).

Visual and auditory stimuli were assumed to produce EEG desynchronization, as demonstrated by several authors (e.g. Adrian and Matthews 1934, Bremer 1935, Rheinberger and Jasper 1937, Lindsley et al. 1950, Sharpless and Jasper 1956), but no specific tests were conducted since the EEG was spontaneously desynchronized during most of the recording session. In one situation, to be described later, V stimulation was seen to suppress cortical spindles.

### Anatomical distribution of reticular unit responses

The locations of 60 of a total of 116 recorded cells were determined from the stereotaxic position of the electrode tip during recording. As seen in Figure 26, these cells were located mainly in the mesencephalic

and anterior pontine tegmentum, while some may lie on the borders of the reticular formation or in specific nuclear regions.

Figures 27 - 30 illustrate the differential distribution of cells responsive to V, A, LPO and MTH stimuli respectively in each of six anterior-posterior planes, five medial-lateral planes, and five dorsal-ventral planes of the brain stem, each bar representing the percentage of cells in each location that responded. There is a slight tendency for V responses to occur in the more medial locations, but no other feature of the distribution is striking (Figure 27). Auditory responses are more clearly segregated in the most posterior and lateral planes (Figure 28), and LPO responses occur slightly more frequently in the medial planes (Figure 29). MTH responses were encountered toward the midline, in either extreme dorsal or extreme ventral locations (Figure 30). In general, however, the distributions appear to overlap considerably, especially in the central regions, and no significant differences between the distributions were observed using chi-square tests (Siegel 1961). Since the subject of this investigation is the interaction of responses evoked by synchronizing and desynchronizing stimuli, it is important to note that those stimuli converge on the same population of neurons.

Convergence of responses

Considering the overlapping distributions of V, A, LPO and MTH responses, it is not surprising to find that stimuli converge on individual neurons as well. In this experiment, 47% of cells tested responded to

at least two modalities of stimulation, 30% responded to at least three, and 12% responded to all four. Conversely, 16% of cells tested with all four stimuli responded to none, at least not in terms of action potential discharges.

The number and percentage of cells responding to each combination of stimuli are given in Table 4. Every combination was effective for some cells, and it is interesting that a greater percentage of cells were responsive to both MTH and LPO stimuli than were responsive to both V and A stimuli. Multimodal cells gave responses similar to responses given by cells responsive to just one modality. For example, a V response would be more or less the same whether it was recorded from a cell responsive only to V stimulation, or from one responsive to other stimuli as well. There was no particular pattern to the anatomical distribution of convergent cells, and a cell responsive to one particular combination of stimuli may be in close proximity to a cell responsive to an entirely different combination. These findings confirm that each afferent system has a wide distribution, but that within that field very fine and selective patterning of terminals is achieved. It may be concluded from Table 4 that a substantial proportion of reticular neurons is contacted by both synchronizing and desynchronizing inputs.

Patterns of reticular unit and field potential responses

Each of the four stimuli was found to evoke clear and consistent

responses, with relatively short latencies, in a large proportion of reticular neurons (see Table 5). In general, responses consisted of early discharge-silent period-late discharge sequences of action potentials correlated with negative-positive-negative transients in the field potential. We will hereafter refer to these as excitatory-inhibitory-excitatory responses, since the weight of the evidence presented below suggests that they reflect underlying EPSP and IPSP activity in reticular neurons. The latency of the early excitatory response, duration of the inhibitory period, and presence or absence of late discharges varied somewhat among response types, but for a given response varied very little between cells.

Of the four response types, V responses showed the greatest variability between cells. Many V responses were similar to the one illustrated in Figure 31a, i. e. a burst of spikes at about 25 ms. In a number of cells, however, there were several bursts of action potentials, beginning about 20 ms after the stimulus, and lasting about 100 ms, followed by a suggestion of spike suppression and then rebound excitation. Only one cell gave an initially inhibitory response which had a latency of about 25 ms and a duration of 100 ms. The V field potential varied from a simple monophasic wave, to the complex waveform seen in Figure 31a. The most consistent feature of V responses was their relatively long latency compared to the other response types.

Auditory responses were more uniform than visual responses,

differing from the latter in three respects: unit responses consisted of only one spike or one brief burst of spikes; spike latencies were usually shorter; and A field potentials were always simple, monophasic negative waves (see Figure 31b). Only two cells deviated from this pattern, giving initially inhibitory responses which began about 10 ms after the stimulus and lasted 65 ms and 250 ms, respectively.

LPO responses were the most uniform, in every cell consisting of a burst of action potentials at 8 - 12 ms followed by a silent period approximately 100 ms in duration (see Figure 31c). Very often the firing rate following the silent period increased above spontaneous levels, strongly suggesting postinhibitory rebound facilitation. There was usually a sharp negative-going field potential during the initial burst of spikes, and a long sloping positive-going field potential during the silent period. In no cell was the initial response to LPO stimulation inhibitory.

MTH responses were similar to LPO responses in that they consisted of a short latency burst of spikes around 10 ms, but they lacked the clear and consistent suppression of activity that always followed LPO responses (see Figure 31d). The MTH field potential consisted of a complex of negative and positive waves during the spike activity, but often did not contain the succeeding long duration positive wave characteristic of LPO responses. One cell responded by inhibition of unit firing lasting about 250 ms.

It appears from these descriptions that the predominant, or initial, effect of stimulation in each modality was excitatory, only a small number of cells giving initially inhibitory responses. However, we cannot conclude from these data that excitation was necessarily the first response of the cell, or that excitation was more frequently evoked than inhibition, only that it was more frequently detected because of the low spontaneous firing rates of many of the cells. In fact, as will be shown in the following pages, responses were found to be far more complex than was evident in the extracellular discharge patterns. For example, we found both latent excitatory and latent inhibitory components to these responses which might occur before and/or after the unit discharges. Latent effects could also be demonstrated in cells ostensibly unresponsive to all stimuli. So excitation (i.e. spikes) was not necessarily the most significant response, only the most easily detected with the present techniques.

Interactions of responses to paired stimuli

The convergence of V, A, LPO and MTH afferents on single reticular neurons confirms that a substrate exists for interactions of synchronizing and desynchronizing influences at the neuronal level. Using the technique of paired conditioning and test pulses, we have been able to observe some of the interactions, and their major features are illustrated below.

A. Blocking following excitatory responses to conditioning stimuli.

As stated previously, many reticular responses consisted of excitatory-inhibitory sequences evident in both the pattern of unit discharge and polarity of the field potential. The most frequently detected interaction between paired stimuli, as might be expected, was the suppression, or blocking, of the excitatory response to the second (test) stimulus when it occurred during the inhibitory period following the first (conditioning) stimulus. For example, Figure 32 shows a cell that responded to V stimulation with a burst of action potentials at about 25 ms (Figure 32a) and to LPO stimulation with a burst of action potentials at about 8 ms (Figure 32b). Although in this case there is no reason to suspect that the period following the V response is inhibitory, all but one spike of the LPO unit response, as well as the negative wave of the LPO field potential, are blocked when the LPO stimulus is presented following the V response (Figure 32c). The blocking period in this cell began immediately after the V spikes, and lasted approximately 20 ms. LPO stimuli applied at later delays evoked full responses.

Figure 33 shows a very similar blocking interaction between V and LPO responses recorded in a different preparation. It is included at this point, however, to illustrate our previous statement about there being little response variability within modalities. Visual and LPO stimuli evoke in this cell unit responses identical in latency and pattern to V and LPO unit responses in Figure 32, and field potentials of

similar shape, latency, and duration. The blocking interaction, seen in Figure 33b, also occurred during the same 20 ms period. These similarities suggest the neural substrate for synchronizing-desynchronizing interactions is well established and of some functional significance, since it occurs consistently not only in different cells, but, as seen here, in different animals.

Blocking interactions like those in Figures 32 and 33 were fairly common and occurred following stimulation in each modality. However, the latency and duration of the effects varied with each stimulus pair. Before presenting these data in tabulated form, illustrations are given below.

Figure 34 shows two examples of blocking produced by LPO stimuli. In one cell, the V response is a burst of spikes with a latency of about 20 ms (Figure 34a). However, those spikes do not occur when V and LPO stimuli are presented simultaneously, i.e. when the V response would have occurred following the shorter latency LPO response (Figure 34b). In this case the blocking occurred just at the peak of a positive wave in the LPO field potential. In another cell, a short latency A response (Figure 34c) is blocked when it follows an LPO response (Figure 34d). Note the similarity of LPO responses in Figure 34b and d recorded from different cells.

In another cell, all three test responses are blocked when they follow an auditory stimulus at various delays (Figure 35a-f). The V response was

blocked between 32 ms and 53 ms after the A stimulus, the MTH response between 40 ms and 65 ms, and the LPO response between 28 ms and 38 ms, the onset and offset of these periods being very abrupt in this cell. In other cells where more than one test response could be blocked by the same conditioning stimulus, there was usually greater variability in the latency and duration of blocking periods.

The effectiveness of conditioning stimuli in blocking responses to test stimuli, in all cells, is summarized in Table 6. For each stimulus pair, the table gives the number and percentage of cells in which the conditioning stimulus ($S_1$) blocked the response to the test stimulus ($S_2$), and the mean latency and mean duration of those blocking interactions. The data are further divided into two categories: cells which responded to $S_1$ with action potentials (N% $S_1$ response) and cells which did not exhibit action potentials following $S_1$ (N% no $S_1$ response). So far we have given examples only of the first type of cell. The second type is illustrated below.

B. Blocking in the absence of a response to the conditioning stimulus

As mentioned above, a number of cells in this experiment failed to respond with action potentials to a conditioning stimulus, but did respond to a test stimulus. In some of these cases, the ostensibly ineffective conditioning stimulus nevertheless produced blocking interactions that had latencies and durations similar to those observed in responsive cells. The phenomenon occurred commonly following V conditioning stimuli, and seldom following other conditioning stimuli (see Table 6).

A clear example can be seen in Figure 36. The cell does not respond to V stimulation (Figure 36a) but gives a short latency burst of spikes following LPO stimulation (Figure 36d). When the LPO stimulus is presented 16 ms after the V stimulus (Figure 36b), the LPO response is not altered. But when it is presented 20 ms after (Figure 36c), the LPO response is blocked. A tentative conclusion from these data is that some stimuli may be exerting powerful inhibitory influences on reticular cells that are not manifest in extracellular recordings. The data also prove that the cell being recorded need not generate action potentials for blocking interactions to occur.

C. Blocking of inhibitory response components

The above examples demonstrated blocking of the excitatory response (action potentials and negative field potential) to the test stimulus, but did not comment on the fate of the inhibitory response component

(silent period and positive field potential). In some cells, the excitatory and inhibitory components were similarly affected, as can be seen in the attenuation of both the negative-going and positive-going field potentials when the LPO unit response is blocked in Figure 33. In other cells, however, the negative field potential could be blocked while the positive field potential was unaltered (see Figure 32). It seems from these data, and others to be reported below, that no consistent relationship exists between excitatory and inhibitory components of a response, suggesting that they may be evoked by parallel as well as sequential processes, and that interactions involving one may occur independently of interactions involving the other.

D. Blocking of spindle trains

While recording from 10 cells, single LPO or MTH stimuli evoked spindle trains, rather than the shorter duration field potentials seen above, in the reticular formation and in the cortex. The spindle responses were more likely to occur during periods of spontaneous EEG synchronization, and the field potential responses during spontaneous EEG desynchronization. About half the reticular spindle responses were blocked following V conditioning stimuli at the same time that the reticular unit responses were blocked.

In the set of photographs demonstrating this effect (Figure 37), the top trace of each frame represents reticular multiple unit activity and the middle trace the reticular field potential, both

photographed during a slow sweep rate. The bottom trace is an expanded version of the first 100 ms of unit activity (following the test stimulus in frames showing paired stimuli) photographed at a faster sweep rate to show the action potentials more clearly. The stimulus occurs at the beginning of the bottom trace.

The responses to V stimulation (Figure 37a) and to LPO stimulation (Figure 37b) both consist of excitatory-inhibitory sequences having different latencies and durations; the LPO stimulus also evokes a spindle train. When the LPO stimulus is placed within the V excitatory response, the excitatory responses of both stimuli overlap, and the spindle is still evoked (Figure 37c). However, when the LPO stimulus is placed in the V inhibitory period, the LPO unit response and spindle response are both blocked (Figure 37d). The blocking period ends about 300 ms later at which time both responses are again evoked (Figure 37e).

The spindle response seems to be an all-or-none process which once begun goes to completion regardless of subsequent inputs. This result is shown in Figure 37f, where the order of the stimuli is reversed, the V stimulus occurring after the LPO stimulus and after the spindle response has begun, and the V stimulus is no longer effective in terminating the spindle.

It seems from these data that the reticular spindle is a local phenomenon, closely related to, or generated by, the LPO unit responses The unit responses must be simultaneously evoked in a large number of

cells for the spindle to be generated, and simultaneously inhibited for the spindle to be blocked. The large number of cells found to be responsive to LPO stimuli, and their almost identical response latencies, support this conclusion.

E. Summation of inhibitory response components

When inhibitory periods of conditioning and test stimuli coincided, the effect was to prolong the silent period and delay rebound excitation. The multiple unit recordings illustrating this effect (Figure 38) need be described in some detail. The V stimulus (Figure 38a) appears to evoke a long latency excitatory response; no inhibitory component is manifest. The LPO stimulus (Figure 38b) evokes a short latency excitation (action potentials and negative-going field potential) followed by inhibition (reduction of background activity in top trace and long duration positive wave in bottom trace). Since the LPO excitatory response is blocked when it follows the V stimulus, but precedes the V response (Figure 38c), the V stimulus must exert a latent inhibitory influence prior to the excitatory influence. The excitation may, therefore, be more accurately described as rebound excitation. The early V inhibitory period seems to summate with the late LPO inhibitory period when the stimuli are paired since rebound excitation is considerably delayed in Figure 38c compared to Figure 38a.

F. Reciprocity of blocking interactions

Blocking in a given cell produced with a given stimulus pair could

not always be obtained if the order of the pair was reversed. In fact, in no cell did A-V pairings, A-MTH pairings, or A-LPO pairings produce mutual suppression. Each blocking interaction involving these pairs was unidirectional. However, V-LPO pairings and V-MTH pairings did produce mutual blocking in 50% and 70% of cells, respectively.

An example of reciprocal V-MTH blocking, which includes some evidence of facilitation of MTH responses by V stimuli as well, is shown in Figure 39. Mutual blocking is illustrated by the following: 1) MTH responses are absent following each spike of the V response up to about 80 ms after the V stimulus (Figure 39e, g, h). MTH responses gradually return at longer interstimulus intervals (Figure 39i), and are fully evoked by 112 ms (Figure 39j). 2) V responses are blocked whenever they follow an MTH response, providing the MTH response is intact (Figure 39c, d). They are not blocked when the MTH response is absent (Figure 39e).

Facilitation is indicated by the shortened latency of the MTH response, from 12 ms when MTH is presented alone (Figure 39b) to 9 ms (Figure 39c) and to 6 ms (Figure 39d) when MTH follows V by different delays. In Figures 39c and d, the V and LPO excitatory responses overlap, and, therefore, may be summating to produce the quicker response. Other examples of facilitatory interactions are given below.

G. Poststimulus histograms

Poststimulus histograms were drawn for three cells in which a large number of responses were recorded for each stimulus pairing. The histograms provide a more quantified measure of many of the interactions described above, and also reveal effects that were not apparent in the individual photographs.

The first set of histograms (Figure 40) shows both blocking and facilitation of V responses by LPO stimulation. V stimuli applied alone produced in this cell four peaks of excitation between 20 and 130 ms (Figure 40a). LPO stimulation produced excitation-inhibition-rebound excitation (Figure 40b). When the V stimulus was presented 14 ms after the LPO stimulus (Figure 40i) or 28 ms after (Figure 40j), all four peaks of the V response occur in the same temporal relationship to each other, but there are many more spikes per peak. This facilitation of the V response occurs approximately at the time of the LPO rebound excitation, suggesting a summation of excitatory inputs from the two systems. At a slightly greater interstimulus interval of 40 ms (Figure 40k), the first three peaks of the V response are facilitated, but the fourth peak is about the same as in the control response. Therefore, the facilitatory interaction ends just as the LPO rebound excitation is ending.

In addition to facilitation, LPO stimuli produced in this cell a blocking interaction that began immediately after the LPO response and

lasted about 20 ms. It can be seen in Figures 40c-g where the V-LPO interstimulus interval is progressively shortened. As each peak of the V response falls into the LPO inhibitory period immediately following the LPO response, it becomes totally suppressed. It is interesting that one peak of the V response may be suppressed at the same time that subsequent peaks are facilitated (e.g. Figures 40c-f), for it may indicate some independence of inputs generating the four response peaks.

From these poststimulus histograms, the amplitude of each peak of the V response was measured at each interstimulus interval and expressed as a percentage of the control response in Figure 40a. The percentages obtained for peak 1 are plotted in line 1, for peak 2 in line 2, etc., in Figure 41a. The data are plotted on a time scale relative to the occurrence of the LPO stimulus, and consequently relative to the occurrence of the LPO response which is superimposed on the graph. It can be seen that peak 1 of the V response is reduced to 14%, peak 2 to 0, and peak 3 to 0 when they occur between 22 ms and 30 ms after the LPO stimulus, i.e. in the LPO inhibitory period. This confirms the previous conclusion that LPO stimulation produces a blocking interaction with a latency of about 20 ms and a duration of 10 ms. The blocking is immediately followed by a period of facilitation, corresponding to the LPO rebound excitation, during which V responses are increased over 200% of their control values.

Although it was not apparent in the histograms, this cell also

became relatively unresponsive to the LPO stimulus during a short period following the V stimulus (Figure 41b), since the LPO response is reduced to 80% of control values just following the first peak of the V response. This suggests a short period of visually evoked inhibition at this point. Such an interpretation could explain, in Figure 41a, why peak 1 is reduced by LPO stimulation to 14% while peaks 2 and 3 are reduced to 0. That is, when V peaks 2 and 3 are in the LPO inhibitory period, the LPO inhibitory period is coincident with the V inhibitory period. If these inhibitory inputs summate, V peaks 2 and 3 would be subject to greater suppression effects than V peak 1.

Interactions between V and LPO responses in two other cells were analyzed with the above methods. The histograms are given in Figures 42 and 44, and the graphs in Figures 43 and 45.

One cell gives a V response consisting of three excitatory peaks (Figure 42b) and an LPO response consisting, typically, of excitation-inhibition-rebound excitation (Figure 42a). We see in Figure 43a that the first and second peak of the V response are reduced to 0 immediately following the LPO excitatory response, and then gradually increase to over 100% at later delays. Thus this cell, like the previous one, shows blocking of a V response for a short period following LPO stimulation, and then facilitation for a longer period.

Similarly, V stimulation reduces the cells' response to LPO for a short period following the first peak of the V response, and thereafter

facilitates the LPO response (Figure 43b).

In the third cell, LPO stimulation reduces V responses to a minimum of 30% of control values (Figure 45a), but V stimulation has little effect on the amplitude of LPO responses (Figure 45b).

Responses to repetitive stimulation of one modality

MTH and LPO stimuli were applied at rates from .5 Hz to 100 Hz while recording from 11 cells in order to observe reticular unit responses during EEG recruiting responses, and to observe the effects of paired stimuli of the same modality.

The general finding was that LPO stimuli blocked responses to subsequent LPO stimuli (and MTH stimuli blocked responses to subsequent MTH stimuli) in about the same percentage of cells, and at about the same latencies as they blocked responses to test stimuli of other modalities. One example is given in Figure 46 showing the responses of one cell to 5, 6, 8, 10, 13, and 33 Hz stimulation. Each pulse at the lowest frequencies (5 and 6 Hz) evokes a burst of action potentials, but only the first pulse of the train evokes a complete field potential response, the later responses lacking the late positive wave. At 8, 10, 13, and 33 Hz, the cell responds with the full burst of action potentials only to the first pulse of the train, and then to subsequent pulses if they occur beyond 250 ms. The positive wave of the field potential is always absent beyond the first pulse, and the negative wave is also absent if the spikes are absent. During 33 Hz stimulation, we also see that the field potential

response to the first pulse runs to completion even though other stimuli intervene. This effect is reminiscent of the LPO spindles in Figure 38 that could not be blocked by V stimuli applied after the spindle had begun.

Summary of results

We have observed a great number and wide variety of effects produced in reticular neurons by V, A, MTH and LPO stimulation. We have been able to illustrate only the more striking and commonly observed effects, which, even so, occurred with different probability, latency, and duration in different cells. The results may be summarized as follows:

1) Reticular neurons were generally very responsive to the stimuli used in this experiment, many responding to two, three, or all four modalities.

2) Stimulation in each modality evoked a characteristic response type, and each response type included both excitatory and inhibitory components.

3) When stimuli were paired, responses to the test stimulus were often modified by the conditioning stimulus, whether or not the conditioning stimulus evoked action potentials in the cell.

4) Typically, the excitatory component of the test response was facilitated during the excitatory component of the conditioning response, and blocked during the inhibitory component. These effects occurred during brief, discrete intervals, or could be very long lasting.

5) When a test response had more than one excitatory component, each component could be modified independently of the other.

6) Inhibitory components of the test response could also be modified, i.e. blocked or prolonged, under certain conditions in some cells.

7) Entire spindle trains could be blocked by conditioning stimuli.

8) The array of interactions occurring in one cell might be quite different from the array occurring in a nearby cell.

9) Some stimuli could modify their own subsequent responses as well as subsequent responses to other modalities of stimulation.

These findings unquestionably demonstrate the existence of synchronizing-desynchronizing reciprocal interactions in the reticular formation that are considerably more complex than predicted by simple hypotheses of mutual inhibition. We will discuss below the various interpretations of synaptic relations that may account for some of the response interactions observed in this experiment, and later speculate about the role of these interactions in controlling reticular output, and in the genesis of the cortical EEG.

## Discussion

The present experiment was carried out to determine whether excitatory and/or inhibitory inputs from EEG synchronizing structures could effect changes in reticular unit responses to sensory stimulation. In the course of obtaining confirmatory results, it became clear that sensory stimuli were also effective in altering reticular responses to synchronizing stimuli. Thus an anatomical and physiological substrate was demonstrated for reciprocal interactions between synchronizing and desynchronizing influences in the reticular formation, one function of which may be regulation of the cortical EEG. In the following pages we will discuss the evidence for reticular response interactions, from the present and previous studies, and suggest possible neuronal mechanisms.

### Convergence of inputs

The first important result of this experiment was to show that inputs from MTH, LPO, V and A systems converge on single reticular neurons. To our knowledge, there are no published reports of convergence from these particular sources with which to compare our results. However, convergent reticular responses to sensory stimuli alone have been reported frequently (Bell et al. 1963, Groves et al. 1973, Scheibel et al. 1955) and are in close agreement with the present results (see Table 7). These comparisons show that V responses tend to be encountered

more frequently than A responses (if sampling is limited to the mesencephalic reticular formation), and they have longer and wider ranges of latencies. The degree of convergence of A and V responses varied from 6% to 21% among the different studies, not a large difference considering the variations of response definition and sampling techniques. Another common finding, not listed in the table, was the tendency to encounter V responses in the dorsal and anterior regions of the reticular formation, and A responses in the lateral and posterior regions, a difference that was statistically significant in Groves et al. (1973) but not in the present study, possibly because of the smaller number of cells recorded. The differential distribution of V and A responses agrees well with anatomical data showing a distribution of tectoreticular (secondary visual) fibers to the anterodorsal mesencephalon (Rasmussen 1936, Kawamura et al. 1974, Pearce and Glees 1956), and lateral lemniscal (secondary auditory) fibers to the lateral sleeve of the reticular core in the more caudal planes (Rasmussen 1946, Brodal 1957, Stotler 1953). All authors agree that V-A convergent cells are most likely encountered in central areas where visually responsive cells and auditory responsive cells overlap. Regarding temporal response characteristics, several authors have reported, in confirmation of the present results, that reticular formation neurons characteristically respond to peripheral sensory stimulation with a brief burst of excitation followed by a period

of reduced responsiveness typically lasting about 150 ms (Bell et al. 1963, Amassian and Waller 1958, Amassian and DeVito 1954, Fox and Wolstencroft 1975, Bowsher et al. 1968, Peterson and Felpel 1971, Scheibel et al. 1955).

Reticular response to MTH and LPO stimuli, when compared to responses reported by Mancia et al. (1974, 1976), Lineberry and Siegel (1971), Bremer (1970), and Grantyn et al. (1973), are generally consistent except for the smaller percentage of LPO responses given by Mancia et al. (1976) (see Table 8). There is no apparent explanation for this discrepancy at this time. Unfortunately, there are no comparative statistics for the anatomical distribution of MTH or LPO responses, nor for their degree of convergence. The present study demonstrated a wide distribution for both systems, and frequent convergence. MTH and LPO unit response patterns have been described by others as they have been described here. For example, Bremer (1970) and Lineberry and Siegel (1971) describe the LPO response as a short latency burst of spikes associated with a sharp negative-going field potential, followed by a long duration suppression of activity and positive-going field potential lasting about 100 ms. Similarly, during low frequency stimulation of MTH, reticular responses consist of bursts of spikes in phase with negative peaks of recruiting waves in the reticular formation, and silent periods between (Purpura 1970).

From the present data it is clear that synchronizing and desynchronizing inputs have wide and overlapping spheres of influence in the reticular formation, but that within those spheres, each system makes selective and asymmetrical contact with a large number of neurons. We can conclude that convergence, though extensive, is not complete since some cells have inputs only from synchronizing systems and others only from desynchronizing systems, and since some combinations of inputs occur in one cell that do not occur in an adjacent cell. In addition, interactions that occur between convergent inputs are not always reciprocal, and this too indicates some imbalance of synchronizing and desynchronizing influences. These differences indicate an extraordinary variability in convergence patterns from cell to cell which may account for the variability in the nature and magnitude of the response interactions to be described below, but at the same time they confirm that a physiological substrate of sufficient complexity and proportion exists in the brain stem for the integration of various and competing inputs.

Interaction of inputs

The second important finding of this experiment was that concurrent stimulation of convergent synchronizing and desynchronizing pathways could produce distinct changes in the firing patterns of reticular neurons. For example, we found that summation of excitatory inputs

from two sources could prolong a reticular discharge, or evoke a spike response sooner than either stimulus alone would have, that summation of inhibitory inputs from two sources could prolong a silent period and delay rebound excitation, and that summation of excitatory and inhibitory inputs frequently resulted in the total suppression of the excitatory input, for short or relatively long periods of time. These effects are not fundamentally different from those seen in other parts of the nervous system where various pathways converge on a single neuronal pool (e.g. Dunner and Rutledge 1965, Purpura 1970, Hagbarth and Kerr 1954, Thompson et al. 1963). However, their occurrence in the reticular formation, between stimuli producing cortical synchronization and those producing cortical desynchronization, may be of some functional significance.

For example, MTH and LPO structures were shown to exert powerful and long duration effects over the responsiveness of reticular neurons to sensory inputs. Both the degree and nature of the effects depended very critically on the temporal parameters involved (i.e. the timing of the interval between the conditioning and test stimuli). Also, the same conditioning stimulus might produce quite different patterns of interaction in one cell compared to an adjacent cell. In as much as the interactions were evident in both the unit activity and field potentials, we may suppose that they probably take place within the reticular

formation.

Previous data, mainly from evoked potential studies, have also demonstrated that sensory responses in, and conduction along, the reticular core can be influenced by stimulation of extrareticular structures, i.e. hypothalamus, hippocampus, cerebral cortex (Adey et al. 1957, Lindsley et al. 1972, Zilov 1970, Rabin 1966, Darien-Smith and Yokota 1966). In each of these reports both phasic facilitation and phasic inhibition could be produced depending on the particular parameters of stimulus pairings. Similarly, in the present experiment, blocking, augmentation, or both, could be evoked in complex temporal sequences by systematically varying the interval between stimuli. The present single unit study confirms that these patterns are reflected in the firing patterns of individual neurons as well as in the configurations of evoked potentials.

Our observations also are important for demonstrating that sensory stimuli as well as central stimuli could exert competing influences over the responsiveness of reticular neurons. Adey et al. (1957) alluded to this possibility after finding that inadvertant auditory stimulation during their tests could alter transmission in the reticular formation as well as the cortical stimuli they were applying. Similarly, the characteristics of interactions produced by V and A stimuli in this experiment were not different in type, latency, or duration from the interactions produced

by MTH and LPO stimuli.

These observations lead to several interesting conclusions about the role of synchronizing and desynchronizing influences in the reticular formation. Since sensory and central synchronizing stimuli have the capacity to exert such powerful and long lasting effects on reticular excitability, they may be presumed to normally have some measure of regulatory control over brain stem functions. This control does not seem to be specific to specific stimuli, i.e. synchronizing inputs do not have primarily inhibitory effects on reticular excitability, and desynchronizing inputs do not have primarily excitatory effects. The nature, temporal characteristics, magnitude, and duration of alterations imposed by any source varied according to the unique patterns of convergence on each individual cell. We may presume these processes to be of some functional significance in reticular control of EEG rhythms in remote parts of the brain, but we cannot imply, on the basis of these data, a simple or linear cause and effect relationship with such complex functions. We may only leave open the possibility that the shifting temporal patterns of reticular discharges, influenced to a large degree by the temporal interleaving of excitatory and inhibitory influences from synchronizing and desynchronizing sources, may carry information regarding the effect of reticular output on other brain structures. In this regard it is interesting to note that synchronizing and desynchronizing

systems which influence brain stem activity are themselves in turn modified by the very changes they induce in the brain stem.

Mechanisms of interactions

Synchronizing and desynchronizing inputs to the reticular formation may gain access to reticular neurons through direct and/or indirect channels. We cannot know for sure at what point in these channels the interactions take place, or what the cellular integrative mechanisms are. However, we may postulate several alternatives, and examine the evidence for and against each. We will find that, on the basis of the available data, none of the alternative mechanisms can be definitely excluded, and possibly all are involved, but some appear to be more likely than others.

If we consider first the possibility that interactions occur outside the reticular formation, at some point where the respective afferent systems converge before converging in the reticular formation, we find there are two inconsistent observations. One is that the reticular excitatory responses to MTH and LPO stimulation have relatively short latencies, so the pathways involved must have few synapses. With few synapses, the opportunities to alter transmission are limited. One possible extrareticular site of interaction is in the thalamus where internuclear communication between MTH and lateral and medial geniculate (V and A relay nuclei) has been demonstrated (Scheibel and

Scheibel 1966). But it is not likely that such powerful and specific effects could be generated by intrathalamic mechanisms. In any case, there are no known points of intersection between LPO and sensory pathways that could account for those extrareticular interactions. On anatomical grounds, therefore, there is little support for integration of synchronizing and desynchronizing inputs prior to their arrival in the reticular formation.

The other incompatible observation comes from field potential data. It may be assumed that the large positive and negative field potentials generated in the reticular formation in response to central and peripheral stimuli represent postsynaptic inhibition and postsynaptic excitation, respectively, on reticular soma and dendrites. This assumption is possible since the reticular formation is an anisotropic structure with postsynaptic elements randomly oriented in the vicinity of the electrode and axons projecting for considerable distances rostrally and caudally. So the electrode "sees" the summation of soma and dendritic potentials probably relative to the axons rather than relative to some other synaptically active region of the cell. Consequently, negative potentials may unambiguously represent net excitation to the neuronal pool, and positive potentials net inhibition. Such interpretations are not possible in laminar structures like the cortex where, for example, positivity recorded from the region of the cell body may represent either

excitation at the apical dendrites, or inhibition at the soma. The significance of being able to more clearly interpret reticular field potential is that excitatory and inhibitory influences of various inputs can be seen to already have representation within the reticular formation, so there is no need to postulate that their effects are exerted elsewhere.

If, therefore, we consider the alternative hypothesis, that integration mechanisms occur within the reticular formation, we have several alternatives to explore. Briefly, they are direct afferent control, indirect afferent control, recurrent reticular inhibition, and presynaptic inhibition. We will not consider here more complex and indirect processes such as disfacilitation and disinhibition since it is even more difficult to distinguish between these alternatives with the present data.

Direct afferent control implies that each modality sends both excitatory and inhibitory projections to the same reticular neuron, the excitatory inputs increasing the responsiveness of the neuron to excitatory inputs from a second source (facilitatory interaction), and the inhibitory inputs decreasing the responsiveness (inhibitory interaction). The inputs may synapse on or near the soma where they may exert a control over the general output of the cell, or on distal dendrites where they may selectively influence V and/or A responses. This hypothesis implies that the cell's excitability may be both increased and decreased directly by the structure being stimulated.

None of the data from the present study contradict this model. The short latency and consistent pattern of excitatory responses certainly suggest direct excitatory input, and there is no reason to believe that the later inhibitory responses couldn't arrive over longer pathways from the same source. The occasional observation of excitatory response components being blocked while inhibitory components remain intact support the idea that each component is generated by a separate independent input from an extrareticular source.

The second proposed mechanism, of indirect afferent control, means, in the case of inhibitory reticular responses, that afferent fibers from the stimulated structure terminate on reticular inhibitory interneurons which in turn generate IPSPs in other reticular neurons. There is no practical difference between this mechanism and the one described above, only the theoretical distinction between inhibition produced directly by the stimulated structure, and indirectly by reticular neurons. Actually, the intercalated inhibitory interneuron may be placed anywhere in the afferent pathway, and as it approaches the stimulated structure, this model approaches the first. It is not possible to determine from the present data whether afferent inhibition, if it exists, is direct, or through an inhibitory interneuron in the reticular formation or elsewhere.

The third possibility, of recurrent inhibition, is fundamentally different from the first two since it implies that afferent input is solely

excitatory, and that any subsequent inhibitory responses are generated by reticular neurons through excitation of reticular inhibitory interneurons. This model predicts that the latency of inhibition must always be greater than the latency of excitation, and that inhibition should not be expected in the absence of excitation. In many cells, these predictions were confirmed, but in other cells, contradictory observations were made. For example, some interactions indicated that inhibition occurred prior to the excitatory response of the cell, so it could not have been generated by that excitatory response. Second, inhibitory responses were seen in the absence of excitatory responses, as when the excitatory component of a test response was blocked while the later inhibitory component was not. It is possible that inhibition in this case could have been generated recurrently by other cells whose excitatory responses were not blocked, but in as much as the negative field potential was suppressed at the same time that the spikes were suppressed, we may assume that excitatory responses were generally absent, and so could not have been responsible for the inhibition. Since recurrent inhibition has never in fact been demonstrated in the reticular formation (Magni and Willis 1963), although it is a recognized phenomenon in other parts of the nervous system (see Eccles 1957), we may conclude that it is one of the less likely interpretations for the present results.

The final possibility to be considered here is presynaptic inhibition,

which describes the situation where collateral fibers from one afferent system terminate on afferent fibers of another afferent system as they both converge on a particular neuron, thereby depolarizing those afferents and reducing the amount of transmitter substance they release. Eccles and colleagues showed that presynaptic inhibitory mechanisms played an important role in reducing responses to sensory afferents in the spinal cord (see Eccles 1964). It is possible that presynaptic inhibition might also explain the observation in the present experiment that input from one source could reduce the responsiveness of reticular neurons to input from a second source, except for two considerations. The first is that presynaptic inhibition, since it is generated by membrane depolarization, should not be expected to be correlated with large amplitude positive waves, which are in fact frequently seen in relation to inhibitory events. It is more likely, therefore, that inhibitory events involve IPSPs somewhere in the system. Second, presynaptic inhibition, like recurrent inhibition, has not yet been observed in the reticular formation, although Bowsher and Westman (1971) do report a considerable number of axoaxonic synapses.

In summary, we cannot be sure which of the mechanisms described above, if any, are responsible for response interactions recorded extracellularly in the reticular formation, although direct afferent excitation and direct afferent inhibition seem the most plausible. It is possible to

resolve some of the questions of cellular mechanisms of V, A, MTH and LPO influences using intracellular recording and stimulation techniques, and that was our intent in Experiment III, described below. For example, it would be important to know whether spike discharges and silent periods were correlated with EPSPs and IPSPs as well as with negative and positive waves in the field potential; and during response interactions, whether the PSPs of each response summated in predictable ways. However, these observations would not be conclusive, and some questions would still remain. It could not be determined from intracellular recordings, for example, whether IPSPs were generated by direct afferent input or recurrent reticular input except by antidromic stimulation techniques, which are not easily applied in as complex a structure as the reticular formation. Also, it may turn out that certain inputs are suppressed without manifestation of IPSPs. Therefore, the inhibition may be "remote", taking place on dendrites or other portions of the membrane far from the site of recording, or presynaptic, taking place completely outside the recorded neuron (see Cook and Cangiano 1972). Another problem is that excitability changes (blocking and/or facilitation) may be achieved through changes in membrane excitability not reflected in changes in membrane potential. In that case current pulses could be applied to measure membrane resistance and time constant. Some of these problems are dealt with in Experiment III

reported below.

In conclusion, the present results confirm that elements normally classified as part of the reticular activating system on the basis of their sensory responses, clearly also receive input from synchronizing structures. Further, both synchronizing and desynchronizing inputs have qualitatively similar effects on reticular excitability, producing either phasic excitation or phasic inhibition depending on the temporal sequence of convergent responses. It seems that these inputs exert their effects directly on reticular neurons, although other cellular mechanisms are possible. The changes in reticular firing patterns produced by synchronizing-desynchronizing interactions may be relevant to determination of the cortical EEG. These conclusions will be discussed in relation to theories of reticular deactivation in the general discussion following Experiment III.

## Experiment III

### Background

The purpose of this experiment was to record from mesencephalic reticular neurons intracellular potentials that might underlie some of the responses and response interactions observed extracellularly in Experiment II. Given the limited time an intracellular recording may be maintained, only LPO and V stimuli were applied since they evoked the most consistent responses in Experiment II.

Previous authors (Mancia et al. 1976, Mancia 1975, Grantyn et al. 1971) have reported that low frequency LPO stimulation induces in mesencephalic neurons short latency, slowly rising EPSPs with or without spikes, thus confirming the general result of Experiment II that LPO regions have a primarily excitatory effect on the reticular activating system. However, Mancia et al. (1976) also report that no IPSPs were ever observed, a result inconsistent with the long duration inhibitory effects so clearly seen in a large percentage of cells in our study. IPSPs may not have been observed because the inhibitory influences were not exerted directly on the recorded neuron, because the inhibitory synapses were some distance from the recording electrode, or because cells responding with IPSPs were not sampled. We are not aware of

reports of intracellular reticular responses to V stimulation.

In view of the lack of intracellular data relating to the influence of synchronizing and desynchronizing influences in the reticular formation, and the question of the origin and location of extracellularly recorded events appearing to represent inhibition, the present experiment further investigated postsynaptic responses of reticular neurons. In addition to observing potential changes evoked by V and LPO stimuli, we measured changes in neuronal excitability before, during, and after V and LPO responses, by applying depolarizing current pulses to the cell through the microelectrode.

We were surprised to find that little or no postsynaptic potential activity could be recorded from many of these cells, despite evidence of successful impalements, and despite the appearance of action potential responses to V and LPO stimuli. We could, however, ascertain in some cells, by applying current pulses, that neuronal excitability was increased during the excitatory phase of the LPO or V response, and decreased during the presumed inhibitory phase. Therefore, some support was found for the hypothesis of direct afferent control of reticular neurons by LPO and V inputs.

## Methods

### Subjects

Four adult female cats obtained from McMaster University Medical Center were used as subjects in this experiment. All experiments were acute and performed under chloralose anesthesia since the results obtained in Experiment II in chloralose-treated preparations did not differ from the results obtained in curarized preparations.

### Surgical preparation

After anesthesia was induced with a gaseous mixture of flouthane and nitrous oxide, the femoral artery and femoral vein were cannulated, an endotracheal tube was inserted, and EKG electrodes made from size 00 insect pins were placed in the skin of each forepaw. The gas anesthesia was then discontinued and chloralose (60 mg/kg dissolved in 25 cc of physiological saline) was injected into the cannulated vein. A blood pressure gauge was attached to the valve of the cannulated artery. After respiration, blood pressure, and EKG has stabilized following the injection of chloralose, the cat was placed in a Kopf stereotaxic head holder after Xylocaine ointment was applied to the ear bars and nose clamp.

The skull was exposed through a midsaggital incision and removal of the temporal muscles. To reach the brain stem area from which recordings were made, a bone flap approximately 1 $cm^2$ was removed unilaterally just anterior to the tectum. In successive stages, the dura below was cut away, and the brain tissue overlying the dorsal hippocampus and superior colliculus was aspirated. In some preparations

the posterior hippocampus was also removed and in all preparations the dura and pia overlying the tectum were carefully dissected away. Bleeding was controlled with electrolytic coagulation and Gelfoam. The operative site was then covered with saline or mineral oil warmed to 37° C.

Bipolar stimulating electrodes made of insulated nichrome wires .01" in diameter twisted together and separated at the tips by 1 - 2 mm were implanted in LPO bilaterally (see Methods, Experiment I, for details) and cemented to the skull. For recording reticular field potentials, a monopolar tungsten electrode etched to a tip diameter of 75 micrometers was introduced through a hole in the cranium at an angle calculated to place the tip in the contralateral hemisphere near the site of intracellular recording. An indifferent reference electrode (jeweler's screw) was placed in bone over the frontal sinus. A second reference electrode, a cotton wick soaked in .9% sodium chloride, was also placed over the frontal sinus to record a reference potential for the intracellular microelectrode.

The animals' eyes were covered with transparent contact lenses and irrigated periodically with physiological saline.

At this point in the preparation, two animals were supplied with an I. V. drip containing isotonic saline and dextrose at a rate of 5 cc per hour and left overnight (approximately eight hours). The remaining surgical preparations to be described below were, in these cases,

performed on the next morning. In the other two subjects they followed immediately.

After the brain stem was exposed and all stimulating and recording electrodes were implanted, the animals' vertebral column was suspended and immobilized with stainless steel pins inserted into the spines at approximately $T_4$ and fixed to a rigid frame attached to the stereotaxic head holder. Then the animal was connected to a respirator and artificially ventilated through the endotracheal tube. A bilateral pneumothorax was performed to reduce breathing movements. The final step of the surgical preparation was injection of 2 cc of Flaxedil through the cannulated vein. Flaxedil injections were repeated approximately every three hours to reduce spontaneous movements that would disrupt the intracellular recordings.

Stimulation apparatus

The LPO area was stimulated with single rectangular pulses .1 to .4 ms in width generated by Tektronix waveform (type 161) and pulse (type 162) generators. The pulses were isolated from ground through a photon-coupled isolation unit (W. P. Instruments, model PC-1) which delivered the pulses at constant currents ranging from .5 to 5 mA to the two LPO stimulating electrodes wired in series. Visual stimuli were single light flashes produced by a Grass photostimulator model PS 22 (see Methods, Experiment II).

Recording apparatus

Intracellular recordings were obtained through glass micropipettes, less than 5 micrometers in diameter at the tip, pulled with glass fibers inside and filled with 3 molar potassium chloride or 2.2 molar potassium citrate. The tip resistence of these electrodes ranged from 10 - 60 meg ohms. The potentials recorded through the potassium microelectrode were referred to a sodium chloride filled cotton wick electrode placed on the cranium over the frontal sinus. To reduce junction potentials in the recording circuit, the upper portion of the microelectrode was filled with .9% sodium chloride in agar gel and fitted into a silver-silver chloride half cell containing .9% sodium chloride. The half cell was attached to the high impedence probe of a model M-4A Electrometer amplifier (W. P. Instruments). This instrument operates as a unity gain DC amplifier with an input impedance greater than $10^{12}$ ohms, and includes controls for DC offset balance, negative input capacity compensation, measurement of electrode resistance, and current injection with DC and transient balance. The current pulses were triggered by input from a pulse generator. Details of the electronic circuitry are schematized in Figure 47.

Recording procedure

The microelectrode, secured in a Narigishe micromanipulator, was placed under visual control through a surgical microscope on the

surface of the exposed brain stem, and then lowered in micron steps through the first 1 - 2 mm while its resistence, capacitance, and stability were checked. Microelectrodes were discarded when their resistence fell outside the 10 - 60 meg ohm range, and when they became unstable and failed to pass current linearly.

With acceptable microelectrodes, successive penetrations of the brain stem were made in micron steps in an attempt to impale spontaneously active reticular neurons. An electrode was considered to be intracellular when the recorded voltage dropped 30 millivolts or more and remained stable. Often the impalement would evoke a short burst of positive-going action potentials (injury discharges) which confirmed the intracellular placement.

After impalement, and after injury discharges had ceased, LPO stimuli were applied once every two seconds, the intensity being varied until a response threshold was reached. Then, stimulation at that and slightly higher intensities was continued while the responses displayed on Tektronix 565 oscilloscope were photographed on 35 mm film through a Nihon-Kohden camera (model PC-2A).

After LPO stimulation, the preparation was stimulated with light flashes once every two seconds and again responses were photographed. The effects of pairing LPO and V stimuli were also observed in some cells.

To anticipate some of the results, we found very few PSP responses

to V or LPO stimuli. The resting potential of the majority of cells was very level and remained so regardless of what stimuli were applied. However, failure to record PSPs in the vicinity of the microelectrode does not necessarily mean that the membrane there was inexcitable or that responses were not being evoked. Excitability changes could still have been produced by altering membrane resistence and conductance independently of the potential. A technique commonly used to measure those other variables is the application of rectangular current pulses across the membrane between the intracellular electrode and extracellular medium. The membrane potential generated by this current will rise exponentially to a steady level and then decline with a similar time course. Current in one direction will depolarize the membrane, while current in the other will hyperpolarize it.

Several characteristics of the current response give information about the excitability of the cell. For example, the cell's input resistence can be calculated by dividing the maximum induced voltage change by the applied current ($r_m = dV/dI$). Membrane resistence is the reciprocal of membrane conductance, and membrane conductance describes the rate of exchange of certain ions, particularly $Na^+$, $K^+$, and $Cl^-$, across the cell membrane. As the ionic concentrations change, the excitability of the cell is either increased or decreased. The time constant of the cell is another measure of excitability roughly proportional to the input

resistance, since high input resistance impedes and hence slows the electrotonic spread of current. The time constant is equal to the time required for the voltage change induced by the current pulse to reach approximately 2/3 of maximum value. A third measure of excitability, and the one most frequently used in this experiment, is the number and latency of action potentials evoked by the current pulse. If the current evokes a threshold depolarization, action potentials will be generated, and the latency to the first action potential, total number of action potentials per pulse, and the interspike intervals all reflect the membrane impedance and membrane excitability at that particular time. With increased excitability the number of evoked action potentials will be greater and/or their latencies shorter. During decreased excitability, the reverse relationship holds.

In the course of this experiment depolarizing rectangular current pulses 20 ms in duration and .5 mA to 9 mA in intensity were applied to 14 cells at various times after the V and LPO stimulus on the same sweep in some cells and on alternate sweeps in other cells.

Data display and analysis

Six signals were simultaneously displayed on a Tektronix 565 oscilloscope:

1) reticular field potential

2) intracellular potential at lower gain than in 4)

3) extracellular DC potential and time calibration pulse

4) intracellular potential

5) rectangular current pulses

6) intracellular potential, AC coupled, at higher gain than in 4).

The first two traces were synchronized with each other and displayed at a slow sweep rate, the stimulus being delayed approximately 100 ms from the beginning of the sweep. A 200 ms portion of these traces, beginning about 20 ms prior to the stimulus, was brightened on the screen to indicate the part of the signal that appears in an expanded version in the bottom four traces. Trace 3 showed a positive-going 16 ms pulse used as a time calibration for traces 3, 4, 5, and 6, which were all synchronized. The top excursion of the 16 ms pulse marked the extracellular DC potential. The intracellular potential, shown in trace 4, was 20 mV/vertical division more negative than the extracellular potential shown in trace 3. Trace 5 showed the rectangular current pulses when they were applied, and trace 6 showed the intracellular potential again, AC coupled, at a very high gain.

From these photographs were measured the membrane potential, height of intracellular spikes, latency of V and LPO spike responses, and current responses (number and latency of evoked spikes, membrane input resistence, and time constant).

Histology

At the end of the experiment animals were injected with 5 cc of

Nembutal, and their brains perfused through the carotid artery with 50 cc of physiological saline followed by 50 cc of formalin. The brains were later removed, cut in 40 micrometer sections, and every 10th section was mounted and stained with cresyl violet. The sections were examined to locate the sites of the LPO stimulating electrodes, the tungsten field potential electrode, and the tracts made by the glass micropipettes.

# Results

## Location of electrodes

LPO stimulating electrodes in all four subjects were located in the basal forebrain area at AP levels 13 through 16 (atlas of Jasper and Ajmone Marsan 1954), approximately 2 mm from the midline, i.e. loci corresponding to LPO stimulation sites in Experiments I and II (see Figure 48).

Intracellular recording sites were not marked, and therefore not determined in histological sections. However, in gross examination of the tissue, slight damage to the dorsal surface of the right superior colliculus indicated the points of entry of the glass microelectrodes to overlie the mesencephalic and anterior pontine reticular formation. Fine tracks left by the penetrations confirmed the general extent of recording sites. The tungsten electrode recording field potentials was located in the right pontine reticular formation as well.

## General properties of cells

Intracellular recordings were obtained from 44 brain stem cells for periods lasting 2 - 10 minutes approximately. Visual stimuli, LPO stimuli, or both were applied during that time, alone or in combination with intracellularly applied current pulses.

A. Resting potentials

As the electrode was advanced toward the cell, negative DC potential shifts up to 70 mV were encountered. They appeared gradually or stepwise, lasted for variable periods of time, and ended with a return to the baseline. In most cells, a transition from small, multiphasic negative-going to large, monophasic positive-going action potentials was also seen. Stable resting potentials upon impalement exceeded -30 mV in 75% of the cells (range of -12 mV to -70 mV), and so were comparable to those reported for reticular neurons in other studies (Segundo et al. 1967a, Magni and Willis 1963a, b, Limansky 1965). The membrane potential sometimes drifted several millivolts positive or negative during the recording, and when it rose to less than 10 mV negative, the recording was terminated.

In some cells it was possible to obtain estimates of membrane input resistence and time constant by measuring the rise time and peak amplitude of responses to intracellularly applied current pulses. Very high values of membrane resistence, 6 - 14 meg ohms, and long time constants, approximately 4 ms, suggested the electrode was recording from a small diameter structure, possibly a dendrite.

B. Postsynaptic potentials

One of 44 cells showed spontaneous oscillations of the transmembrane potential clearly identifiable as EPSPs or IPSPs. These

potentials, 5 - 10 mV in amplitude, sometimes reached firing threshold and suggested the presence of complex, summated inputs. No post-synaptic activity could be recorded from the other 43 cells despite the fact that the majority of these cells had large and stable resting potentials and action potentials, and were responsive to intracellularly applied current. An example may be seen in Figure 49a in which five superimposed responses to LPO stimuli are represented. There are clearly defined field potential responses containing negative and positive components (trace 1) and 6 mV positive-going action potentials at a latency of about 8 ms (trace 4). Yet the spikes are not superimposed on a depolarizing membrane shift, and the silent period following the spikes, and corresponding to the beginning of the positive-going field potential, is not accompanied by a hyperpolarizing shift. Failure to record postsynaptic activity in cells giving action potentials most likely implies a lack of active contacts in the vicinity of the recording electrode, although other interpretations, discussed below, are also plausible. The large proportion of recordings lacking postsynaptic activity suggests a sampling bias of some sort, or a real difference in the synaptic organization of reticular neurons compared to the classical model presented for the spinal motorneuron (see Eccles 1964).

C. Action potentials

Thirty-nine of 44 impaled cells exhibited purely positive-going,

all-or-none action potentials with fast rise times and variable, slower decay times. The five cells without action potentials, and without PSPs, may have been inactive neurons, or glia. Typically large amplitude spikes, greater than 25 mV, were seen in only 16% of cells, and in one of these cells having a membrane potential of about -50 mV, the action potential approached the 0 level. More commonly, very small size spikes, 3 - 10 mV, were seen. They occurred in 60% of the cells, but there was no distinct cut-off between large and small spikes for their amplitudes among cells showed a continuous gradation from one extreme to the other. Small spikes had the same fast rise time as large spikes, but relatively longer durations. They occurred singly or in bursts, and appeared to show temporal summation at high rates of discharge (see Figure 50a). However, summated small action potentials did not trigger large action potentials. The small spikes appeared to be a real phenomenon, not an artifact of recording. Spikes of this type have been reported by several previous authors (e. g. Segundo et al. 1967a, Limansky 1965, Spencer and Kandel 1961, Llinas and Nicholson 1971) and are sometimes interpreted as spikes originating in dendrites. For the most part, spike amplitude was constant, so large and small spikes were never seen in the same cell, though such a coincidence has been reported by Segundo et al. (1967a). Also, spike amplitude was not correlated with membrane potential since small spikes could appear on large or small membrane

potentials.

With the exception of spikes occurring in the one cell with PSPs, impulse initiation in large and small spikes was characterized by an absence of depolarizing prepotentials. Action potentials took off directly from the baseline, and returned to it without evidence of after hyperpolarization (see Figures 49a, 50b). Also, it was occasionally observed that action potentials arose from levels of relative membrane hyperpolarization as well as depolarization, as if there was no causal relationship between the membrane potential at the recording site and the potential at the site of impulse initiation (see Figure 49b). Each of these events resembles those in the brain stem and elsewhere where "fast pre-potentials" associated with small or large spikes have been reported (e.g. Spencer and Kandel 1961, and Maekawa and Purpura 1967b).

Taken together, these general observations imply that the most active regions of the cell, and the sites of impulse initiation and propagation, were relatively distant to the intracellular recording electrode since the amplitude of the postsynaptic activity by the time it had spread to the membrane from which we were recording was negligible, and since spike potentials were also attenuated and their slow components absent. Other possibilities are that the electrodes consistently impaled inexcitable patches of membrane where electrotonic conduction was blocked, or that

the impalements were poor or incomplete, and the cell deteriorating. The various interpretations and implications of these findings will be discussed after adding the following observations regarding effects of V and LPO stimuli on reticular neuronal excitability.

Intracellular responses to V stimulation

While V stimuli evoked field potential responses in the reticular formation that were very similar to field potentials evoked in Experiment II, they evoked spike responses in only one of 18 cells tested (6%), a smaller percentage than in Experiment II. The one visually responsive cell happened also to be the one cell from which PSP activity could be recorded. This may have been coincidental, or it may have indicated that visually responsive cells, or cells with any substantial synaptic input, were not regularly impaled with our techniques, and that the high frequency of unresponsive cells, and cells without PSPs, resulted from a sampling bias. In any case, this one cell did give a response very typical of V responses seen in Experiment II, and the PSPs underlying the response were clearly evident.

For example, Figure 51 shows that V stimulation evoked in this cell slowly rising 5 - 10 mV compound EPSPs with approximate latencies of 15, 35, and 60 ms, the second peak only attaining threshold and generating two spikes. In other trials spikes were generated at the other two EPSP peaks as well, but not as often. The increase in excitability

produced between 15 and 60 ms corresponds in latency to the single to quadruple bursts of action potential responses recorded extracellularly in Experiment II, and to the facilitatory periods following V stimulation. Following the EPSPs is a 6 mV IPSP with an approximate latency relative to the V stimulus of 80 ms, and duration of 120 ms. It corresponds to the V blocking period of Experiment II. As can be seen in traces 1 and 2, the evoked depolarizations correspond to the negative waves in the field potential, and the evoked hyperpolarization to the positive wave, the spikes at 36 ms occur exactly at the peak of the negative extracellular response.

These data suggest that facilitatory and blocking interactions seen in Experiment II following V stimulation might have been related to similar membrane potential fluctuations, the cells becoming more responsive during depolarizing shifts and less responsive during hyperpolarizing shifts. Unfortunately, this interpretation is limited by the fact that no other cell gave either action potential or PSP responses to V stimulation. However, the results of applying current pulses, as described below, suggest some of those other cells may have been subliminally responsive.

Anodal currents (tip positive) were applied to two cells neither of which gave action potential responses to V stimulation or displayed PSP activity. In one cell, 7 nA pulses applied at various delays after the

V stimulus (20 ms - 120 ms) consistently evoked one spike per pulse having a latency of 4 - 5 ms. The regularity of the responses over this long interval suggests that the cell's excitability was not altered by the V stimulus, either because the cell had no input from the V system, or because V inputs synapsed on portions of the membrane distant from the recording site.

In the second cell, however, also lacking a V response and PSPs, current pulse responses did change over a 120 ms interval as can be seen in Figure 52a. Plotted is the percentage of pulses, at each of several delays following the V stimulus, that evoked an action potential. Above that is the approximate latency of the spikes.

It can be seen that the likelihood of spike responses was 100% except at two places in the graph where spike numbers, and therefore membrane excitability, were reduced. The first reduction occurred between 5 and 15 ms after the V stimulus, i.e. preceding the time that most V responses are expected. The second decrease occurred over a relatively longer interval, peaked at about 66 ms, and closely corresponded to the blocking period following V stimulation in Experiment II. Between these two periods of reduced excitability, an increase in excitability can be inferred at 15 ms. The percentage of spikes is not increased above 100% at this point, but the latency of spikes is slightly decreased, suggesting some increase in responsiveness occurring at the

typical latency of the V excitatory spike response.

This result implies first that V stimulation can evoke a very short latency, covert inhibition prior to the time that excitatory responses are seen extracellularly. The inhibitory influences must arrive in the reticular formation over very short pathways, while excitatory influences are traversing longer pathways, and may be very long lasting, accounting for the second but smaller decrease in excitability at 66 ms. On the other hand, the second inhibitory period may represent a second, later inhibitory input, or recurrent reticular inhibition.

The major significance of this result is that even though the membrane potential is not changing at the recording site, there is a synaptic input to the cell apparently some distance away, altering the cell's excitability to current pulses. The synaptic input and the stimulating/recording electrode must, however, be close enough to each other for the electrotonic spread of current from the V postsynaptic potential and the current pulse to interact to either increase or decrease the likelihood or latency of spikes. Although such a configuration is not impossible, an alternative interpretation is that the electrode is near the synaptic input, but in a relatively inexcitable patch of membrane. While we cannot distinguish between these alternatives, the result confirms the observation in Experiment II that latent inhibitory influences can be exerted directly on reticular neurons both before and after overt excitatory responses are

seen extracellularly, and these inhibitory influences may be responsible for blocking interactions.

In summary, V stimulation evoked action potential and field potential responses in one cell that matched the V responses seen in Experiment II, and suggested they could be related to EPSP and IPSP activity in the neurons. Two of the ostensibly unresponsive cells were tested with depolarizing current pulses. One of them seemed unaffected by V stimulation since no changes could be detected in membrane excitability from 20 - 120 ms after the V stimulus. This cell may not have been part of the reticular activating system. The other cell showed increased excitability at the time the V excitatory response is expected, and decreased excitability before and after that time. In general, the results of intracellular reocrding during V stimulation support the conclusion from Experiment II that V stimuli have both excitatory and inhibitory influences on reticular neurons, that inhibitory influences may precede excitatory, and that both influences may be exerted directly on the recorded neuron.

Intracellular responses to LPO stimulation

Forty cells were recorded during LPO stimulation. Five cells (13%) gave action potential responses at latencies of 8 - 10 ms as in Experiment II (see Figure 49a). Fourteen other cells (35%) responded at latencies ranging from 15 - 40 ms, and three cells appeared to give

long duration (200 - 600 ms) inhibitory responses. Neither of these effects was observed in Experiment II. In no cell were PSPs clearly evident even though the field potentials consisted of the same high amplitude, positive- and negative-going waves seen in Experiment II. The small number of responsive cells, and even smaller number giving responses like those so frequently seen in Experiment II, supports the suggestion made previously in relation to V responses, that the intracellular electrode selectively sampled a different population of neurons.

Depolarizing current pulses .5 - 9 mA in intensity were applied to 12 cells following LPO stimulation. In half of these cells, no change in membrane excitability could be detected for periods up to 120 ms following the LPO stimulus. That is, current responses consisted of the same number of action potentials having the same latencies, or the consistent absence of action potentials, no matter what the delay of the current pulse. The responses were also equal to responses evoked by identical current pulses applied before the LPO stimulus on alternate trials. Half the cells that did not show excitability changes (3 of 6) did not give action potential or PSP responses to the LPO stimulus either, so it is not surprising that excitability changes were not evoked following these stimuli. These three cells, like the cell found to be unresponsive to V stimuli, may simply have belonged to different reticular systems not receiving V or LPO input. However, the other three cells did

generate action potentials in response to the LPO stimulus, yet their membrane responses to internally applied currents were not altered before, during, or after the unit responses. Apparently the LPO synaptic activity and site of impulse initiation were both a considerable distance from the site of stimulation, and therefore relatively insensitive to the depolarization produced by the current pulse. Such a situation would exist if the electrode was inside a dendrite, and the LPO synapses were on a different dendrite some distance away.

The other six cells tested with current pulses did give current responses that varied as a function of delay. The most reliable effect, seen in five of the six cells, was a decreased excitability at extremely short delays, less than 10 ms, after the LPO stimulus--preceding the time that most LPO responses are expected to occur. This result is similar to the one described following V stimulation, and is subject to the same interpretation regarding an early covert inhibitory input. Following the decrease in excitability was an increase just prior to the typical latency of LPO responses, and a second decrease corresponding to the blocking period seen following LPO stimulation in Experiment II. As mentioned previously, these effects were seen in five cells, two of which also gave spike responses to the LPO stimulus and three of which did not. Data from one of the "unresponsive" cells are given in Figure 52b.

In this cell, maximum responsiveness to current pulses occurred when they were presented 6 ms after the LPO stimulus, i.e., just before the LPO spike response is expected. Before and after that time, responsiveness is reduced implying an early as well as a late inhibitory input, or a long lasting inhibition, upon which excitation is superimposed. After the second inhibitory period, responsiveness increased again, although, as far as was measured, it had not yet returned to maximum levels The latencies of spikes evoked by the current pulses varied predictably, being longest when the probability of response was lowest, and shortest when the probability was greatest.

Thus, intracellular recordings during LPO stimulation lead to many of the same conclusions expressed previously in regard to V stimulation. Briefly they are that 1) some changes in membrane excitability could be demonstrated in apparently unresponsive cells by applying current pulses at various delays after the LPO stimulus; 2) excitability was increased when action potential responses occurred or were expected, and decreased before and after that time; 3) the electrodes were either not located near the active parts of the membrane or not able to record certain types of excitability changes; and 4) nevertheless it was possible in some cases for current pulses and postsynaptic effects to interact.

Summary of results

1) The general properties of reticular neurons impaled in this experiment were in some respects unusual: only one cell exhibited spontaneous or evoked postsynaptic potential activity; many of the intracellular spikes were of small amplitude; and both large and small spikes lacked depolarizing prepotentials. These findings may indicate consistent impalement of neurons having few synapses and/or large areas of inexcitable membrane. Alternatively, the synaptically active areas of the cell, and sites of impulse initiation may have been distant from the recording electrode.

2) A disproportionately small number of neurons gave V or LPO spike responses resembling those of Experiment II, suggesting a sampling bias of some sort.

3) In half of the responsive cells, a sequence of excitability changes could be demonstrated having the same time course as blocking and facilitatory interactions in Experiment II. To this extent our prediction about direct afferent input to reticular cells was supported.

4) The failure to record excitability changes in other responsive cells indicated the electrode was distant from the synaptically active membrane or in a nonconductive membrane.

5) Excitability changes could also be demonstrated in a small additional number of cells that did not give action potential responses to

V or LPO. Those cells may have received only subliminal input from V or LPO systems.

6) The very much larger number of cells that were unresponsive to V and LPO stimuli, that failed to exhibit postsynaptic activity in the region of the electrode, and could not be demonstrated to undergo excitability changes of any kind, suggests selective sampling in this experiment of a relatively homogeneous population of neurons characterized by certain morphological and electrical properties that were unexpected, and deviate somewhat from the classical model of a central neuron.

## Discussion

The intracellular responses reported above may be discussed along two main lines: their relationship to extracellular responses obtained in Experiment II; and their comparison with results of similar intracellular studies.

### Relationship to extracellular responses

The main finding of Experiment II was that large numbers of reticular neurons responded to V and LPO stimulation, and those responses consisted of clearly defined phases of excitation and inhibition. Based on this result, it was expected that intracellular recordings would show: 1) bursts of spikes in response to V and LPO stimulation in the same proportion of cells and at the same latencies as responses recorded extracellularly, and 2) EPSPs during spike responses and facilitatory periods following conditioning stimuli, and IPSPs during suppression of spikes and blocking periods. We were surprised to find that neither prediction was fully confirmed.

First, V and LPO spike responses very similar to those recorded extracellularly were obtained, but in many fewer cells than expected. The likelihood of V responses was reduced from 47% to 6%, and LPO responses from 71% to 13%, constituting an 87% reduction in the first case, and 82% reduction in the second. That is, responses were reduced

proportionately in the two modalities. If we assume that the extracellular and intracellular preparations were equally viable, and that stimuli were of equivalent strengths in the two experiments, then the large difference in the number of responsive cells may indicate that two different populations were sampled. It is unlikely that two different parts of the brain stem were explored because of the histological evidence, and the demonstration in Experiment II that V and LPO responsive cells were widely and uniformly distributed. Therefore, within this general area, there may be two types of neurons, one which is more readily impaled than the other because of its size, shape, membrane structure, or glial covering, but which has few or no V or LPO inputs. The results of Bowsher and Westman (1970, 1971) are interesting in this regard. Through light and electron microscopy they have identified two different cell types: polydendritic neurons with five to six large dendrites, spines on soma and dendrites, and massive synaptic contacts; and oligodendritic neurons with few dendritic branches, no spines, and very few synapses. If oligodendritic cells were the more easily penetrated, a random sample would produce a large percentage of relatively silent cells, and give the impression that reticular neurons had little input.

The second unexpected finding, which tends to support the above interpretation, was that only one of 44 cells showed postsynaptic activity of any kind. Previous authors also have reported surprisingly low levels

of membrane activity in reticular neurons considering the high density of synaptic contacts seen in histological sections. For example, Segundo et al. (1967a) found spontaneous postsynaptic potentials in only 20% of impaled bulbar reticular neurons. Other authors simply exclude from analysis 50 - 75% of recordings mainly because they lacked postsynaptic potentials and, therefore, were considered to result from intraaxonal impalements (see Grantyn et al. 1973). They may, in fact, have been cells with few synapses. Of the remaining intrasomatic recordings, approximately half again were found not to exhibit postsynaptic potentials in response to cortical, spinal, or peripheral sensory stimulation, although they were characterized by some spontaneous membrane noise (see Magni and Willis 1964, Limansky 1965, Grantyn et al. 1971, 1973, Mancia et al. 1974). It is also interesting that mesencephalic reticular neurons gave consistently fewer responses than bulbar reticular neurons, and this difference was suggested by Grantyn et al. (1973) to result from a greater difficulty in penetrating mesencephalic neurons. In summary, it is not entirely uncommon for intracellular electrodes to preferentially encounter reticular neurons that are uncharacteristically quiet in comparison to neurons recorded extracellularly, perhaps because they are more easily penetrated, and this sampling bias may account for the small numbers of V and LPO responses in this experiment.

Fortunately, we were able to penetrate one cell that displayed

membrane potential transients clearly identifiable as EPSPs and IPSPs. The fact that this cell was also the only cell to respond to V stimulation suggests that it was more representative than the others of the population sampled in Experiment II. Because membrane depolarization occurred during the action potential response, and prolonged hyperpolarization during the succeeding silent period, and because those effects had the same time course as blocking and facilitatory interactions following V stimulation in Experiment II, the result supports the hypothesis that reticular membrane excitability can be influenced directly by afferent input. However, it does not exclude the possibility that other mechanisms, such as recurrent inhibition, disfacilitation, and disinhibition may also be involved in the potential changes. Such mechanisms cannot be easily distinguished in a structure as complex in its synaptic organization as the reticular formation. Therefore, the data from this one cell, although interesting for their demonstration of postsynaptic potential correlates of frequently observed extracellular responses, do not add greatly to the understanding of interactions of convergent inputs to the reticular formation.

Of the remaining 43 cells from which PSPs could not be recorded, 13 were tested with current pulses to determine whether other correlates of membrane excitability, aside from membrane potential, could be observed following V or LPO stimulation.

In half the cells tested with this technique, we continued to see no change in excitability. Absence of excitability changes may be expected in cells unresponsive to V or LPO stimuli, as some of the cells were, but is somewhat puzzling in cells that do generate spike responses. Clearly the cell is receiving synaptic input, but that input is not seen as PSP changes at the recording electrode, and does not alter the excitability of the membrane at the recording site. Apparantly, inputs generating spikes are some distance from the recording electrode and yet close enough to the impulse generating portion of the membrane to be effective. The recording electrode, therefore, might be in a relatively remote dendrite. This conclusion is supported by the high membrane resistence found at some recording sites.

In the other half of cells tested with current pulses, excitability changes were detected even though the membrane potential was steady, implying that the stimulus (V or LPO) was altering some other determinant of membrane excitability. In some cells, a sequence of three excitability changes was observed: a decrease at very short delays, preceding the latency of most V and LPO excitatory spike responses; an increase during the period of spike responses; and a second decrease during the silent periods following the spike responses. The second and third effects have been discussed previously, and are evidence that facilitatory and blocking interactions may be related to postsynaptic

events in the recorded neuron. The first effect, of an inhibitory influence prior to the overt excitatory response, was alluded to in Experiment II where, it will be recalled, some blocking interactions were observed preceding unit discharges. If this is a reliable phenomenon, it suggests that 1) the earliest inputs to the reticular formation from V and LPO may be inhibitory rather than excitatory, and 2) both V and LPO afferent pathways may have fewer synapses than previously assumed, and that the LPO pathway may be monosynaptic. It is not surprising that initially inhibitory influences are not more commonly reported in the reticular formation or elsewhere since they can be difficult to detect with extracellular or intracellular techniques. But Smith (unpublished observations) has also observed, following visual stimulation, initial, short latency depression of cortical unit activity in cells giving later excitatory responses. These results suggest that very short latency inhibition may be an important, and often unrecognized, mode of action in neural transmission.

Comparisons with previous studies

Most previous intracellular studies in the reticular formation have been confined to bulbar and pontine reticular regions, and to responses to cortical or somatic sensory stimulation (Limansky 1965, Magni and Willis 1963a, b, Segundo et al. 1967a, b). However, Mancia et al. (1976) and Grantyn et al. (1973) provide some data comparable to ours

regarding effects of LPO stimulation on mesencephalic reticular units. For example, both of the above studies found that LPO stimuli influenced a small percentage of reticular cells, but in these cells evoked 7 - 10 ms EPSPs with or without spikes. The latency of spikes was the same as latencies we observed, but, as stated previously, we could not detect EPSPs. The same authors also state that no IPSPs were ever observed, and that such a result was surprising in view of the clearly defined silent periods and positive-going field potentials following LPO stimulation in studies by Bremer (1970) and Lineberry and Siegel (1971). We too failed to observe hyperpolarized membrane potentials following LPO stimulation but could nevertheless demonstrate with intracellularly applied currents periods of reduced membrane excitability, suggesting that inhibitory influences may be exerted covertly, as in presynaptic inhibition, inhibition at even earlier points in the afferent pathway, or remote dendritic inhibition.

Regarding the small number of LPO responses during intracellular recording compared to extracellular recording, and the relatively low level of postsynaptic activity, we mentioned previously the possibility of selective sampling from a population of generally unresponsive cells. Others have also found mesencephalic units to be relatively unaffected by stimulation, at least in comparison to more caudal reticular units, and have suggested mesencephalic units receive less input (Mancia

et al. 1976, Grantyn et al. 1973). However, another possibility is that hyperpolarizing and depolarizing potentials occur in about equal proportions in mesencephalic neurons and so cancel each other (Lindansky 1965). A third possibility is that the recording electrode is not near the active synapses and so sees no PSPs. Assuming the electrode is frequently located in the soma, this implies major synaptic input occurs on remote dendrites. Alternatively, it is possible for an electrode to be in an inactive dendrite and fail to record membrane potential changes at the soma or in other dendrites because of electrotonic conduction blocks.

In summary, one interpretation of the absence of postsynaptic activity is that it is a real consequence of sparse synaptic contact. Sparse contact is not characteristic of one population of cells seen in histological sections to be densely covered with synaptic terminals, and another, probably identical population, which responds to a wide variety of stimuli during extracellular recording. It may, however, be characteristic of a different population of cells that happen to be more accessible to impalement and which were sampled by Mancia et al. (1976), Grantyn et al. (1973), and the present author. Bowsher and Westman's (1970, 1971) demonstration of two reticular cell types corroborates this interpretation. On the other hand, the same absence of postsynaptic potentials may indicate that reticular neurons have structural features

which result in frequent impalement of parts of the neuron that have few active synapses, inexcitable patches of membrane, and/or are distant from the site of impulse initiation. There are insufficient data to resolve this question at this time.

As regards the problem of establishing the site of cell penetration, the shape and amplitude of the action potentials are relevant. An interesting result in our experiment was the observation of small size spikes that lacked depolarizing prepotentials. Small spikes like these have been reported previously, in isolation or as components of large spikes in neurons of the cortex, hippocampus, cerebellum, superior colliculus, thalamus, and reticular formation of rats and cats, and in numerous invertebrate and fish preparations (Tasaki et al. 1954, Eccles et al. 1958, Spencer and Kandel 1961, Segundo et al. 1967, Purpura et al. 1965, Llinas and Nicholson 1971, Sandeman 1969, Zucker 1972, Calabrese and Kennedy 1974, Kriebel et al. 1969, Bowsher 1970, Limansky 1965, Maekawa and Purpura, quoted in Purpura 1972). They are clearly all-or-none phenomena, not local graded responses, since they appear with constant amplitude in a given cell. It has been reported that they are not generated in the axon since they are not evoked by antidromic stimulation, or in the soma since they are not blocked by intracellularly applied hyperpolarizing current which blocks large spikes. Nor are they to be considered pathological phenomena since 1) they are

not associated with injury discharges, 2) they coexist with large spikes in some cells, 3) they occur during large and stable resting potentials, and 4) their firing pattern and frequency, recorded extracellularly, does not change after impalement.

While there is still some controversy over their origin, most authors agree that small spikes are action potentials generated within the impaled neuron, but initiated at some point in the dendritic tree rather than at the axon hillock. After being triggered in an excitable dendrite, the action potentials may be propagated distally to dendritic synaptic terminals (see Ramon-Moliner 1975, Reese and Shepherd 1972, Hinds 1970, Famiglietti 1970, Harding 1971, Sloper 1971) and proximally to the soma. Eccles (1957) has suggested that the trigger zone lies at the main bifurcation of the apical dendritic system where PSPs spreading down from upper branches converge and summate. Their small size when recorded from the soma or from another dendrite may be explained by hypothesizing patches of inexcitable membrane between the trigger zone and site of recording which do not propagate the action potential and hence attenuate its amplitude.

Small spikes recorded in the present experiment were similar in many respects to small spikes seen in caudal reticular neurons by Segundo et al. (1967), Bowsher (1970), and Limansky (1965) and in red nucleus neurons by Maekawa and Purpura (quoted in Purpura 1972). In

all of these experiments, low amplitude (3 - 10 mV), long duration action potentials were seen to rise directly from the baseline without depolarizing prepotentials. They rose from relatively stable baselines as well as from levels of depolarization or hyperpolarization, indicating a trigger site distant to the recording electrode since it was not dependent on the potential at the electrode. Very often small spikes were seen in cells from which no PSP activity could be recorded, and they often occurred in high frequency bursts forming "coxcomb" patterns (cf. Figure 50a this thesis, and Figure 8, p. 1203, Segundo et al. 1967). Spikes within a burst often had different (fast or slow) decay times. These findings support the suggestion of Limansky (1965) and Segundo et al. (1967a) that reticular neurons may be provided with several trigger zones and this may apply to mesencephalic neurons as well. The absence of prepotentials in the generation of small and large spikes has been interpreted as pointing to the same conclusion.

Previous authors reporting small spikes in reticular neurons have assumed, or demonstrated, intrasomatic impalements, and so were able to conclude that the spikes originated in dendrites. Since we did not have positive evidence of somatic impalements, we must consider the alternative possibility that spikes were generated in the soma but appeared attenuated because the recording electrode was in a dendrite some distance away. This interpretation would account for the high

membrane resistence we recorded, the absence of PSPs (if the electrode was in an "inactive" dendrite), and the failure to record excitability changes in some cells clearly influenced by V and LPO stimuli (if the electrode was in a dendrite not receiving V or LPO input). Of course, an electrode located in a dendrite would record small spikes whether the spikes were generated in the soma, or in another dendrite. So our data do not preclude the possibility of spikes of dendritic origin in the reticular formation, but rather suggest that electrodes may have been recording from other dendrites as well as from soma.

To recapitulate, we have observed a population of reticular neurons having the following unexpected or unusual properties: they were unresponsive to V and LPO stimulation; they lacked postsynaptic potentials, they lacked depolarizing prepotentials preceding spikes; and they displayed spikes that were very often of low amplitude. One possibility is that cells with little synaptic input, such as "oligodendritic neurons" described by Bowsher and Westman (1970) were preferentially impaled. Another is that synaptically active cells were penetrated, but at a point distant from the current source. Within both of these categories, the possibilities exist of somatic impalement with postsynaptic activity and spikes in dendrites, or dendritic impalement with postsynaptic activity and spikes in soma, or in different dendrites. We cannot further evaluate these alternatives on the basis of the current data, but recent studies

of cytoarchitecture and synaptic morphology in the reticular formation supports two of the more controversial hypotheses--dendritic impalement and dendritic spikes initiation.

Reticular neurons are known to have extensive dendritic fields, oriented coronally, which freely cross nuclear boundaries and may exceed one third the diameter of the brain stem (Scheibel and Scheibel 1958). Five to six main dendrites branch from the soma and follow relatively rectilinear courses without extensive collateralization as seen in many other parts of the nervous system (Bowsher and Westman 1970). Also, whereas dendrites of other systems often diminish in width with distance from the soma, reticular dendrites up to 400 micrometers from the soma are often as thick as proximal dendrites (Kositzyn 1964). Therefore, it is very possible that reticular dendrites, even very remote dendrites, may be regularly penetrated by fine guage intracellular electrodes. Such recordings would be expected to show relatively high membrane input resistences, as we have seen here.

Another significant feature of reticular dendrites is that the number of synapses per unit surface area remains virtually constant with distance from the cell body (Kositzyn 1964). Bowsher and Westman (1970, 1971) have estimated the total number of synapses on a "polydendritic" reticular neuron to be 7560, and these synapses cover up to 80% of the somatodendritic surface. It is also interesting in the light

of Eccles' suggestion of spike initiation at the main bifurcation of the apical dendritic tree of motor neurons, that the density of synapses seems to be greatest at the points of dendritic branching (Kositzyn 1964).

Scheibel and Scheibel (1967) questioned the significance of synaptic terminals so far from the presumed spike generator at the axon hillock, but if spikes may also be generated in dendrites, the abundance of inputs to remote reticular dendrites takes on a new functional significance. We already know from electrophysiological data that neurons of the reticular formation seem specially adapted to integrate information from the numerous heterogeneous inputs that converge there. Major synaptic inputs to dendrites, both proximal and distal, allows for compartmentalization of inputs and selective facilitation or suppression of some inputs while not affecting others, and not significantly altering discharge rate. It is not surprising, therefore, that reticular dendrites should have special structural features, and unusual properties of conduction, such as spike initiation, that may provide for both a tonic control of the cell's excitability and alternately potentiate the effectiveness of competing inputs. Indeed, it would be surprising if a wide variety, and flexibility of mechanisms were not available for the complex functions of reticular neurons.

In conclusion, the present intracellular results suggest that V

and LPO stimulation can, in some cells, increase and/or decrease neuronal excitability, and that the time course of these changes parallels the time course of facilitatory and blocking interactions observed in Experiment II. Unfortunately, there was no unique answer for the question of what inhibitory processes were operating. Despite the demonstration of excitability changes, we could record very few spontaneous postsynaptic potentials in reticular neurons, and the excitability changes that did occur were not manifest in membrane polarization or impedence changes. The absence of postsynaptic activity, in conjunction with the observation of low amplitude spikes and high values of membrane resistence, suggest that the electrode was in a small diameter structure distant from both the active V and LPO inputs and the sites of impulse initiation. In view of similar data from other studies, and the morphology of reticular dendrites, we may strongly suspect that electrodes were often located in one dendrite while afferent inputs and spike initiation were occurring on a different dendrite, or on the soma. The significance of these findings for theories of reticular control of the cortical EEG will be examined in the general discussion below.

## General Discussion and Conclusions

The work reported here has been concerned with behavioral and neuronal functions of MTH and LPO. We have tested the hypotheses that electrical stimulation of MTH and LPO 1) induces sleep in awake, freely moving animals, and 2) functionally inhibits the brain stem reticular activating system. We find no support for the first hypothesis, and qualified support for the second.

### Hypothesis of sleep induction

The present findings confirm the common observation that low frequency stimulation of MTH and LPO elicits cortical rhythmical EEG which others have likened to spindles and slow waves occurring naturally during sleep. Since these responses have been studies and reviewed extensively, we need not discuss them further. However, we may add to the literature our failure to replicate the synchronogenic effects of high frequency LPO stimulation reported by Sterman and Clemente (1962b). In our experiments, synchronization could be observed during high frequency stimulation, but it was not a response since it occurred during unstimulated periods as well, and while the animal was falling asleep. In our opinion, low frequency electrical responses to high frequency

stimulation cannot be obtained easily in this system, and may not have been obtained even in the original reports, since the synchronized potentials illustrated in Sterman and Clemente (1962b) appear to be spontaneous.

Our main finding was that the same stimulation effective in synchronizing the cortical EEG was totally ineffective in producing behavioral sleep. Animals exhibited consistent behavior patterns from trial to trial regardless of the application of MTH or LPO stimulation, and they neither fell asleep significantly sooner, nor slept for significantly longer periods following such stimulation. Furthermore, there were no serious inconsistencies between the behavior we observed on all trials, and behavior others have interpreted as induced sleep. Therefore, our data strongly suggest that sleep observed by previous investigators was spontaneous, not induced. If it is true that sleep has never been produced by electrical brain stimulation, a large and influential literature is invalidated.

In order for a central structure to be called hypnogenic it should be shown not only that its stimulation induces sleep distinguishable from spontaneous sleep, but also that its destruction is followed by insomnia. However, no insomnia occurred after MTH lesions as reported by Andersen et al. (1967) and Angeleri et al. (1969), and the insomnia produced by LPO lesions was reversible in animals whose hypothalamic

regulatory mechanisms were not damaged in the operation. So neither stimulation nor lesion experiments convincingly demonstrate that MTH or LPO play a critical role in the process of falling asleep.

We would not deny that MTH and LPO are part of more extensive neuronal systems regulating sleep onset for they are both areas of convergence of sensory, motor, motivational and homeostatic systems involved in sleep. But it is difficult to imagine how so complex a function could be concentrated in either of these precise locations. If a locus exists which when stimulated with an appropriate pattern of electrical pulses would initiate the neural processes leading to sleep, it has not yet been identified, and probably will not be found in MTH or LPO.

We may in fact question the basic concept that neural "sleep centers" exist at all. The possibility that sleep can be triggered electrically has been discussed by Berlucchi (1970) and Moruzzi (1969, 1972) both of whom conclude that sleep mechanisms are so diffuse as not to be accessible through electrical stimulation. Furthermore, they believe that the hypothesis of separate centers for sleep and waking is very improbable. Sleep is neither a unitary nor a simple phenomenon, and to expect to identify it with a circumscribed set of neurons is, at this point in our understanding, overly optimistic.

A second important conclusion from this work is that EEG

mechanisms and sleep mechanisms need not have identical neural substrates. We described earlier how a distinction is now being made in the literature between EEG desynchronization and wakefulness since under spontaneous conditions they may appear independently, and under experimental conditions they may be dissociated through several techniques. We do not know how or where the mechanisms differ neuronally, but apparently separate substrates are involved. The present data make the point that EEG synchronization and sleep must also have separate substrates since each may occur independently of the other, and that MTH and LPO should cease to be considered sleep structures in favor of their designation as EEG synchronizing structures. It is surprising how few contemporary researchers are rigorous about this distinction, perhaps because until now there have been few experimental refutations of the sleep induction reports.

In summary, we believe that it is justifiable to consider MTH and LPO as EEG synchronizing structures, and to study further their electrophysiological properties as we have done in Experiments II and III. But there is no evidence, given the control data from Experiment I, that behavioral sleep has ever been produced by electrical stimulation of these structures. It is important for both theoretical constructs and the design of future experiments that this distinction be made explicit and more widely acknowledged.

Hypothesis of active reticular deactivation

Soon after Moruzzi and Magoun (1949) discovered a cortical desynchronization mechanism in the brain stem reticular formation, there was speculation as to how the mechanism was suppressed during periods of cortical synchronization. The hypothesis of active reticular deactivation stated that central structures existed whose output to the reticular formation actively modified reticular discharge patterns so as to disrupt the desynchronization process (see Dell et al. 1961, Moruzzi 1964). The most obvious candidates for those control structures are EEG synchronizing systems since they may gain control of cortical rhythms by actively opposing the desynchronizing influence exerted by the brain stem. Since some episodes of cortical desynchronization are initiated by a sudden sensory barrage to the reticular formation, it might be interesting to see whether synchronizing structures can oppose the effectiveness of sensory stimulation in single reticular neurons.

The present studies, after examining reciprocal interactions between synchronizing and desynchronizing inputs to individual reticular neurons, clearly show that although reticular sensory responses may be blocked by LPO and MTH stimulation, these synchronizing systems do not have exclusive control of reticular excitability, and their influences are not purely inhibitory. This conclusion is based on the observation that sensory responses could be facilitated as well as inhibited by LPO

and MTH stimuli, and that sensory stimuli could exert the same excitatory and inhibitory effects on LPO and MTH responses. Therefore, there are no qualitative differences between synchronizing and desynchronizing influences in the reticular formation, and any change in the operation of the reticular activating system brought about by interactions between those influences must result from changes in the spatiotemporal patterning of discharges rather than a simple net excitatory or net inhibitory drive. This interpretation is supported by statistical measures of reticular single unit activity showing that the pattern of firing may change from random discharges during desynchronization to clustered discharges during synchronization, but the rate of firing does not convincingly increase or decrease between these states (Strumwasser 1958, Huttenlocher 1961). The random discharges may indicate that reticular neurons are being influenced more strongly by sensory inputs which under physiological conditions arrive in the reticular formation in a random fashion. Random reticular discharges may be reflected in the cortex as desynchronized EEG. During synchronization, on the other hand, reticular neurons may be more strongly influenced by MTH and LPO inputs which seem to produce in reticular neurons, just as in thalamic and cortical neurons, excitatory and inhibitory sequences of discharge lasting approximately 120 ms, i. e. approximately the frequency of cortical spindles. Thus reticular neurons might be involved in cortical

synchronization by acting in phase with intrathalamic mechanisms.

Since both of these processes, one leading to desynchronization and the other to synchronization, involve excitation of reticular neurons, we may dismiss the simplistic version of the reticular deactivation hypothesis put forward by Sterman and Clemente (1968) and Bremer (1970) which states that MTH and LPO synchronization results from direct inhibition of reticular neurons. Our data indicate that competing or antagonistic relationships may exist between synchronizing and desynchronizing systems, but the cellular mechanisms of the interactions are more complicated than the hypothesis implies. The present results do not permit a detailed analysis of what those mechanisms are, or how they relate to the determination of the cortical EEG, but certain possibilities are suggested.

For example, it is reasonable to infer that synchronizing and desynchronizing inputs have overlapping distributions on reticular dendrites. Since we frequently failed to record the postsynaptic effects of these inputs, they may be relatively localized and remote. We know that at subthreshold levels, each type of input can modulate the tonic level of reticular excitability and also exert modality-specific effects, both facilitatory and inhibitory, on competing inputs. We cannot be sure of the mechanisms of inhibition, but direct postsynaptic inhibition is one of the more likely. Each input is also powerful enough to control the output of the

neuron by evoking or suppressing action potentials, in the soma and possibly in dendrites. Therefore, the interactions we observed may have taken place on reticular dendrites.

In describing V and LPO influences in the reticular formation, and their possible role in determining EEG rhythms, we should at least mention the relevance of numerous other factors too complicated to consider here. For example, reticular neurons are also strongly influenced by corticofugal afferents (see Dell et al. 1961) and ascending afferents from more caudal parts of the brain stem (see Moruzzi 1963). Each of these influences has a demonstrated role in determination of the cortical EEG, and their relationship to MTH and LPO influences is not know. Considering also the range of somatic, sensory, and cognitive factors that affect the pattern of the EEG, we cannot expect to explain the whole process in terms of MTH, LPO, V, and A interactions. However, our results may serve to clarify some specific hypotheses concerning MTH and LPO influences in the reticular formation, their anatomical distribution, and synaptic mechanisms.

Indications for further research

One of the most striking results of these experiments was the widespread and rigid control over reticular unit activity exerted by LPO afferents. LPO stimulation influenced over 70% of neurons sampled in Experiment II, producing in them invariant patterns of excitation and

apparent inhibition. It would be useful at this point to study further the anatomical pathways descending from LPO to the reticular formation since they may be monosynaptic. If they are, lesions of LPO may produce anterograde degeneration of axon terminals in the reticular formation, and electron micrographs may show the distribution of degenerating synapses on the reticular somadendritic membrane. The results of such a study might also determine whether LPO input is exclusively, or mainly, to polydendritic-type neurons described by Bowsher and Westman (1970).

Also, intracellular recordings from LPO neurons might show antidromic spikes following reticular stimulation, confirming a monosynaptic pathway. In the same experiment, it would be of interest to observe orthodromic LPO responses as well since reticulocortical desynchronizing efferents pass through this area, but very little is known about possible collateral terminals on LPO neurons. The demonstration of reciprocal circuits between reticular formation and LPO, as there are between reticular formation and MTH, would be relevant to theories of synchronizing-desynchronizing subcortical interactions.

## Summary

Consistent with many previous reports, we found that electrical stimulation of MTH and LPO of cats evoked cortical EEG spindles and slow waves similar to those appearing spontaneously during sleep. Contrary to numerous other reports, however, we found no convincing evidence that the same stimulation, in unanesthetized animals, could induce sleep behavior. These results strongly argue for a more rigorous separation of electrographic and behavioral functions of MTH and LPO neurons, and for reconsideration of classic concepts of "sleep centers" in the brain.

Second, we have demonstrated that the same MTH and LPO systems described above project caudally and converge along with visual and auditory afferents on single neurons of the brain stem reticular formation. Reticular responses generated by each of these four sources contain both excitatory and inhibitory components, probably generated within the recorded neuron, and appearing to modulate the cell's excitability. Intracellular recordings indicate complex and unusual cellular integration mechanisms in the reticular formation, possibly including spike initiation in dendrites, remote dendritic inhibition, or presynaptic inhibition. The significance of these findings is in demonstrating a subcortical site

for interactions between cortical EEG synchronizing and desynchronizing influences. To the extent that these interactions alter the firing patterns of reticular neurons, it may be said that synchronizing structures deactivate the reticular activating system.

Figure 1

Cortical EEG patterns associated with sleep and wakefulness in the cat. Fr EEG, frontal EEG; EOG, electro-oculogram; W, waking; LSWS, light slow wave sleep; DSWS, deep slow wave sleep; REM, rapid eye movement sleep. a, EEG during wakefulness is characterized by continuous desynchronization; rapid eye movements or eye fixation also indicate that the animal is awake. b, as the animal becomes drowsy, intermittent spindles appear on the desynchronized background, and eyes drift slowly beneath the closed lids. c, when the animal is fully asleep, the EEG displays continuous spindles and slow waves, and eye movements continue to be long and slow. d, EEG of the REM sleep stage is desynchronized, as in wakefulness, and rapid eye movements occur beneath the closed lids.

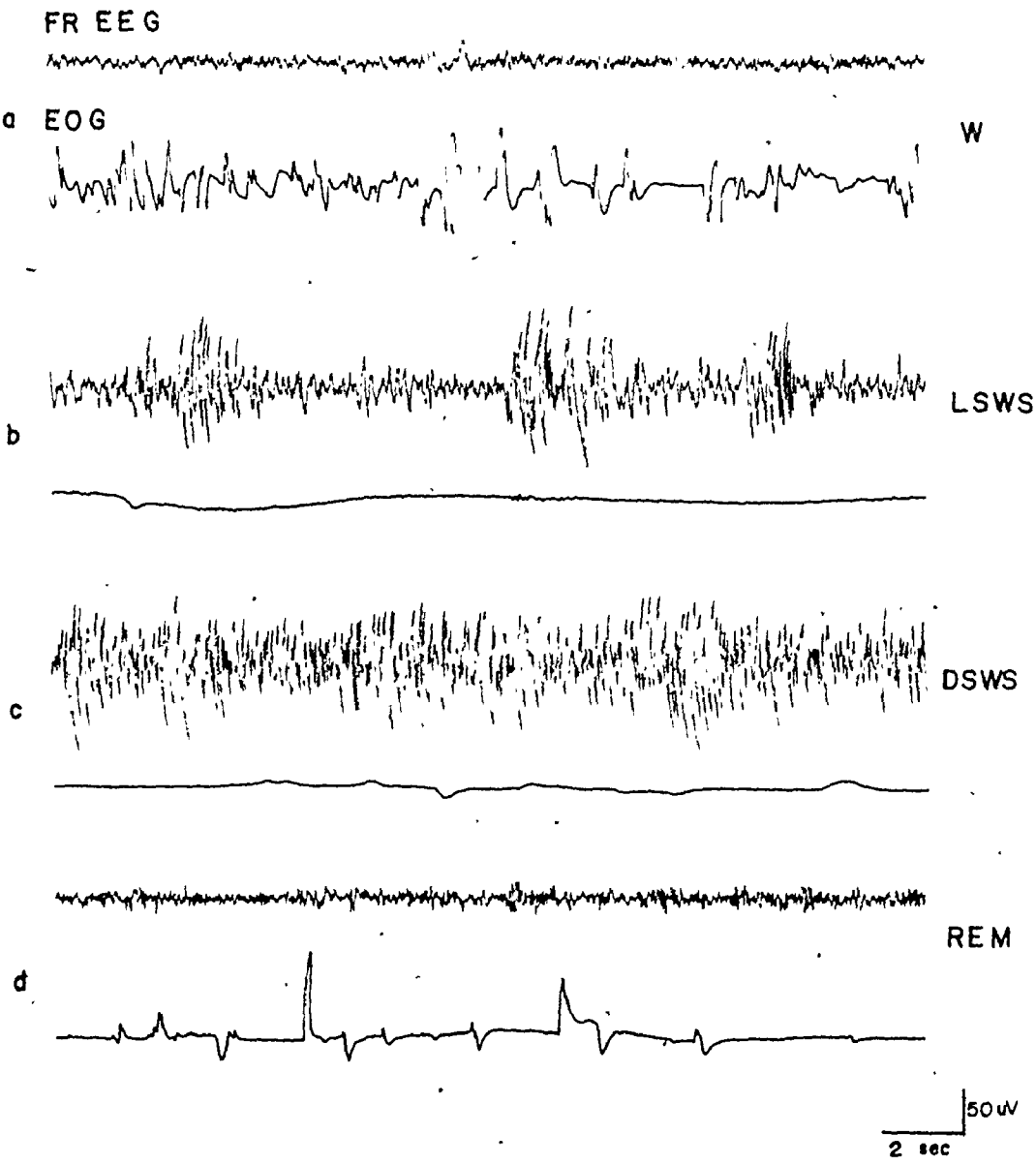



Figure 1

Figure 2

Schematic drawing of anatomical connections between MTH, LPO, and MRF. Axon bundles from mesencephalic reticular formation (MRF) and pontine reticular formation (PRF) ascend through the tegmentum and bifurcate caudal to the thalamus forming a dorsal leaf (1) which terminates in thalamic intralaminar fields (MTH), and a ventral leaf (2) which passes through the subthalamus and lateral hypothalamus and makes contact with neurons of the lateral preoptic area (LPO). Axons of MTH send a caudal contingent back to MRF (3) as well as a rostral contingent to the cortex (6). LPO efferents pass through or near the medial forebrain bundle (4) to make contact with neurons of MRF. It is likely that MTH and LPO have reciprocal anatomic connections (5) but they have not yet been identified. Cb, cerebellum; IC, inferior colliculus; SC, superior colliculus; ON, optic nerve.

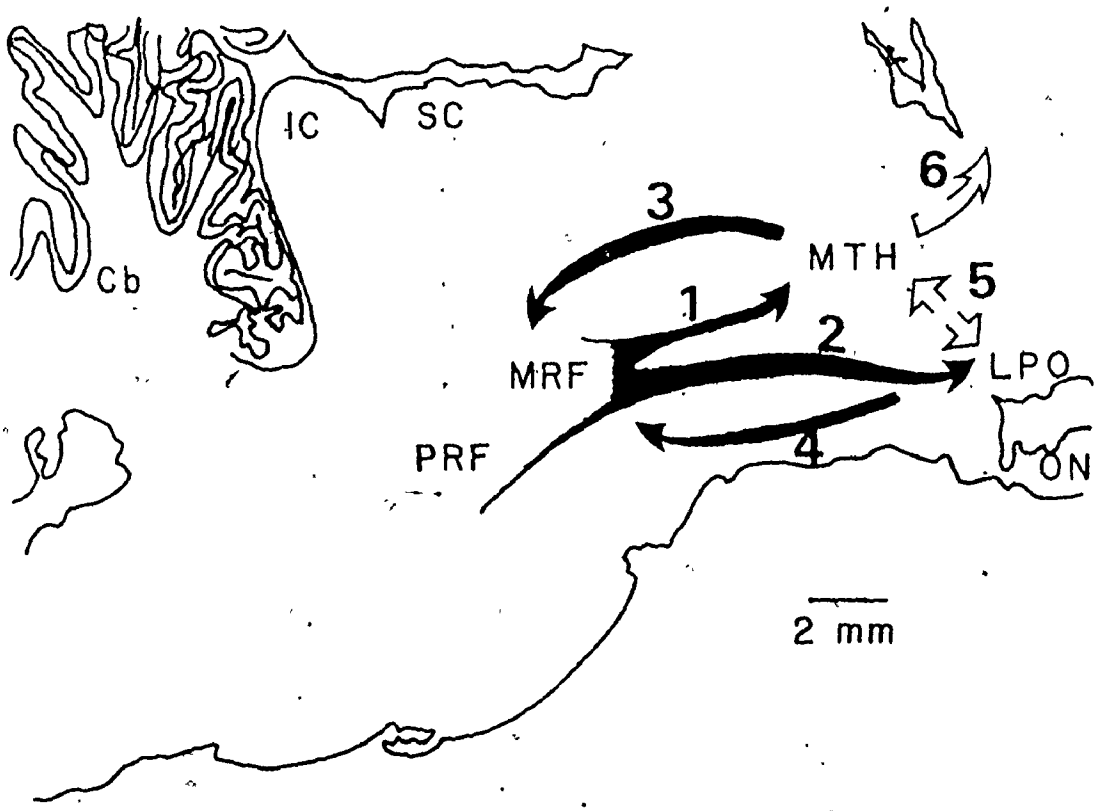
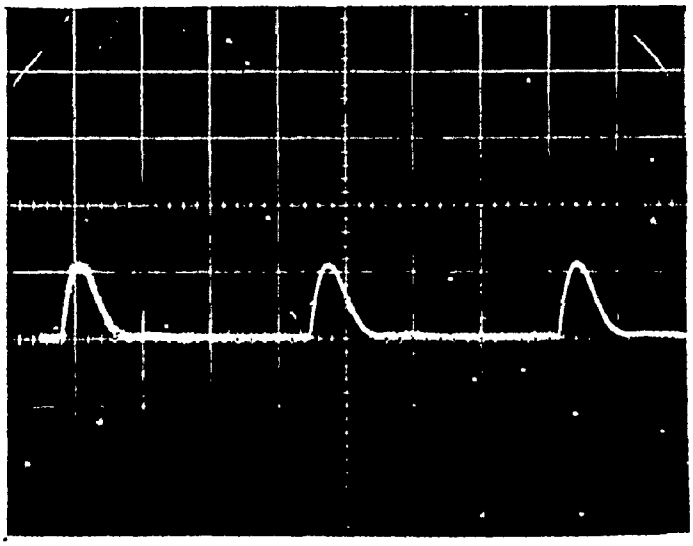



Figure 2

Figure 3

Long duration MTH stimulus pulse. Conforming to the specifications of Wyss (1965) and Hess (1957), this pulse has a slow rising phase and duration of 12.5 ms at approximately half amplitude. It is presumed by Hess to selectively excite high threshold autonomic fibers in the thalamus involved in sleep induction.
(See review by Gloor 1954.)



20 ms

Figure 3

Figure 4

Trials procedure. Counterbalanced order for presentation of stimulation and control trials on two days of testing. The subject receives the same type and intensity of stimulation on both days. A typical pattern of sleep episodes is depicted.

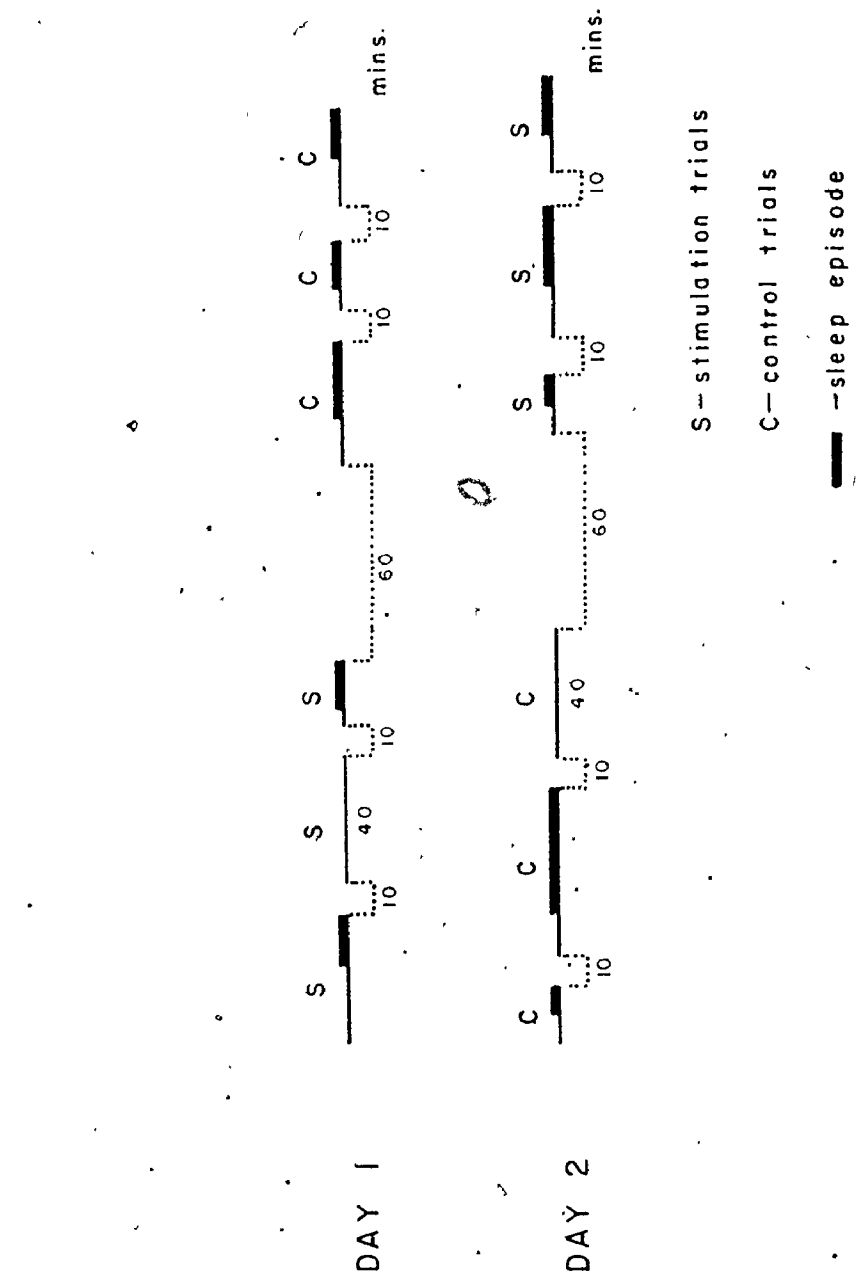
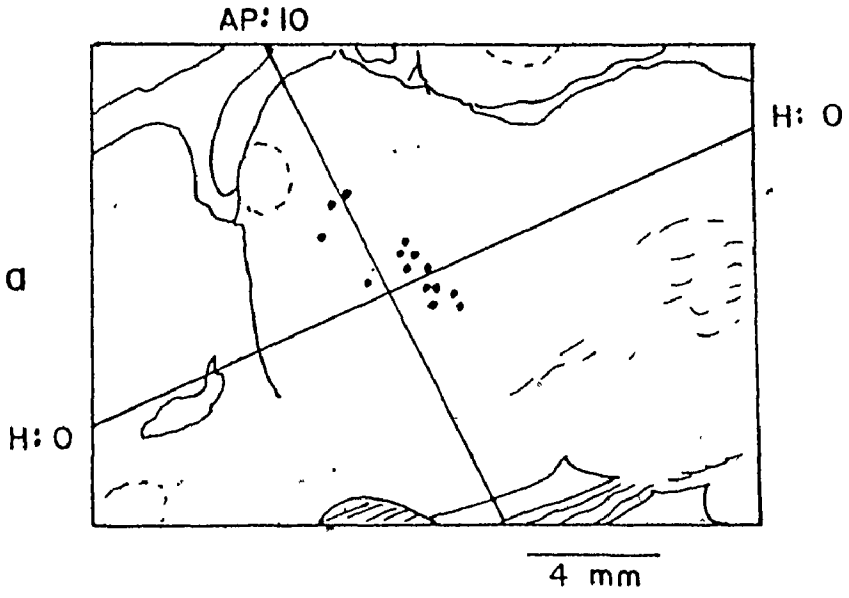

DAY 1
S
10
S
40
10
S
10
60
C
10
C
10
C
mins.
DAY 2
C
10
C
40
10
C
10
60
S
10
S
10
S
mins.
S—stimulation trials
C—control trials
—sleep episode

Figure 4

Figure 5

Location of MTH stimulating electrodes. Sagittal section through the thalamus of the cat 2.5 mm from the midline. a, locations of MTH stimulating electrodes in all subjects in the present experiment. b, stimulation points (black circles) from which sleep responses were obtained, taken from Akert et al. (1952), p. 261. This diagram also contains negative points (open circles) where stimulation was not followed by sleep in that experiment.



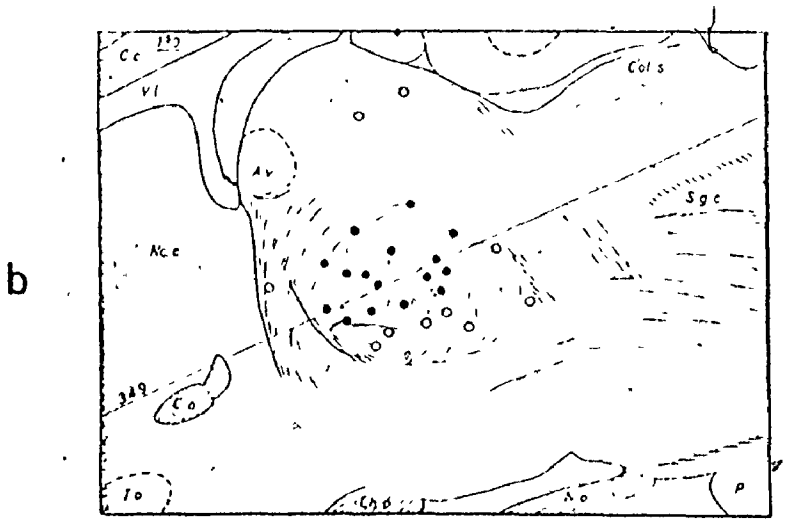




Figure 5

Figure 6

Location of LPO stimulating electrodes. Frontal sections through the forebrain at A15 and A16 (atlas of Jasper and Ajmone Marsan, 1954). a, location of LPO electrodes in all subjects in the present experiment. b, points (black circles) where stimulation was effective in eliciting cortical synchronization and sleep, taken from Sterman and Clemente (1962a), p. 95. Negative signs indicate ineffective stimulation sites.

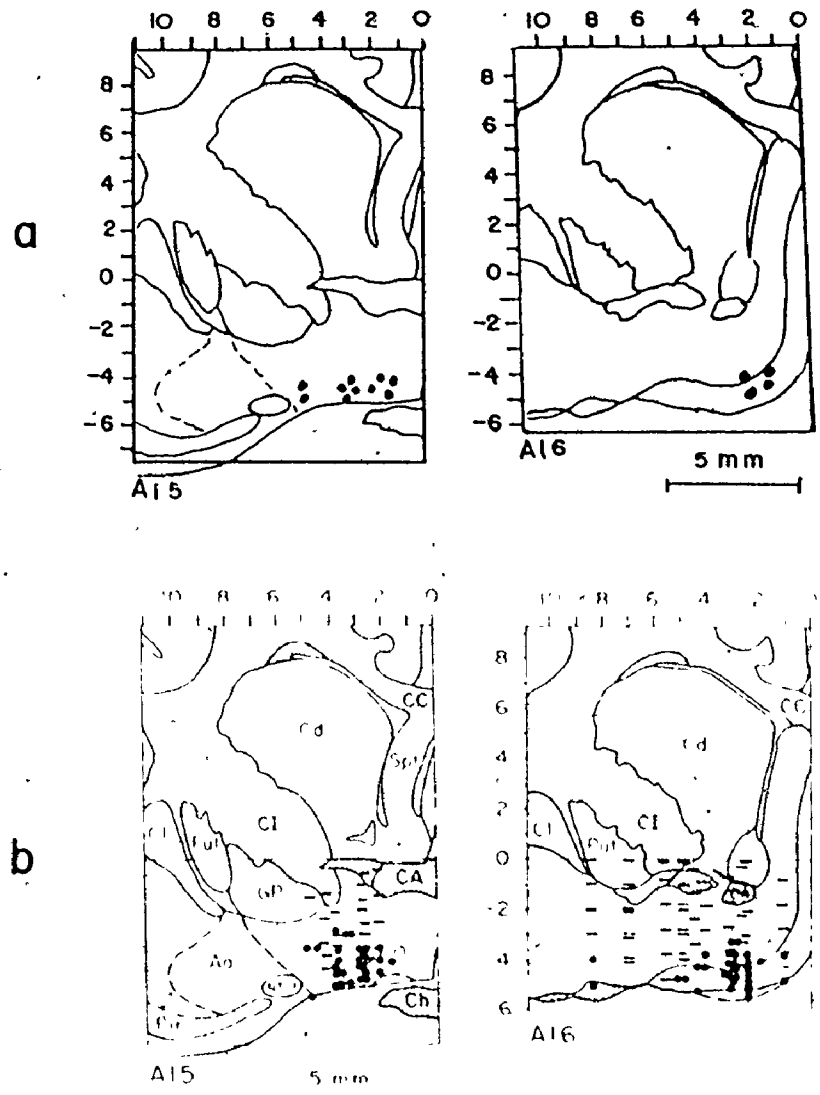



Figure 6

Figure 7

Cortical evoked responses to MTH and LPO stimulation. Recording from right frontal cortex. a, spindle responses to single pulse (at arrows) bilateral stimulation of MTH at 2 mA. b, recruiting responses to continuous 6 Hz MTH stimulation, threshold at 1.5 mA. c, spindle responses to single pulse (at arrows) bilateral stimulation of LPO at 1.5 mA. d, synchronization and recruiting responses to continuous 6 Hz LPO stimulation, threshold also at 1.5 mA. All stimulation pulses were .4 ms biphasic square waves. Recordings obtained in unanesthetized animal.

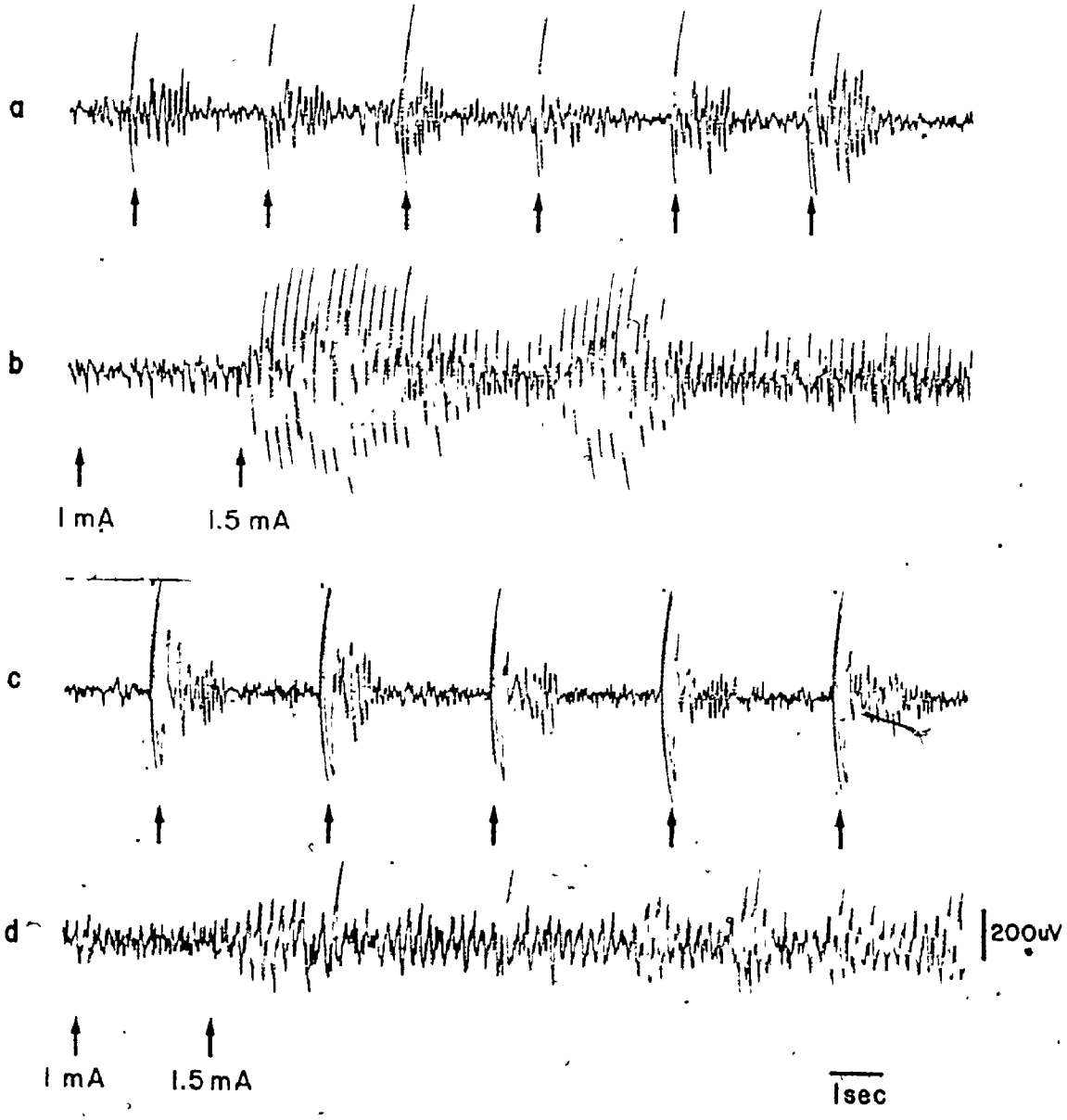



Figure 7

Figure 8

Effects of 150 Hz LPO stimulation on the cortical EEG. Fr EEG, frontal EEG; Occ EEG, occipital EEG; EOG, electro-oculogram. Continuous recording during alternate periods of no stimulation and 150 Hz LPO stimulation at .2 mA. Record begins 2 minutes after beginning of stimulation trial. Stimulus artifacts partially obscure EEG tracings during the 30 second stimulation periods, however suppression of the artifact would have distorted the tracings as well.

In 8a the cortex is desynchronized and stimulation does not appear to evoke slow potentials. In 8b slow waves appear toward the end of the third stimulation period, but may be attenuated because the amplifier is being blocked by stimulus artifacts. The slow waves continue through the rest of the record, their apparently sudden onset being explained by recovery of the amplifiers from partial block during stimulation.

Since the animal showed behavioral signs of falling asleep during these recordings the slow wave activity that began at end of the third stimulation period and continued beyond may have been spontaneous (see text).

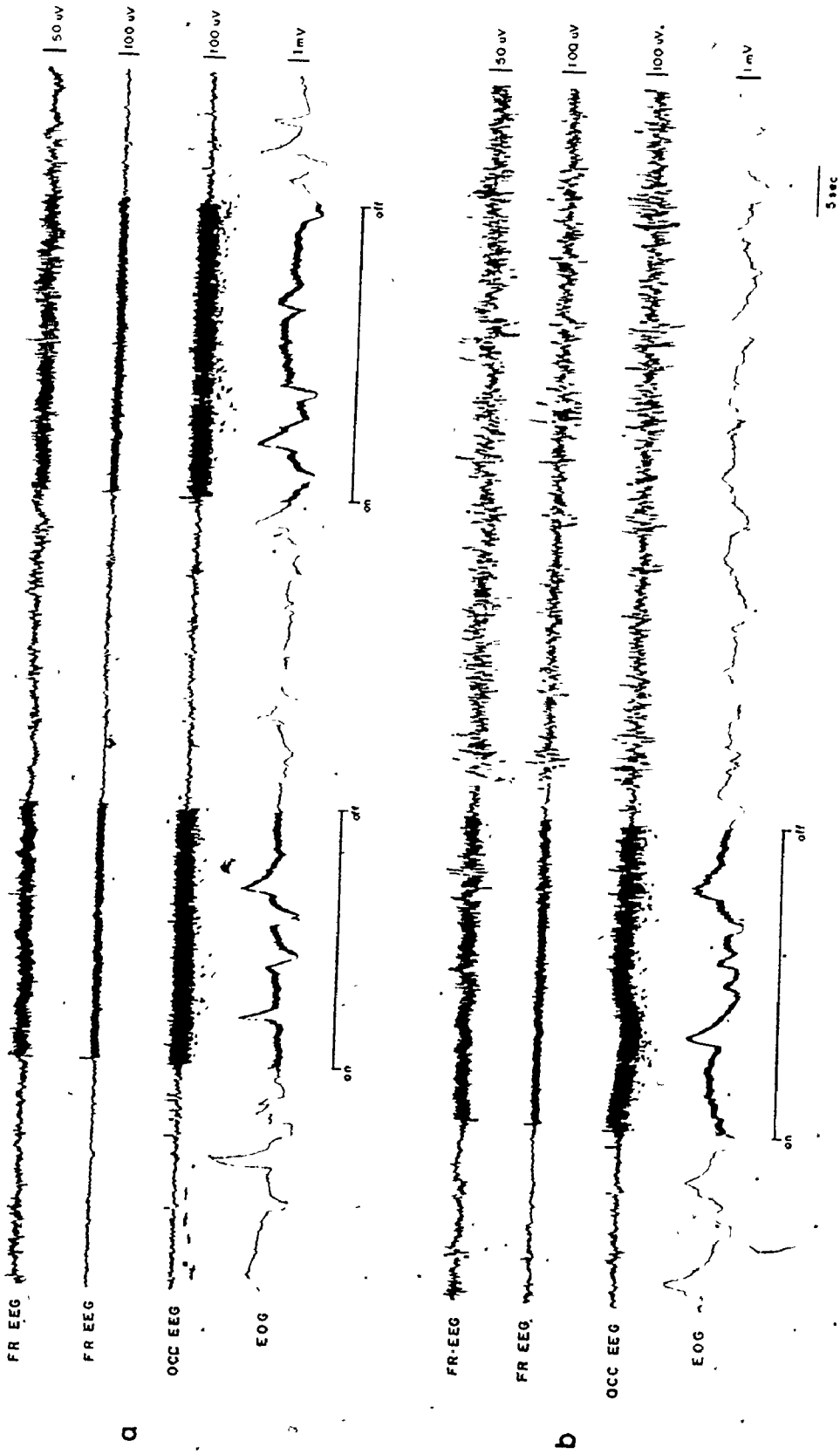

FR EEG
50 uV
FR EEG
100 uV
OCC EEG
100 uV
EOG
1mV
off
on
a
FR EEG
50 uV
FR EEG
100 uV
OCC EEG
100 uV.
EOG
1mV
off
on
5 sec
b

Figure 8

Figure 9

Effect of behavioral state on MTH recruiting responses. Continuous recording from left occipital cortex during ipsilateral 6 Hz MTH stimulation at .2 mA. Stimulation is applied for approximately 30 seconds each minute. Arrow up, onset of stimulation; arrow down, offset. In traces A-F, recruiting responses become more frequent and attain higher amplitudes as the animal passes from wakefulness to drowsiness.

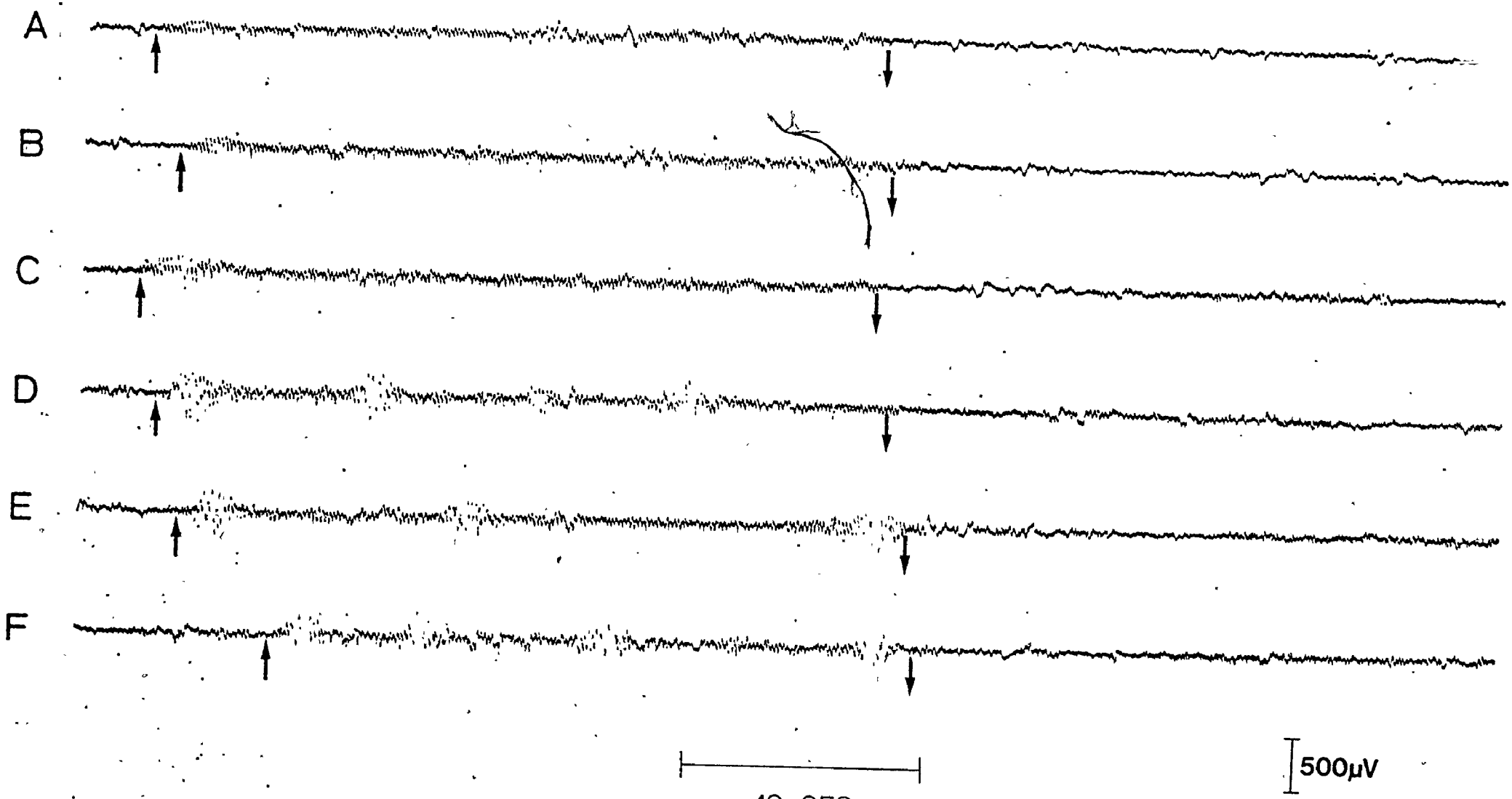



Figure 9

Figures 10 - 15

Latency of SWS on stimulation and control trials for Subjects M14 - M19. Crossed data point indicates first trial of day. Stimulation parameters are given above the data points: L, locus of stimulation; F, frequency in Hz; I, current intensity in mA.

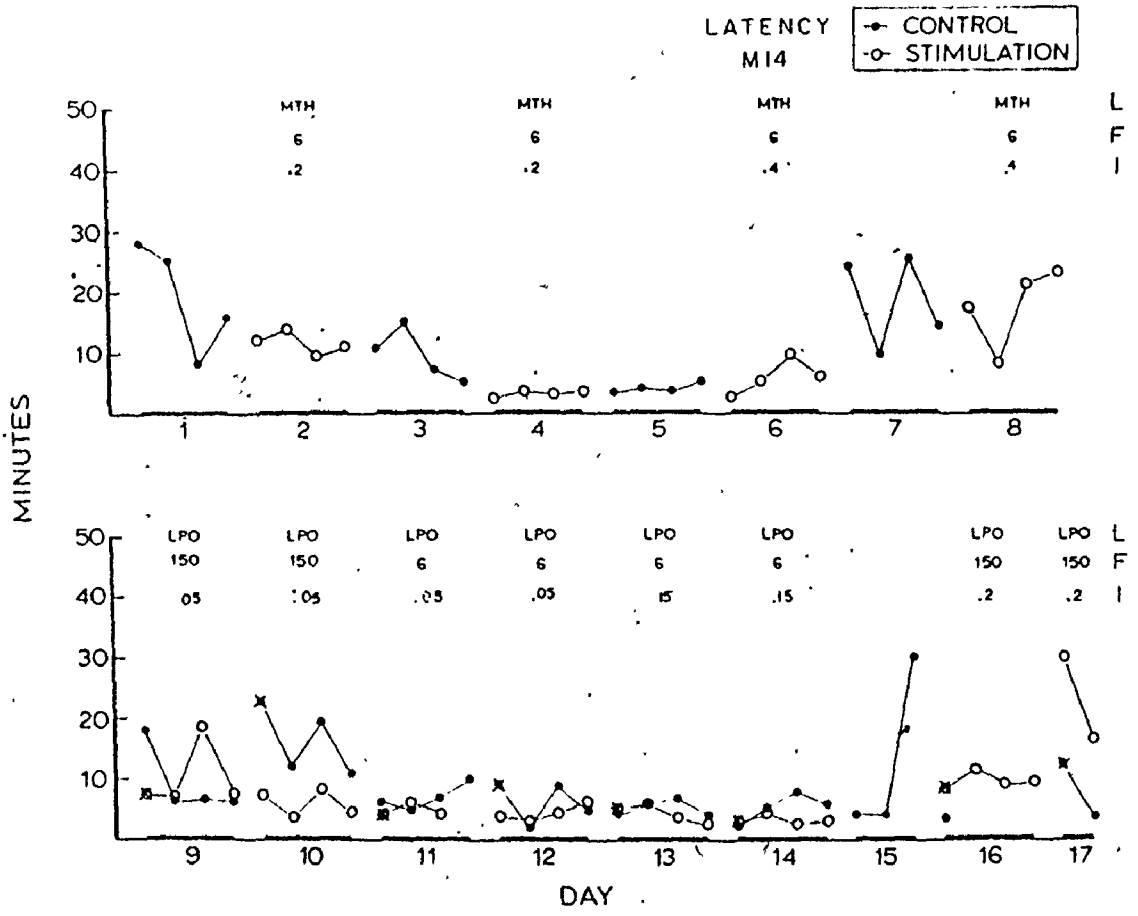



Figure 10

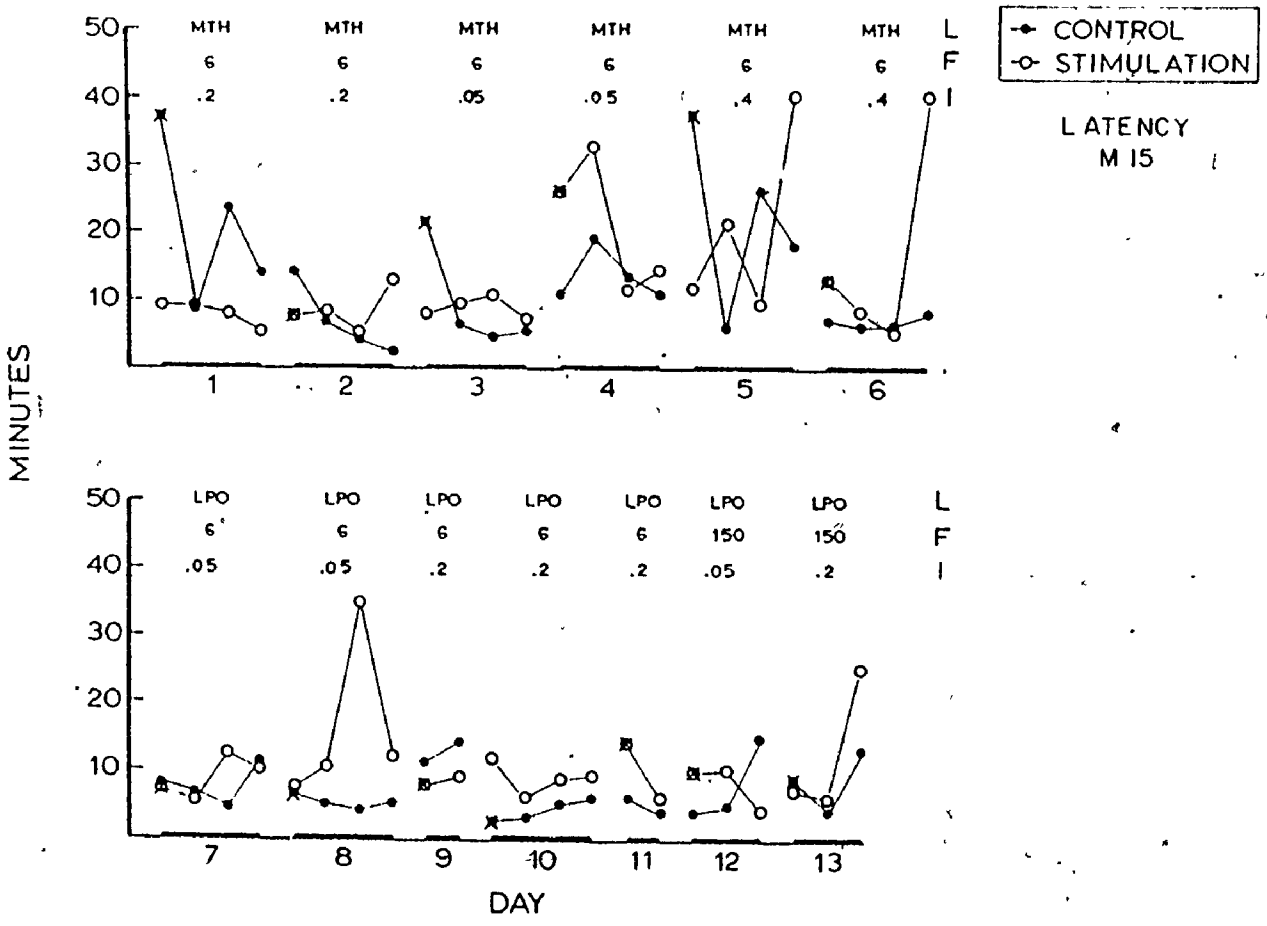

MTH 6 .2
MTH 6 .2
MTH 6 .05
MTH 6 .05
MTH 6 .4
MTH 6 .4
L F I
CONTROL
STIMULATION
LATENCY
M 15
LPO 6 .05
LPO 6 .05
LPO 6 .2
LPO 6 .2
LPO 6 .2
LPO 150 .05
LPO 150 .2
L F I
50
40
30
20
10
MINUTES
1
2
3
4
5
6
7
8
9
10
11
12
13
DAY

Figure 11

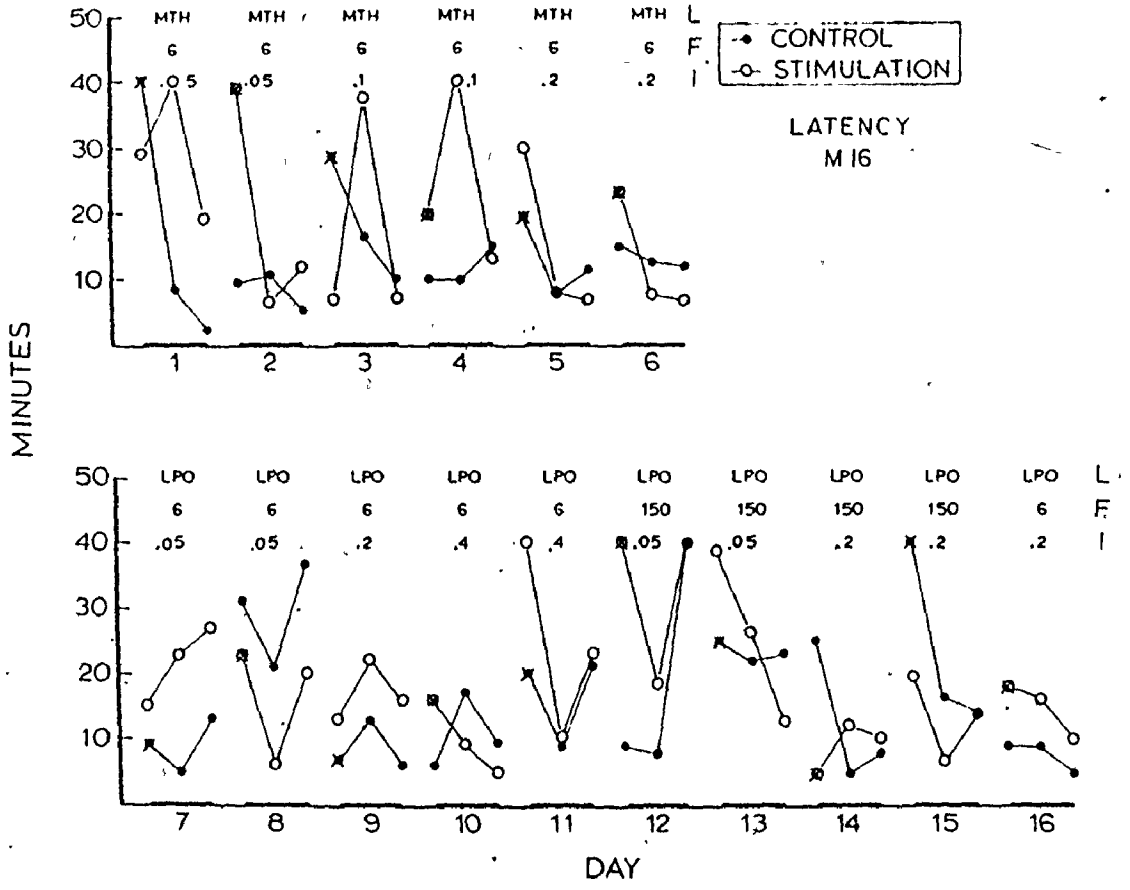

MINUTES
50
40
30
20
10
MTH 6 .5
MTH 6 .05
MTH 6 .1
MTH 6 .1
MTH 6 .2
MTH 6 .2
L
F
I
CONTROL
STIMULATION
LATENCY
M 16
1
2
3
4
5
6
LPO 6 .05
LPO 6 .05
LPO 6 .2
LPO 6 .4
LPO 6 .4
LPO 150 .05
LPO 150 .05
LPO 150 .2
LPO 150 .2
LPO 6 .2
7
8
9
10
11
12
13
14
15
16
DAY

Figure 12

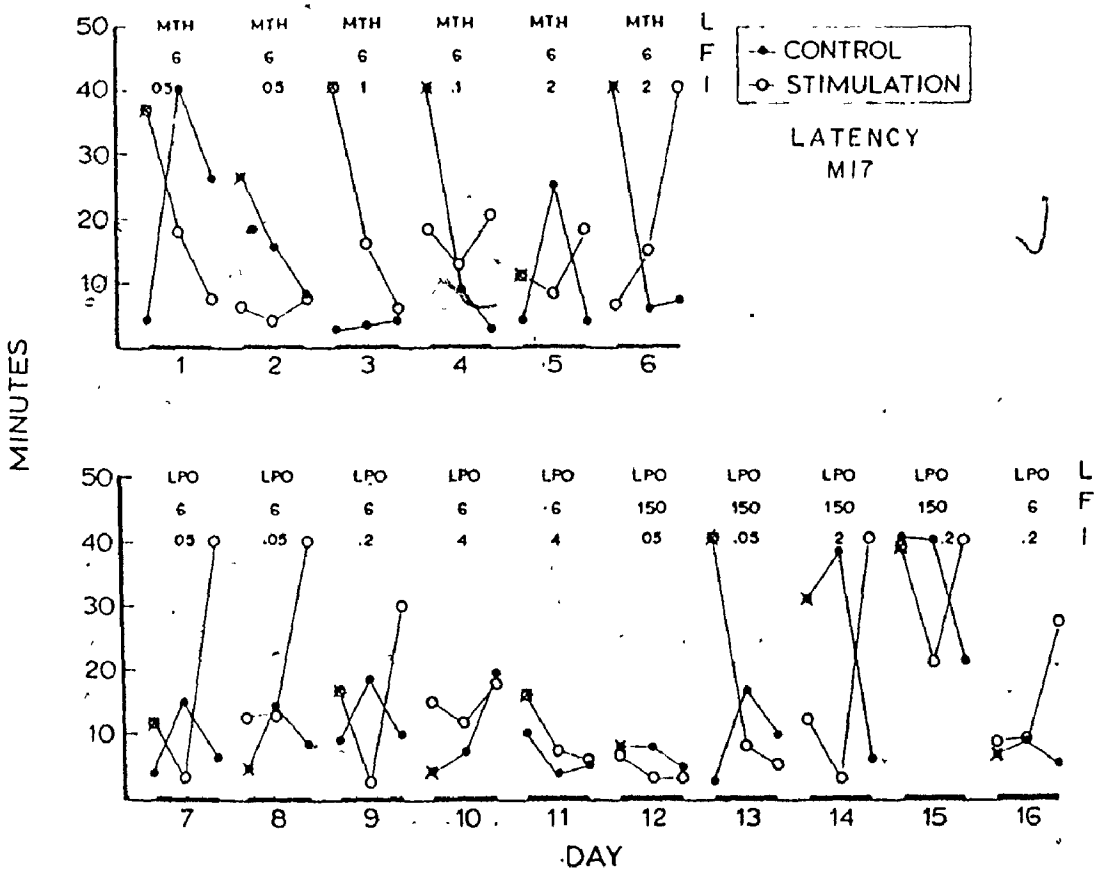

MINUTES
50
40
30
20
10
MTH 6 05
MTH 6 05
MTH 6 1
MTH 6 .3
MTH 6 2
MTH 6 2
L F I
CONTROL
STIMULATION
LATENCY
M17
1
2
3
4
5
6
LPO 6 05
LPO 6 .05
LPO 6 .2
LPO 6 4
LPO 6 4
LPO 150 05
LPO 150 .05
LPO 150 2
LPO 150 .2
LPO 6 .2
7
8
9
10
11
12
13
14
15
16
DAY

Figure 13

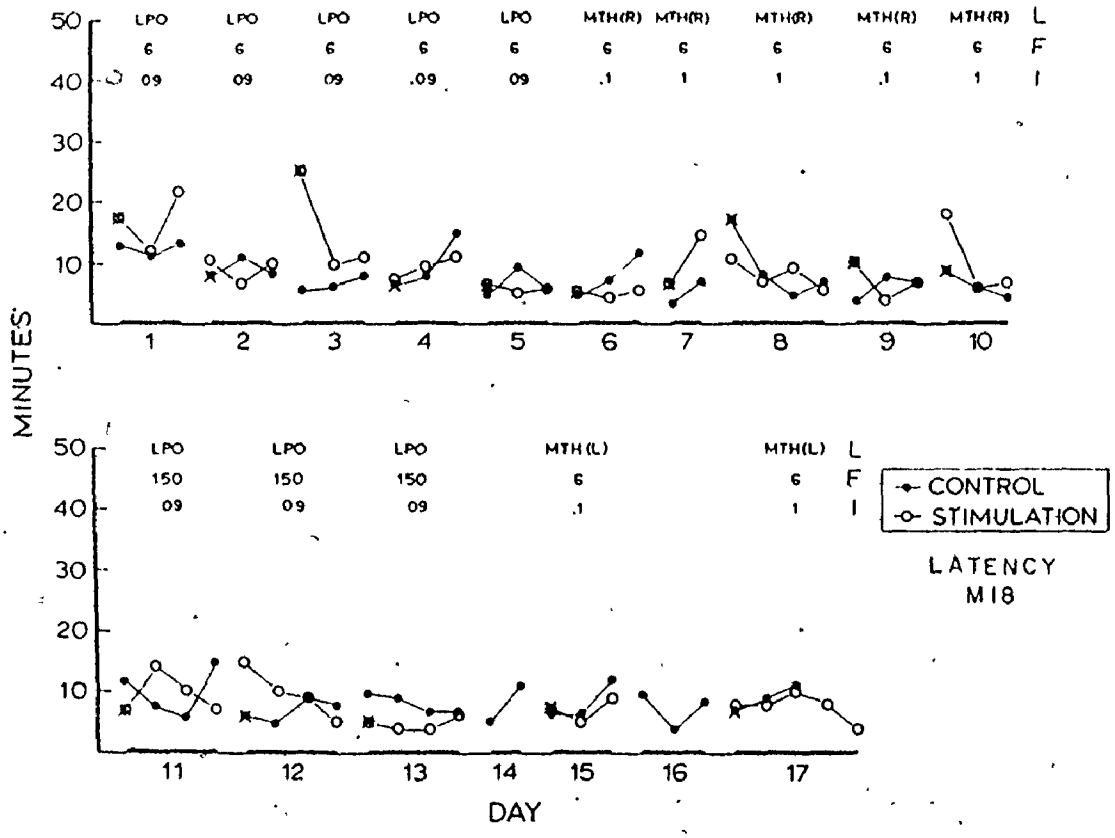

LPO LPO LPO LPO LPO MTH(R) MTH(R) MTH(R) MTH(R) MTH(R) L
6 6 6 6 6 6 6 6 6 6 F
09 09 09 .09 09 .1 1 1 .1 1 I
50
40
30
20
10
MINUTES
1 2 3 4 5 6 7 8 9 10
LPO LPO LPO MTH(L) MTH(L) L
150 150 150 6 6 F
09 09 09 .1 1 I
11 12 13 14 15 16 17
DAY
CONTROL
STIMULATION
LATENCY
M18

Figure 14

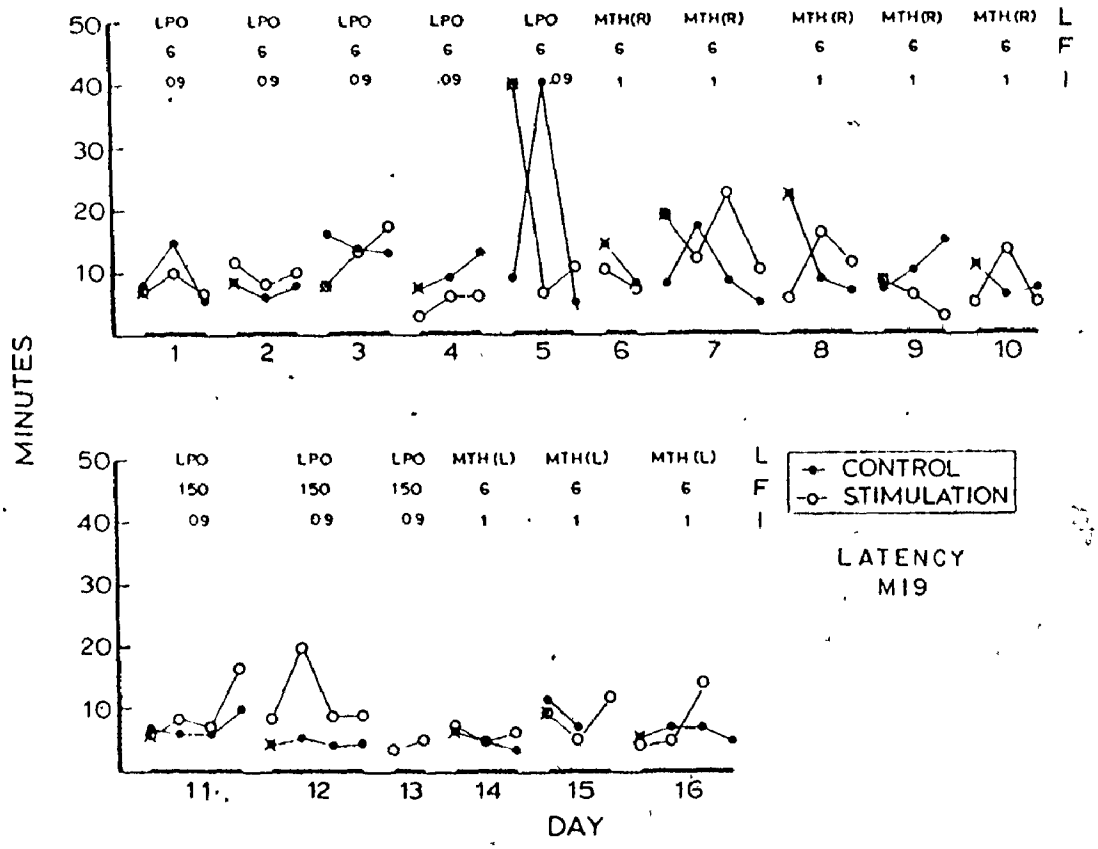

MINUTES
50
40
30
20
10
LPO LPO LPO LPO LPO MTH(R) MTH(R) MTH(R) MTH(R) MTH(R) L
6 6 6 6 6 6 6 6 6 6 F
09 09 09 09 09 1 1 1 1 1 I
1 2 3 4 5 6 7 8 9 10
LPO LPO LPO MTH(L) MTH(L) MTH(L) L
150 150 150 6 6 6 F
09 09 09 1 1 1 I
11 12 13 14 15 16
DAY
CONTROL
STIMULATION
LATENCY
M19

Figure 15

Figures 16 - 21

Duration of SWS on stimulation and control trials for Subjects M14 - M19. Crossed data point indicates first trial of day. Stimulation parameters are given above the data points: L, locus of stimulation; F, frequency in Hz; I, current intensity in mA.

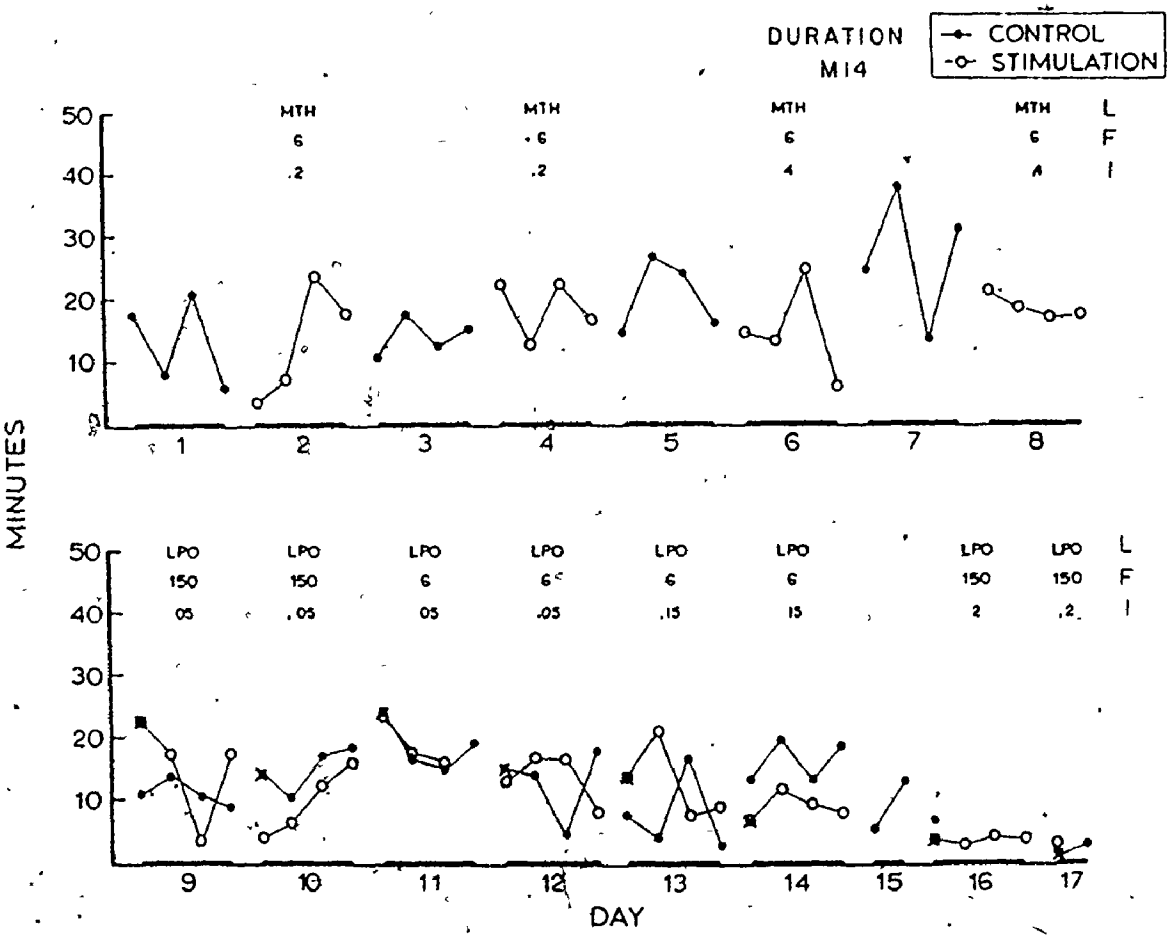

DURATION
M14
CONTROL
STIMULATION
MTH 6 .2
MTH 6 .2
MTH 6 4
MTH 6 8
L F I
LPO 150 05
LPO 150 .05
LPO 6 05
LPO 6 .05
LPO 6 .15
LPO 6 15
LPO 150 2
LPO 150 .2
MINUTES
DAY

Figure 16

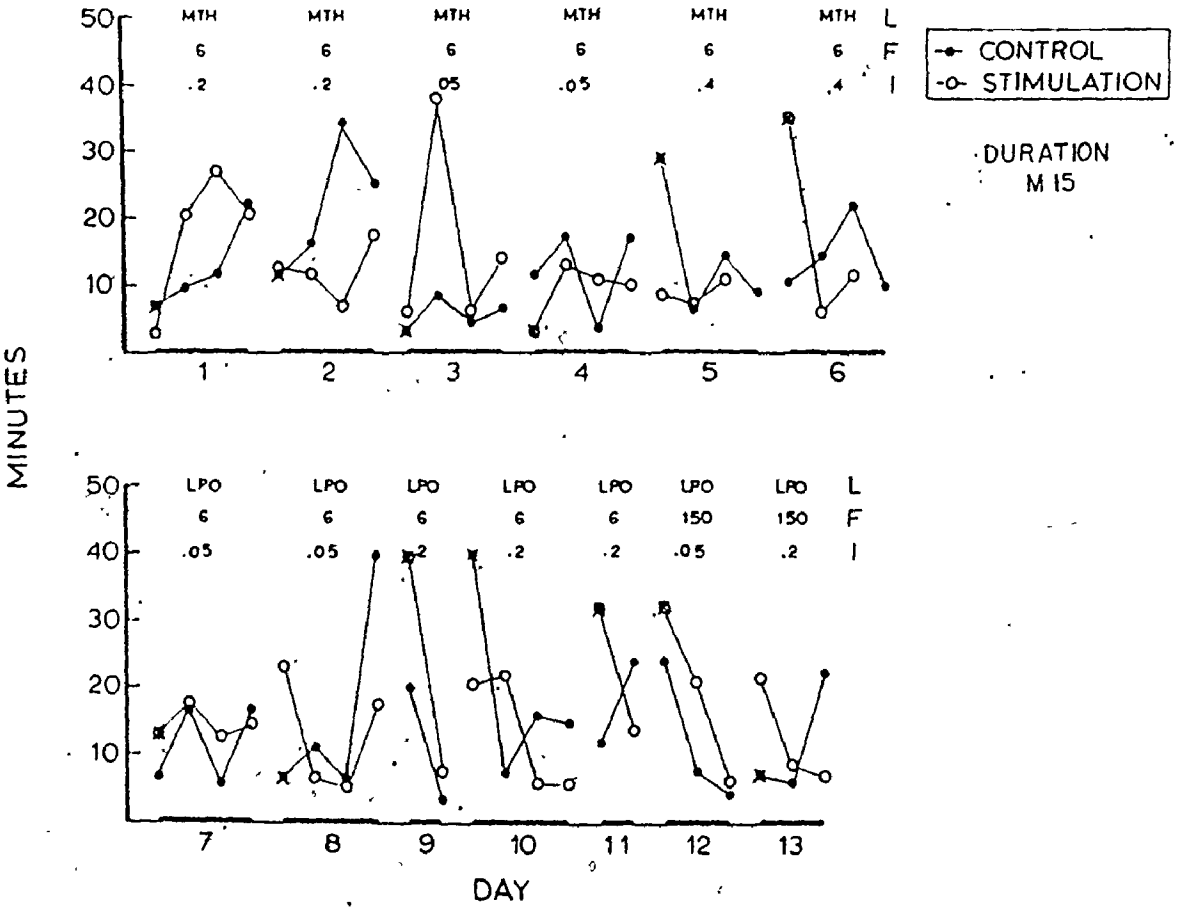

MTH 6 .2
MTH 6 .2
MTH 6 .05
MTH 6 .05
MTH 6 .4
MTH 6 .4
L F I
CONTROL
STIMULATION
DURATION
M 15
50
40
30
20
10
MINUTES
1
2
3
4
5
6
LPO 6 .05
LPO 6 .05
LPO 6 .2
LPO 6 .2
LPO 6 .2
LPO 150 .05
LPO 150 .2
L F I
7
8
9
10
11
12
13
DAY

Figure 17

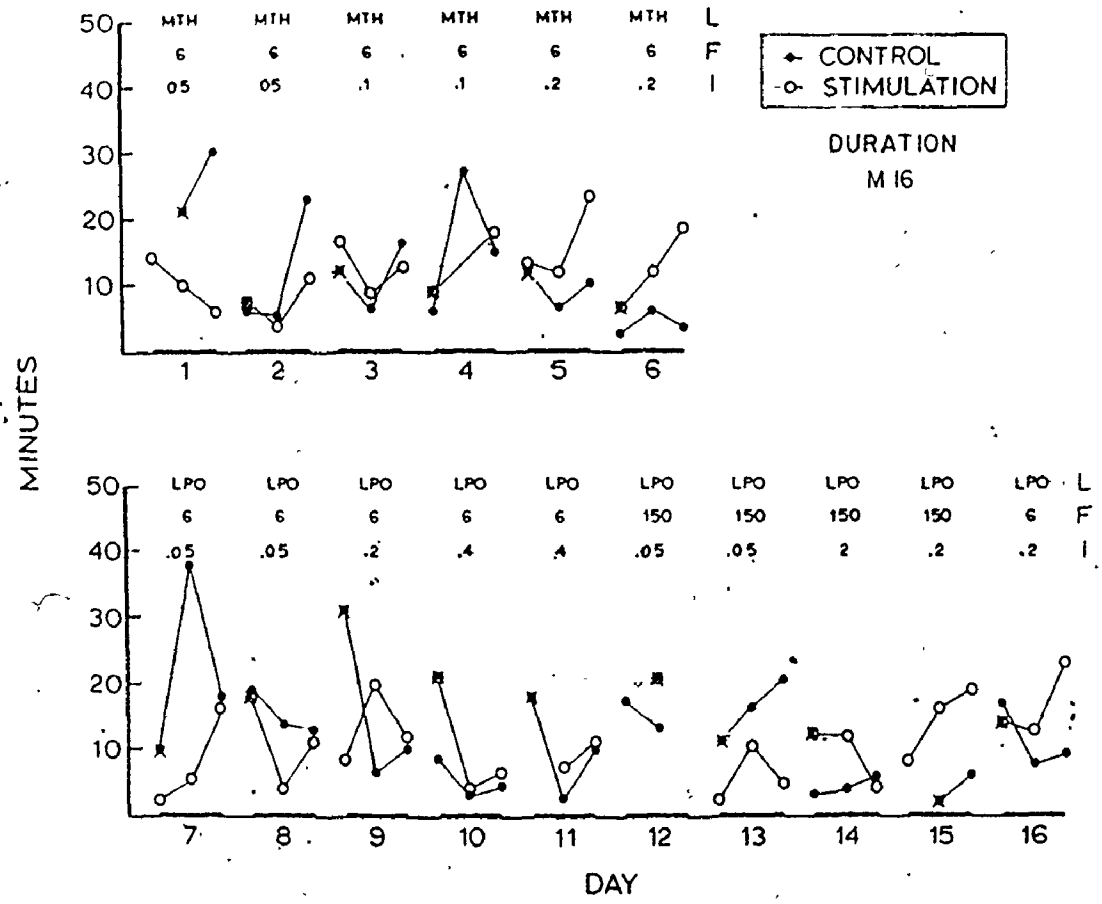

MTH MTH MTH MTH MTH MTH L
6 6 6 6 6 6 F
05 05 .1 .1 .2 .2 I
CONTROL
STIMULATION
DURATION
M 16
MINUTES
50
40
30
20
10
1 2 3 4 5 6
LPO LPO LPO LPO LPO LPO LPO LPO LPO LPO L
6 6 6 6 6 150 150 150 150 6 F
.05 .05 .2 .4 .4 .05 .05 2 .2 .2 I
7 8 9 10 11 12 13 14 15 16
DAY

Figure 18

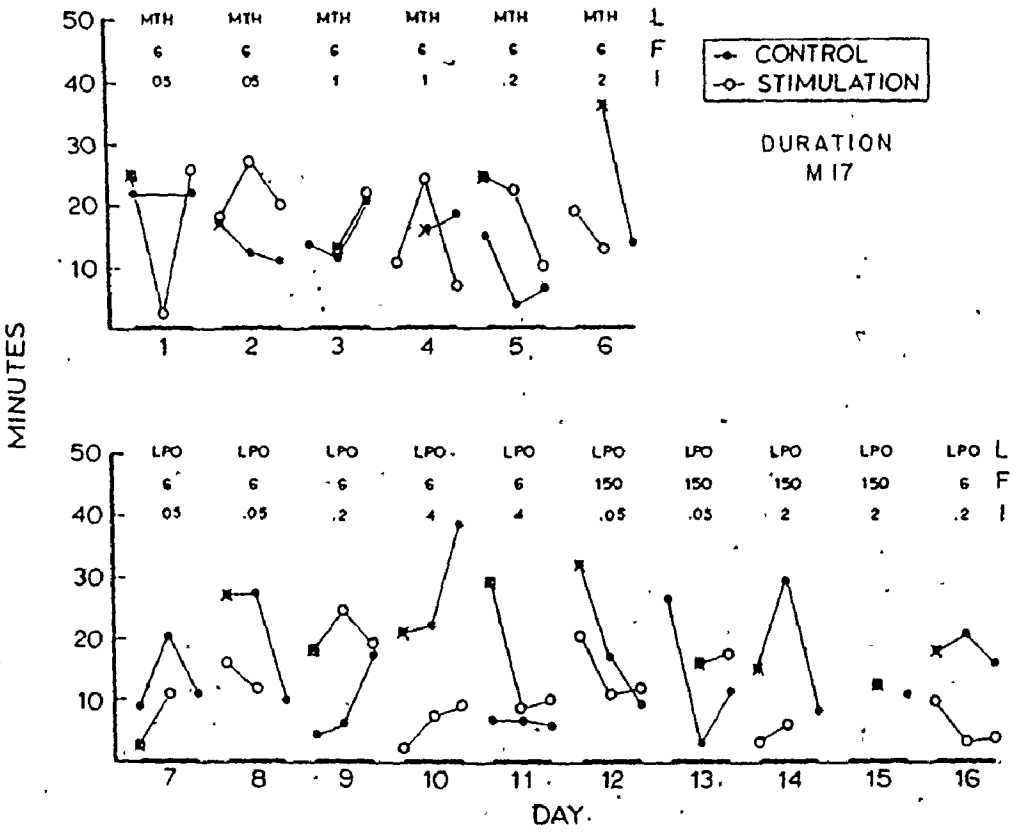

MTH MTH MTH MTH MTH MTH L
6 6 6 6 6 6 F
05 05 1 1 .2 2 I
CONTROL
STIMULATION
DURATION
M 17
MINUTES
50
40
30
20
10
1 2 3 4 5 6
LPO LPO LPO LPO LPO LPO LPO LPO LPO LPO L
6 6 6 6 6 150 150 150 150 6 F
05 .05 .2 4 4 .05 .05 2 2 .2 I
50
40
30
20
10
7 8 9 10 11 12 13 14 15 16
DAY

Figure 19

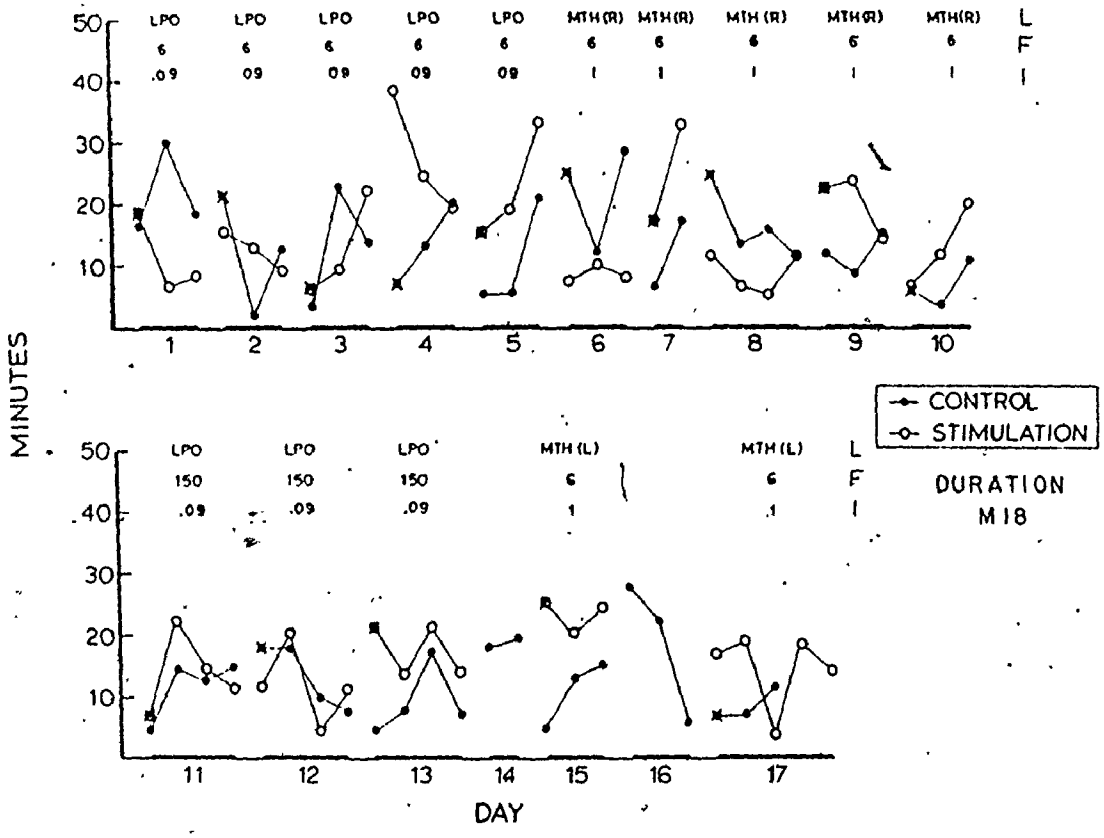

LPO
MTH(R)
MTH(L)
L
F
I
MINUTES
DAY
CONTROL
STIMULATION
DURATION
M18

Figure 20

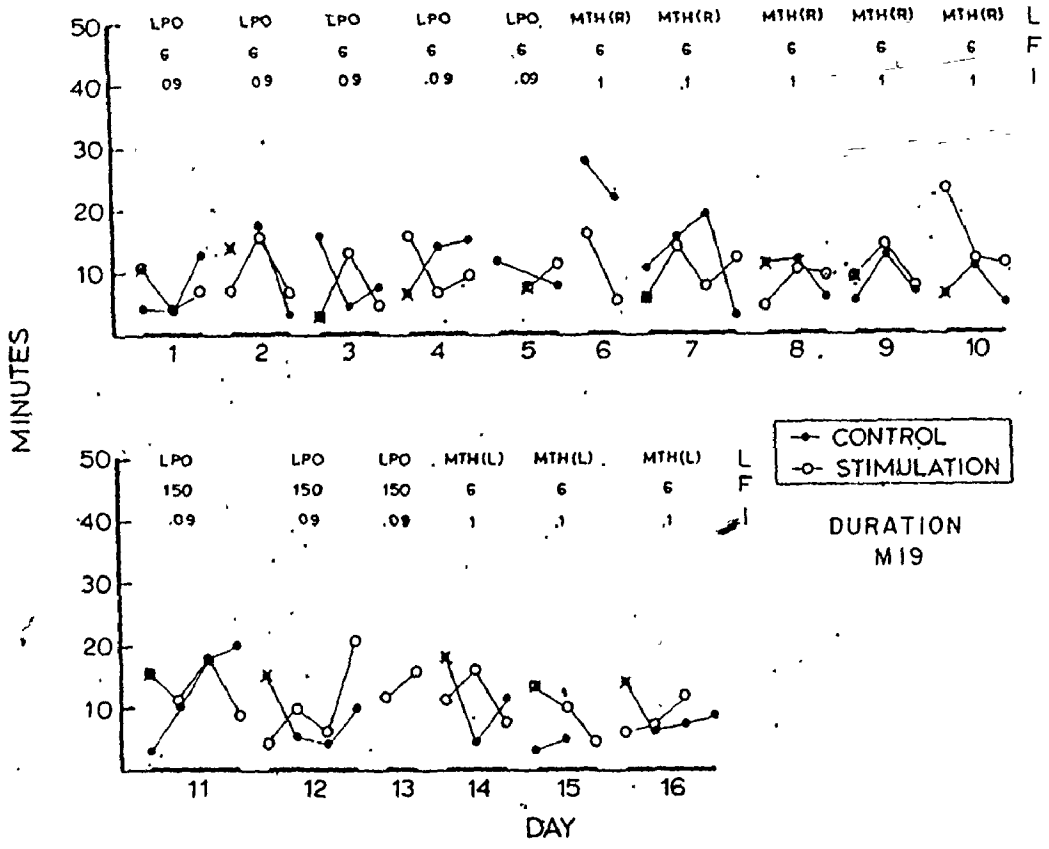

LPO
6
09
MTH(R)
6
1
MTH(L)
6
1
L
F
I
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
MINUTES
DAY
CONTROL
STIMULATION
DURATION
M19

Figure 21

Figure 22

Mean latency and mean duration of sleep episodes. a, mean and standard deviation of sleep latencies for each subject on all MTH stimulation trials and associated control trials. b, same for LPO stimulation trials and associated control trials. c, mean and standard deviation of sleep durations for each subject on all MTH stimulation trials and associated control trials. d, same for LPO stimulation trials and associated control trials.

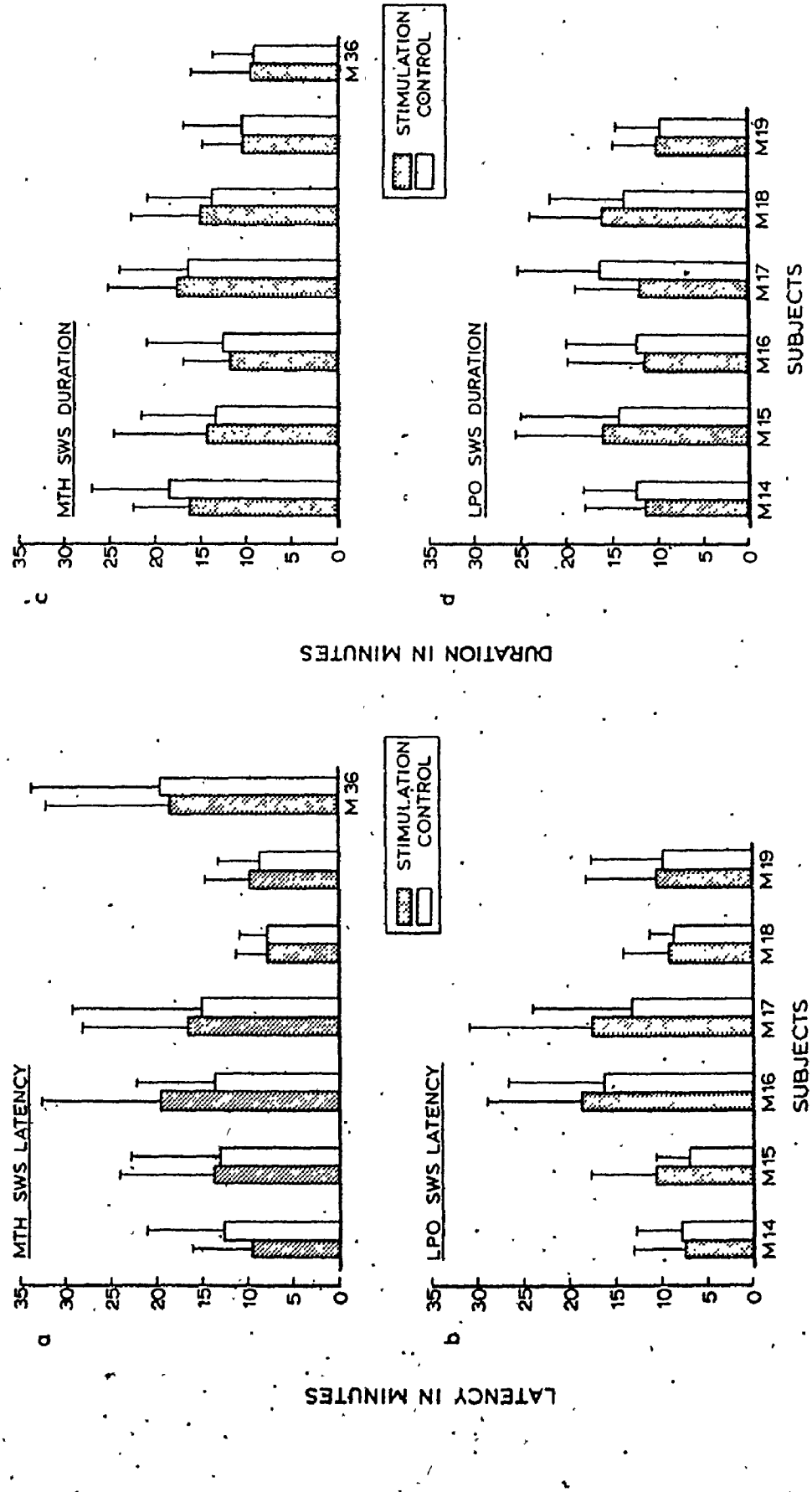

STIMULATION
CONTROL
MTH SWS LATENCY
LPO SWS LATENCY
MTH SWS DURATION
LPO SWS DURATION
SUBJECTS
M14
M15
M16
M17
M18
M19
M36
0
5
10
15
20
25
30
35
a
b
c
d
LATENCY IN MINUTES
DURATION IN MINUTES

Figure 22

Figure 23

REM-sleep latency and duration. Latency (a) and duration (b) of REM sleep episodes in Subject M16. Stimulation parameters are given above the data points: $L_I$, locus of stimulation; F, frequency in Hz; I, current intensity in mA.

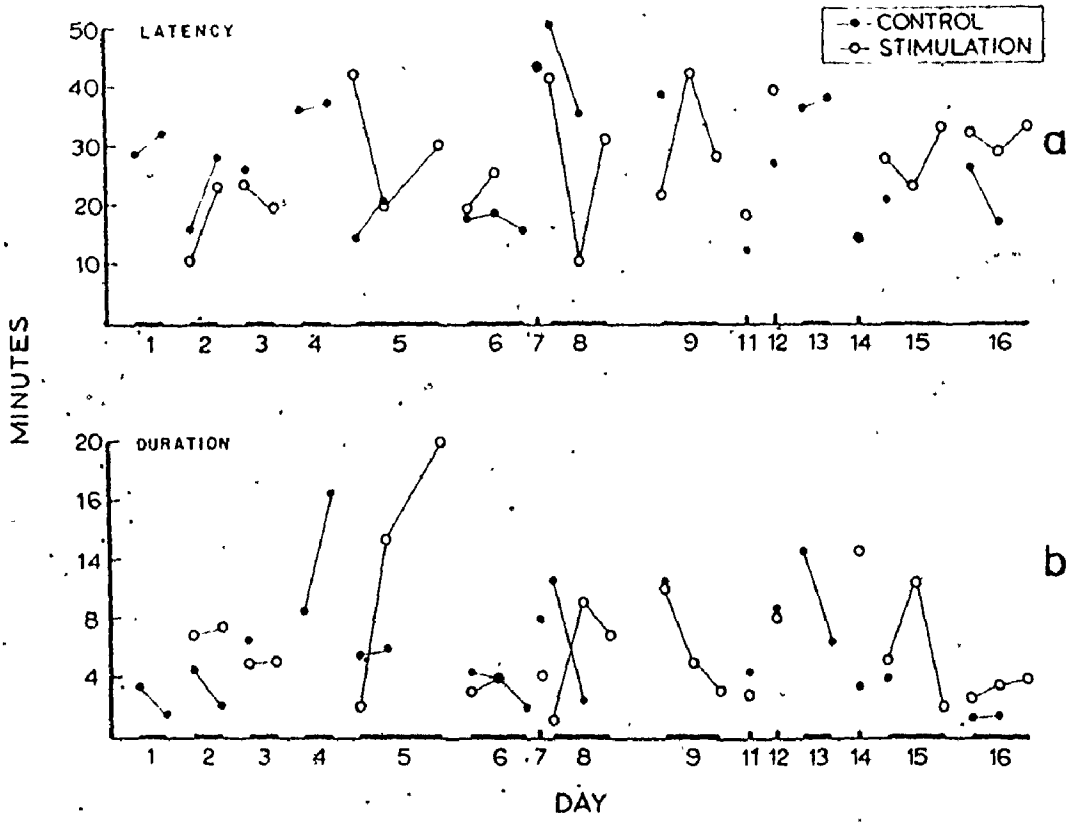

CONTROL
STIMULATION
LATENCY
DURATION
MINUTES
DAY
a
b
50
40
30
20
10
16
14
8
4
1 2 3 4 5 6 7 8 9 11 12 13 14 15 16

Figure 23

Figure 24

Location of MTH and LPO stimulating electrodes. a, sagittal section through the thalamus of the cat 2.5 mm from midline showing the location of MTH electrodes in one of the three subjects receiving MTH stimulation. b, frontal sections through the basal forebrain of the cat at A15, A16 and A17 showing location of LPO electrodes in six of the seven subjects receiving LPO stimulation.

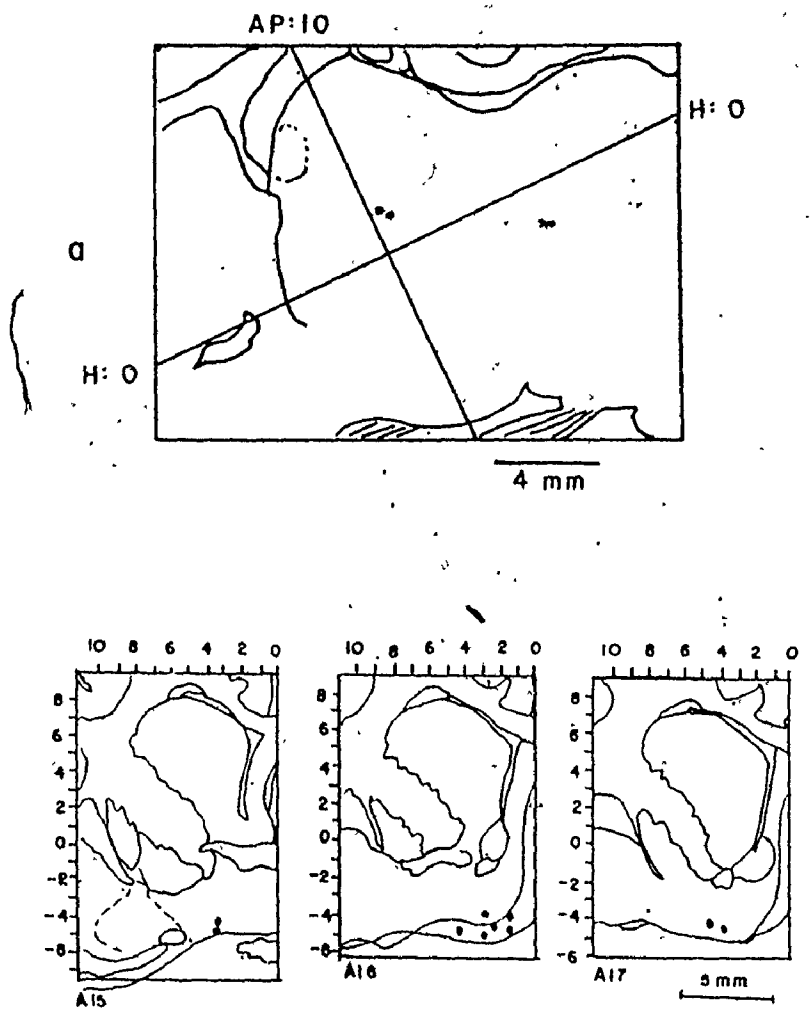



Figure 24

Figure 25

Cortical EEG response to MTH and LPO stimulation. a, recruiting responses obtained in left occipital cortex by bilateral stimulation of MTH at approximately 6 Hz, .9 mA in curarized preparation. b, in same preparation, spindles obtained by 1.6 mA bilateral LPO stimulation. A single LPO pulse is presented at the beginning of each sweep.

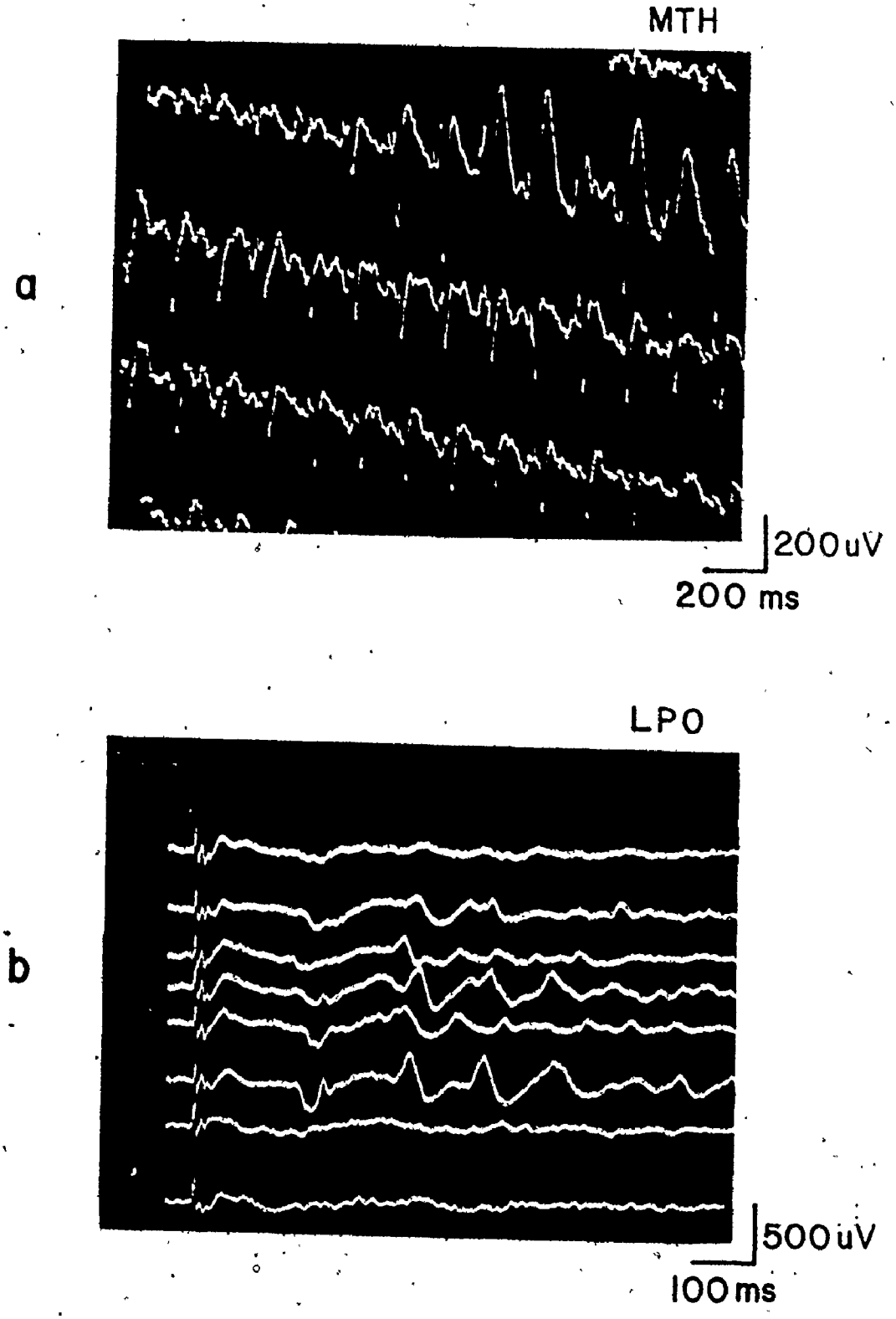



Figure 25

Figure 26

Locations of recorded cells. Sagittal section through the brain stem of the cat 3.3 mm from the midline showing the loci of electrode tips in several penetrations during single unit recording from the reticular formation. CB, cerebellum; IC, inferior colliculus; SC, superior colliculus; PRF, pontine reticular formation; MRF, mesencephalic reticular formation; PG, pontine gray; SN, substantia nigra; R, red nucleus; VMT, ventromedial thalamus; AC, anterior commissure; DB, diagonal band of Broca; OT, optic tract; ON, optic nerve.

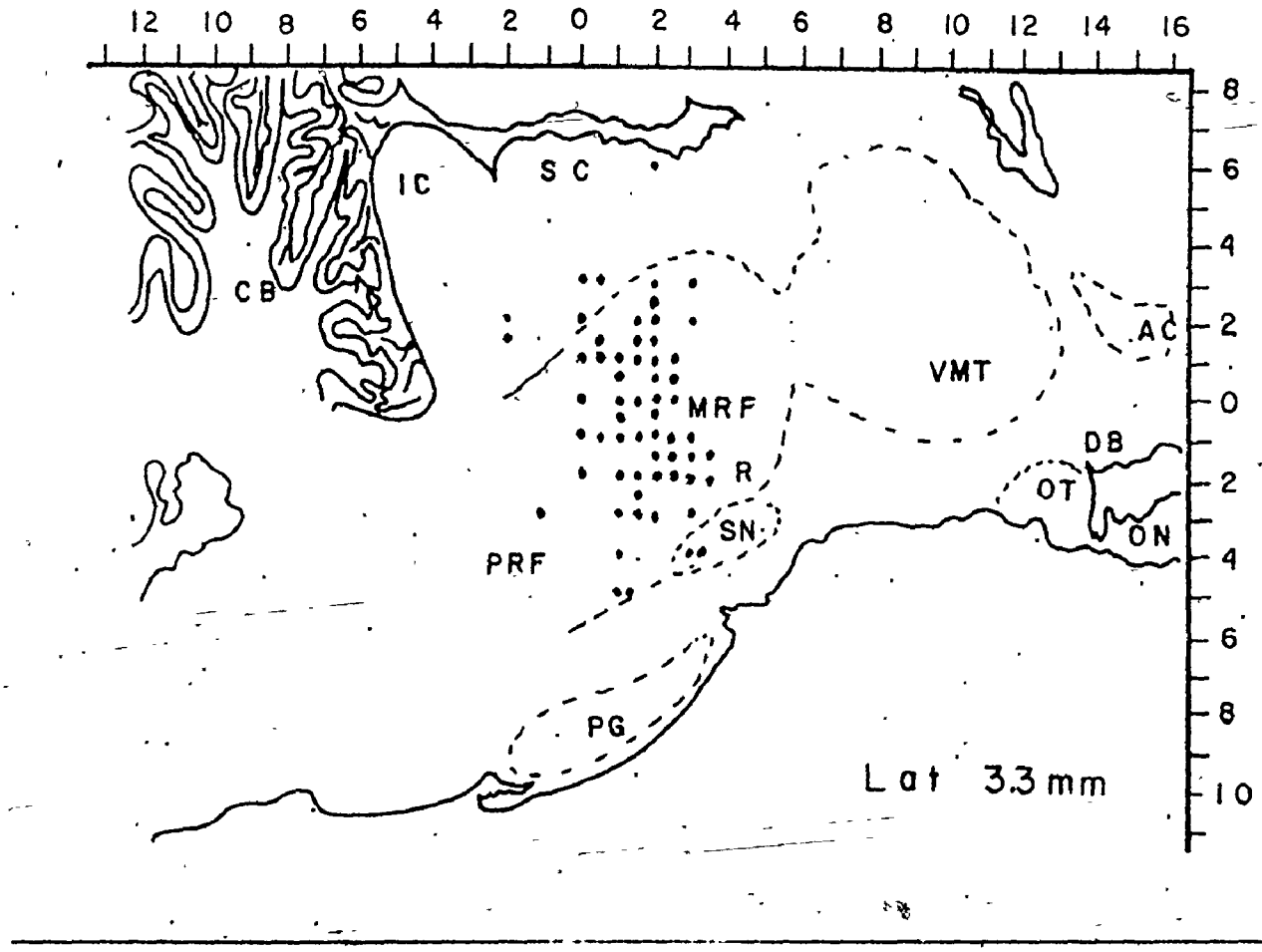

12 10 8 6 4 2 0 2 4 6 8 10 12 14 16
8 6 4 2 0 2 4 6 8 10
IC
SC
CB
AC
VMT
MRF
R
SN
PRF
DB
OT
ON
PG
Lat 3.3mm

Figure 26

Figure 27

Anatomical distribution of visual responses. Percentage of cells tested in each of 6 AP planes (a), 5 ML planes (b), and 5 DV planes (c) that exhibit responses to V stimulation. AP planes 1-6 correspond to AP +3 mm, AP +1 mm, AP 0 mm, AP -1 mm, and AP -2 mm; ML planes 1-5 correspond to ML +1 mm, ML +2 mm, ML + 3 mm, ML +4 mm, and ML + 5 mm; DV planes 1-5 correspond to DV +6 mm through +3.1 mm, DV +3 mm through +1.1 mm, DV + 1 mm through -1 mm, DV -1.1 mm through -3 mm, and DV -3.1 mm through -6 mm. All coordinates are relative to the stereotaxic atlas of Snider and Niemer (1961).

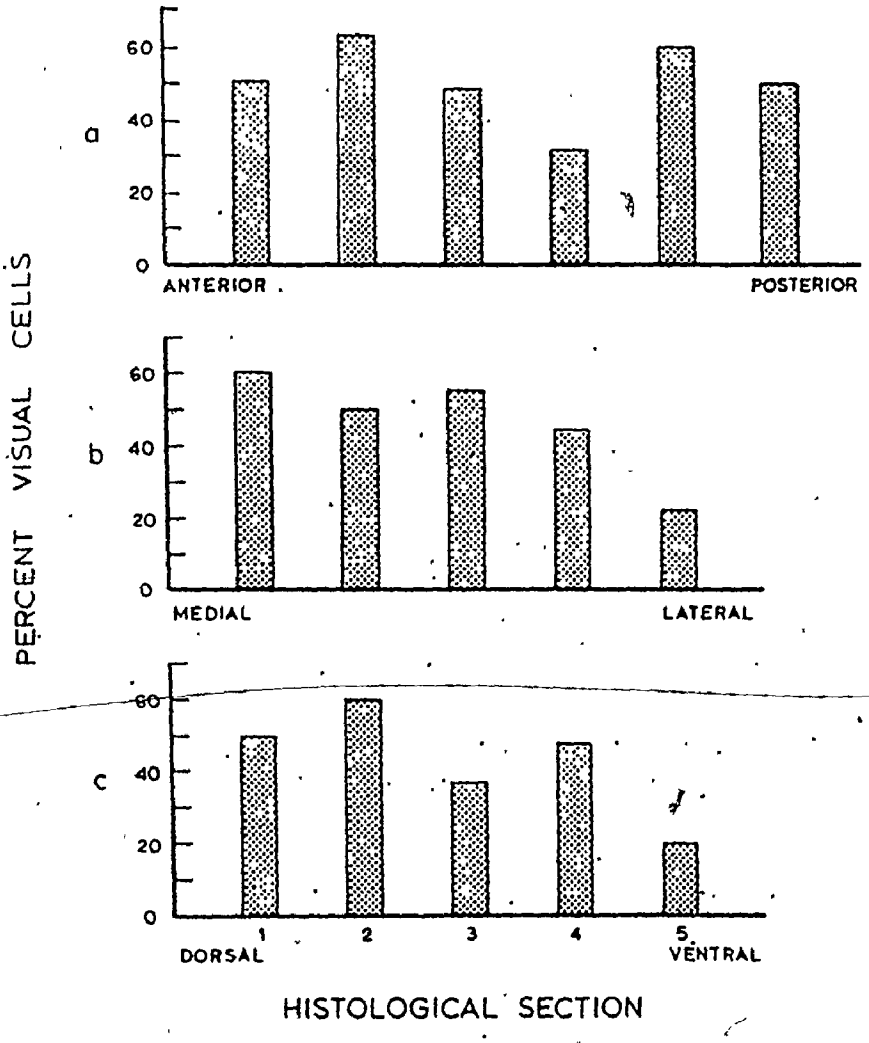

PERCENT VISUAL CELLS
a
60
40
20
0
ANTERIOR
POSTERIOR
b
60
40
20
0
MEDIAL
LATERAL
c
60
40
20
0
1
2
3
4
5
DORSAL
VENTRAL
HISTOLOGICAL SECTION

Figure 27

Figure 28

Anatomical distribution of auditory responses.
See legend to Figure 27.

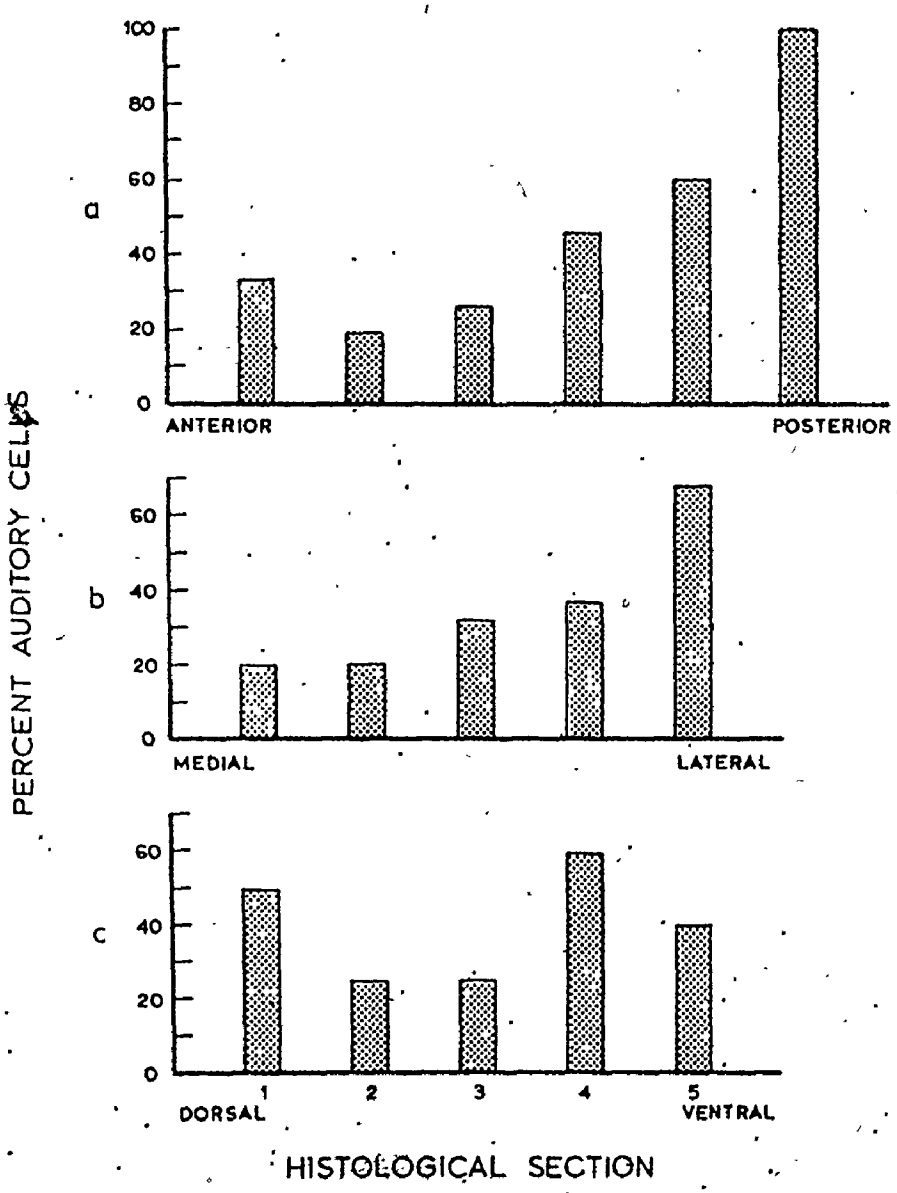

PERCENT AUDITORY CELLS
a
100
80
60
40
20
0
ANTERIOR
POSTERIOR
b
60
40
20
0
MEDIAL
LATERAL
c
60
40
20
0
DORSAL
1
2
3
4
5
VENTRAL
HISTOLOGICAL SECTION

Figure 28

Figure 29

Anatomical distribution of LPO responses.
See legend to Figure 27.

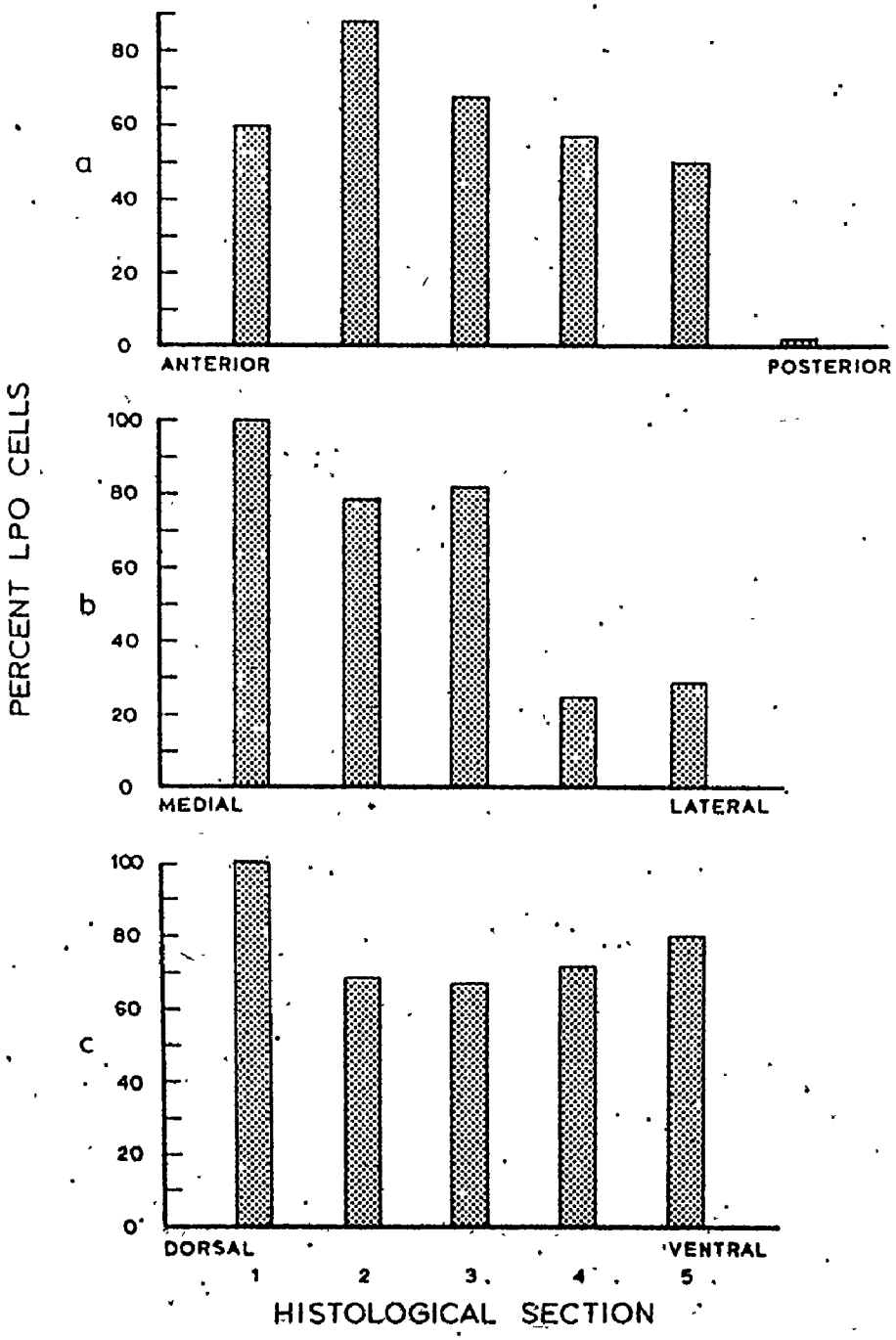

PERCENT LPO CELLS
a
80
60
40
20
0
ANTERIOR
POSTERIOR
b
100
80
60
40
20
0
MEDIAL
LATERAL
c
100
80
60
40
20
0
DORSAL
VENTRAL
1
2
3
4
5
HISTOLOGICAL SECTION

Figure 29

Figure 30

Anatomical distribution of MTH responses.
See legend to Figure 27.

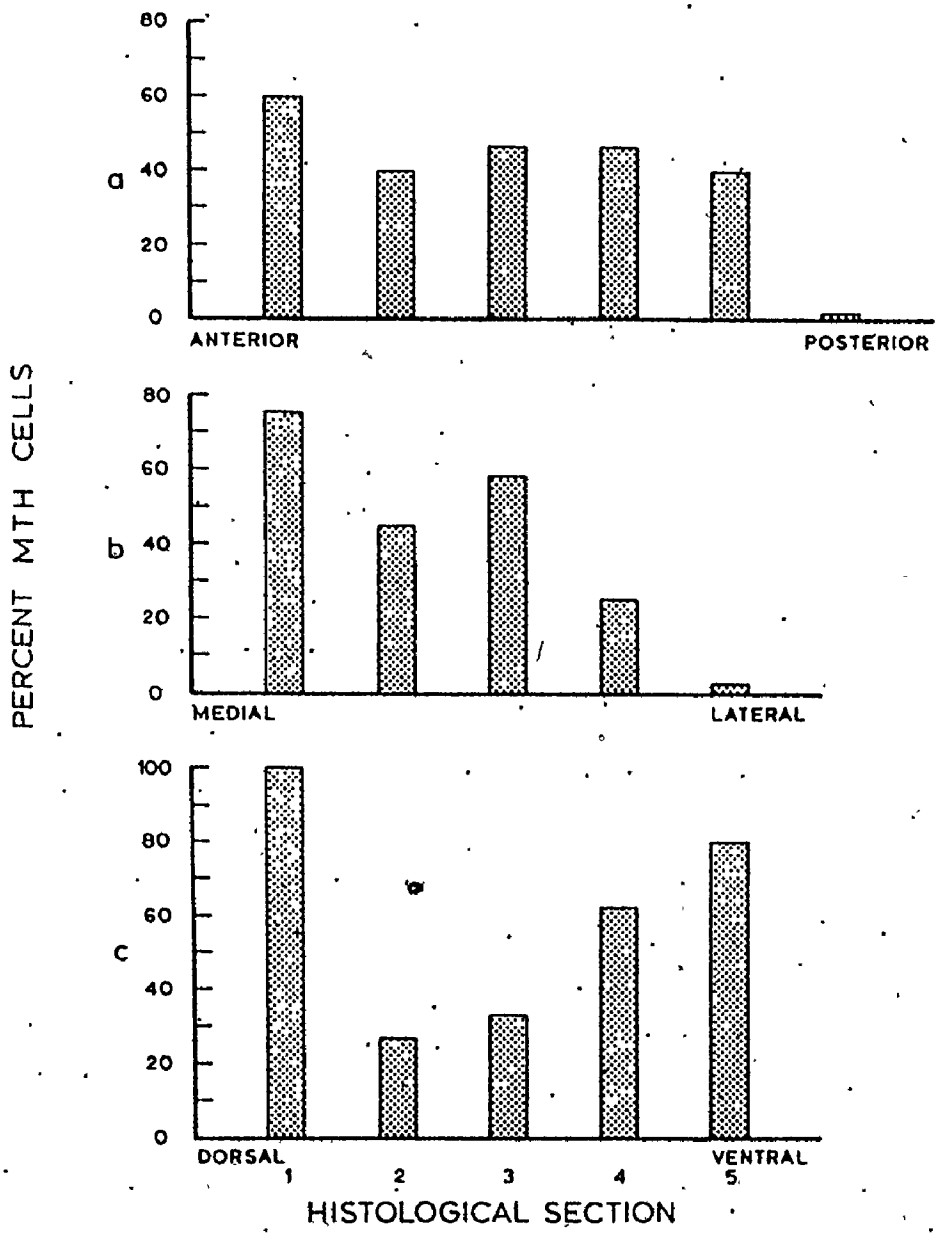

PERCENT MTH CELLS
a
b
c
0
20
40
60
80
100
ANTERIOR
POSTERIOR
MEDIAL
LATERAL
DORSAL
VENTRAL
1
2
3
4
5
HISTOLOGICAL SECTION

Figure 30

Figure 31

Typical unit and field potential responses to V, A, LPO, and MTH stimulation. a, V responses in cell #101. b, A responses in cell #93. c, LPO responses in cell #65. d, MTH responses in cell #110. Stimulus occurs at arrow. Positive deflections up, negative down.

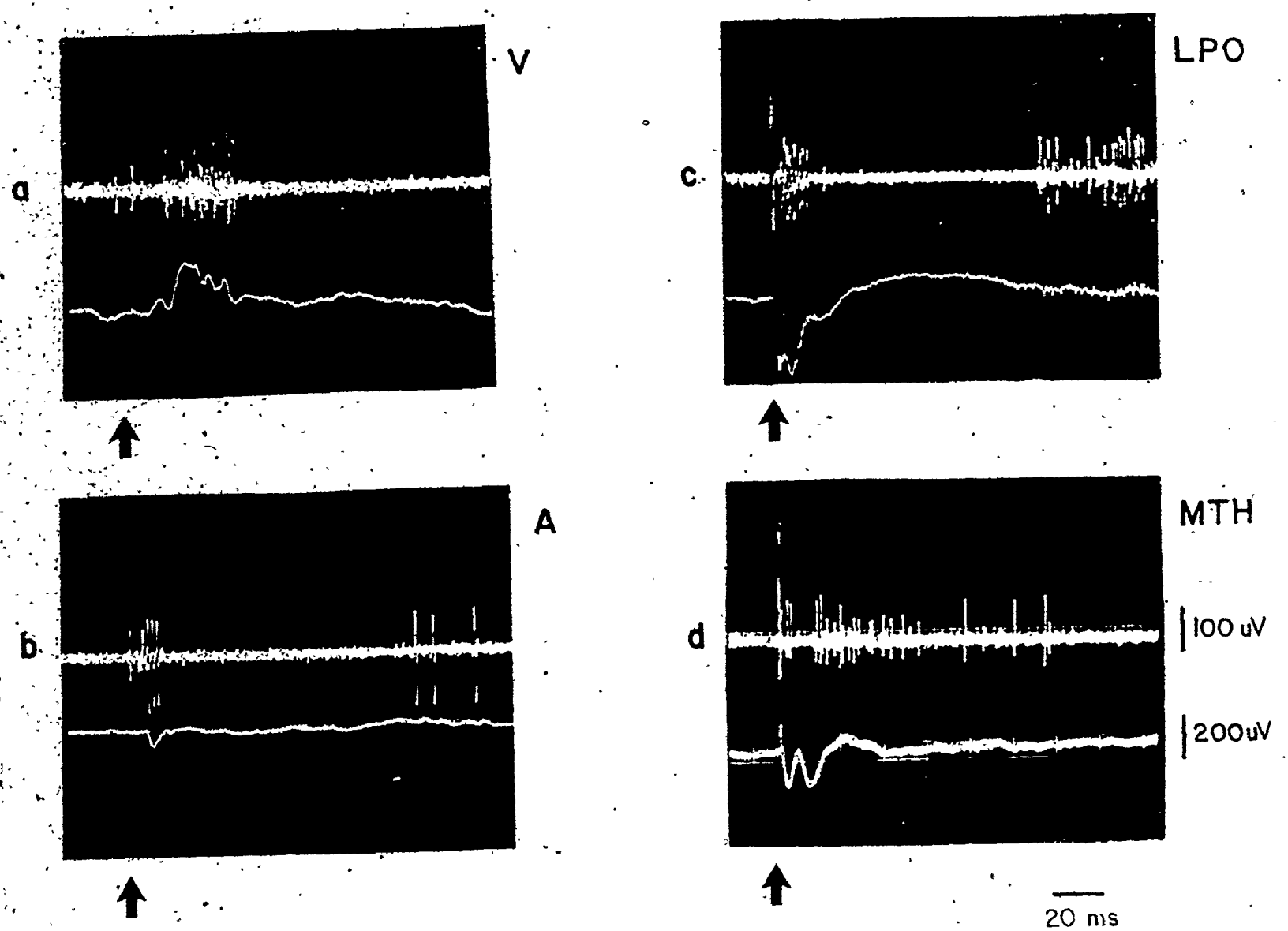



Figure 31

Figure 32

Blocking of LPO responses by V stimuli (cell #65). a, response to V stimulation alone is two bursts of spikes correlated with two negative-going field potentials. b, response to LPO stimulation alone is a shorter latency burst of spikes and complex field potential with negative and positive components. c, when LPO stimulation follows V by 68 ms, the LPO spike response and negative field potential are reduced. Stimuli at arrows. Positive deflections up, negative down.

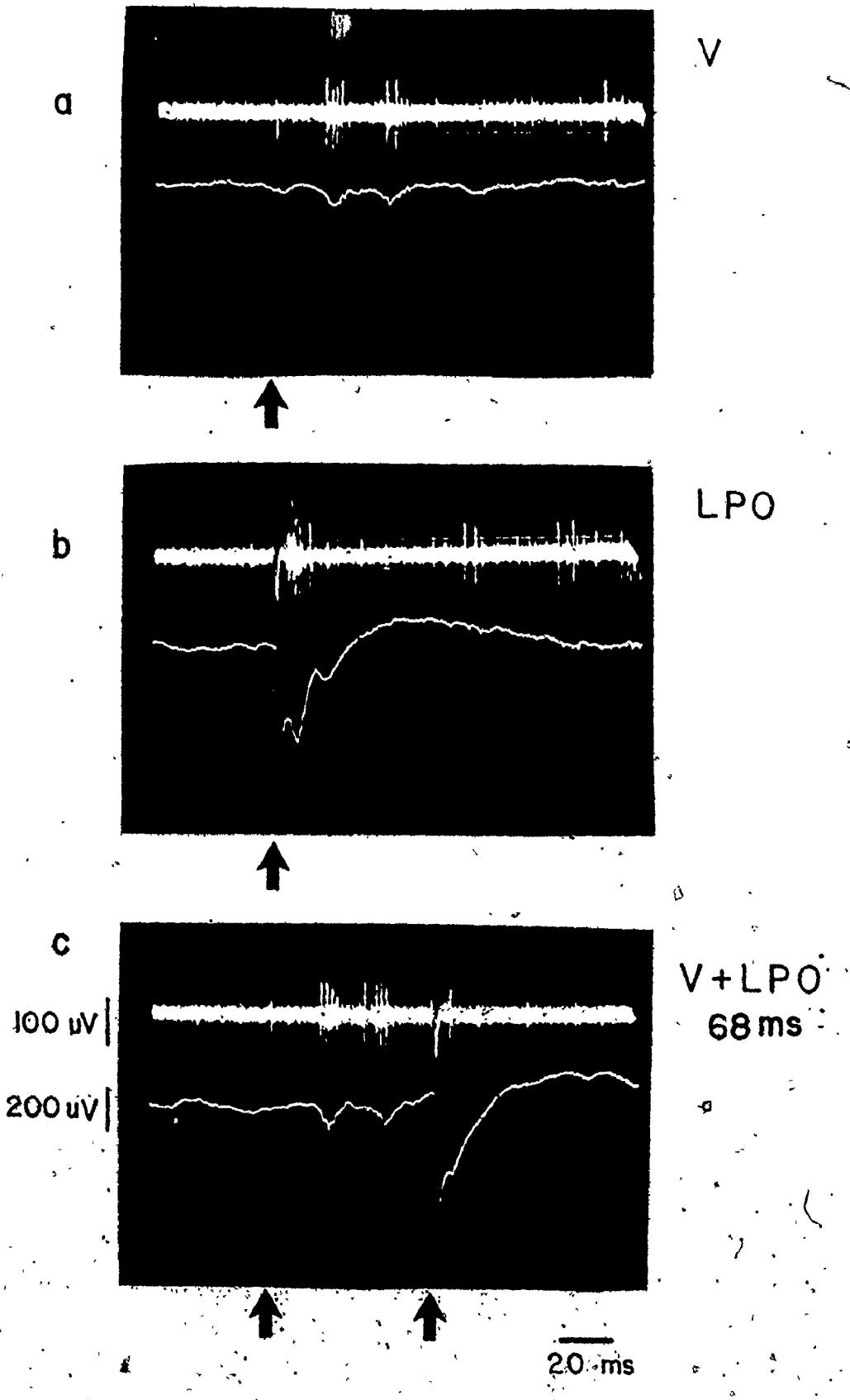



Figure 32

Figure 33

Blocking of LPO responses by V stimuli (cell #18). a, response to LPO stimulation alone is short latency burst of spikes and positive-negative-positive field potential. b, LPO spike response is blocked and field potential reduced in amplitude when the LPO stimulus follows V by 75 ms. Note the similarities in response pattern and blocking latency between this cell and the cell in Figure 32, which was recorded from a different animal. Stimuli at arrows. Positive deflections up, negative down.

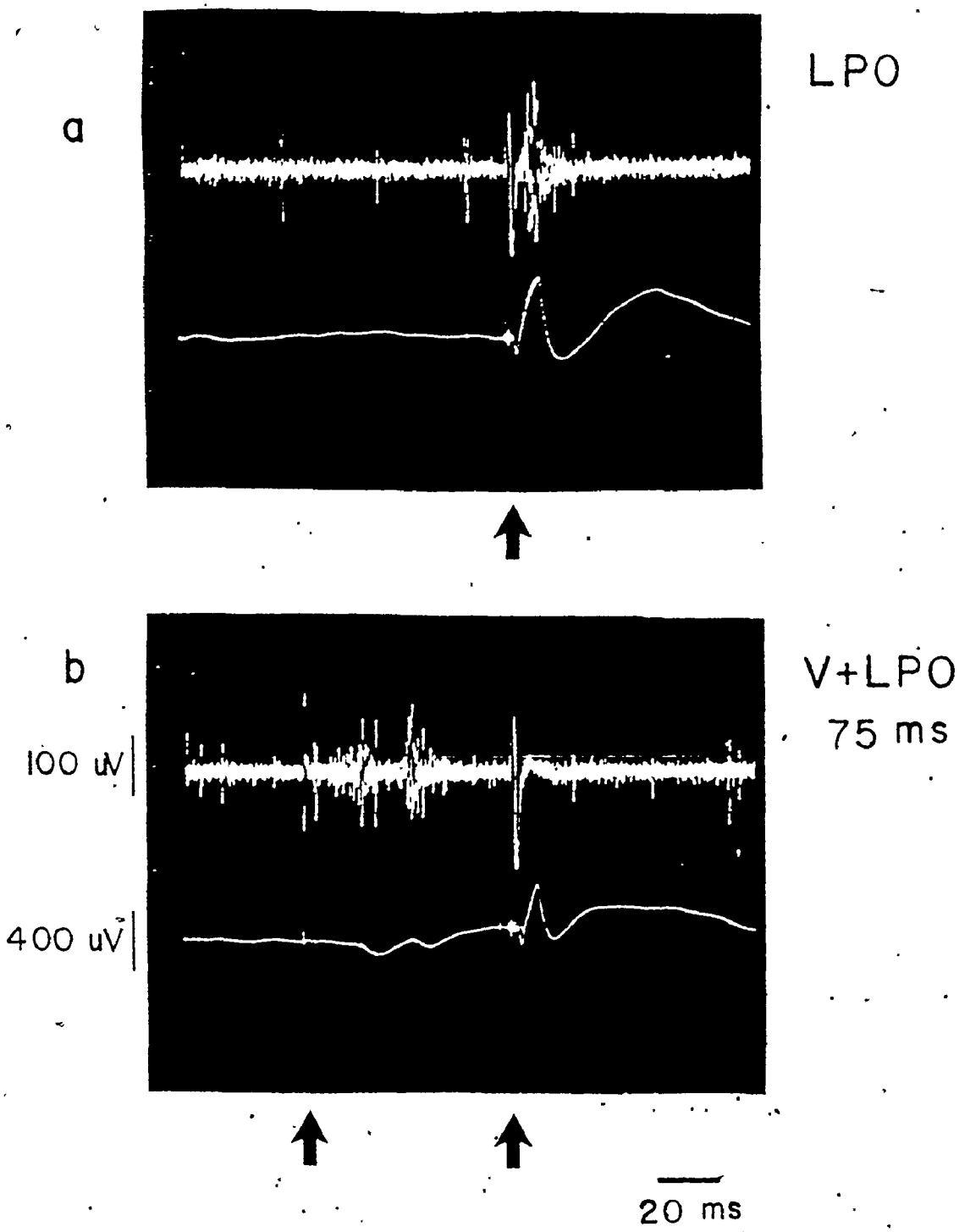



Figure 33

Figure 34

Blocking of V and A responses by LPO stimuli. a, V response of cell #75 is brief burst of spikes at about 25 ms. b, when V and LPO stimuli are presented simultaneously, the LPO stimulus evokes a typical response, but spikes do not occur at the expected time following the V stimulus. c, A response of cell #81 is a burst of spikes at a latency of about 8 ms. d, A response is blocked when it follows LPO response of this cell. Arrows at top of photograph mark occurrence of stimuli in top traces, arrows at bottom mark stimuli in bottom traces. Positive deflections up, negative down.

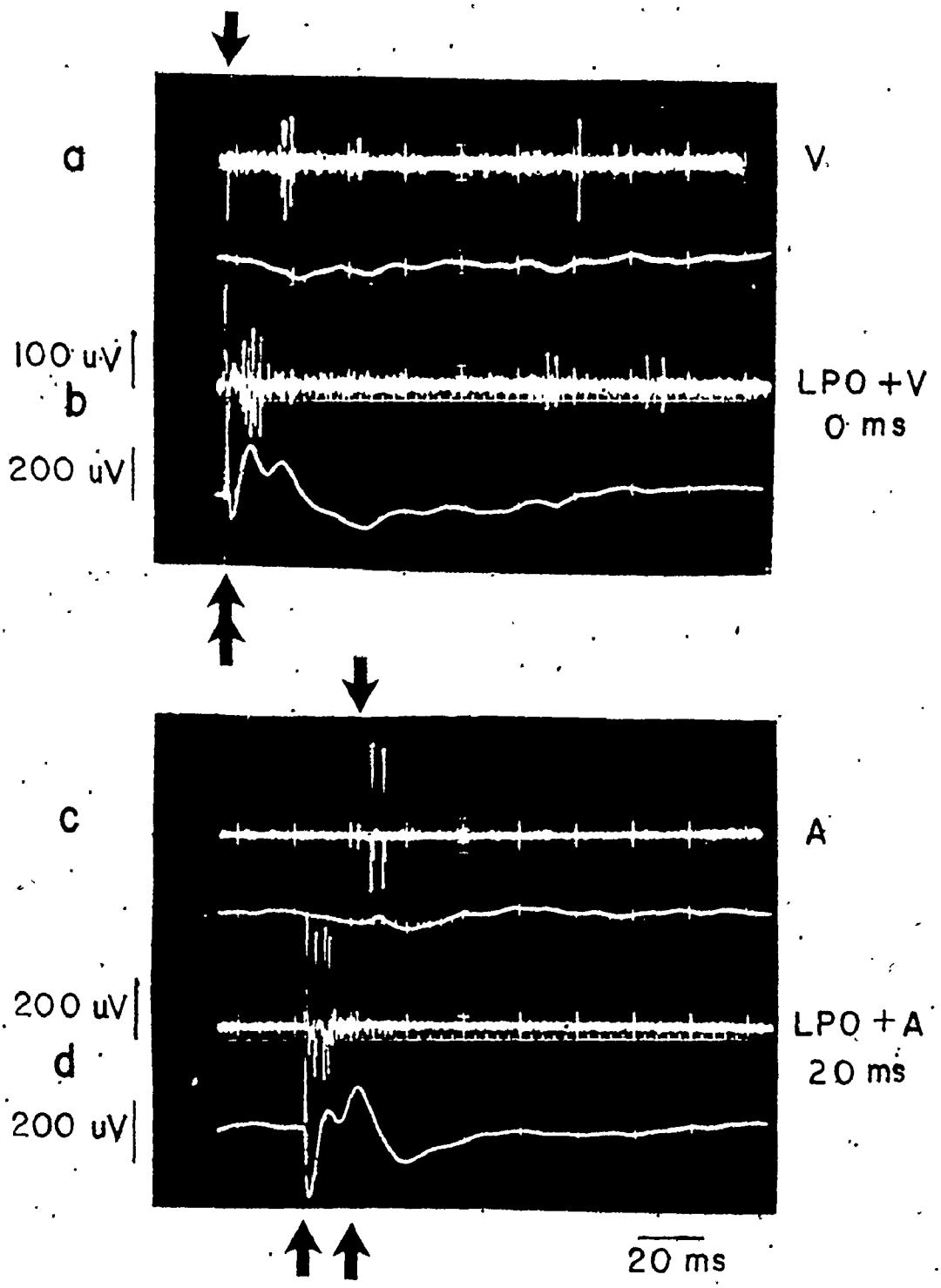



Figure 34

Figure 35

Blocking of V, MTH, and LPO responses by A stimuli (cell #93). This cell responded to each of the four stimuli with a burst of spikes at latencies of 8 - 15 ms. Responses to V, MTH, and LPO stimuli are shown in a, c, and e, respectively. Each of those responses is blocked when it follows an A response, as in b, d, and f. Each blocking interaction occurred at slightly different latencies and lasted for different periods of time. Note the close relationship of V and A spike responses to negative waves in the field potential. MTH and LPO field potentials are largely artifact. Arrows indicate occurrence of stimuli. Positive deflections up, negative down.

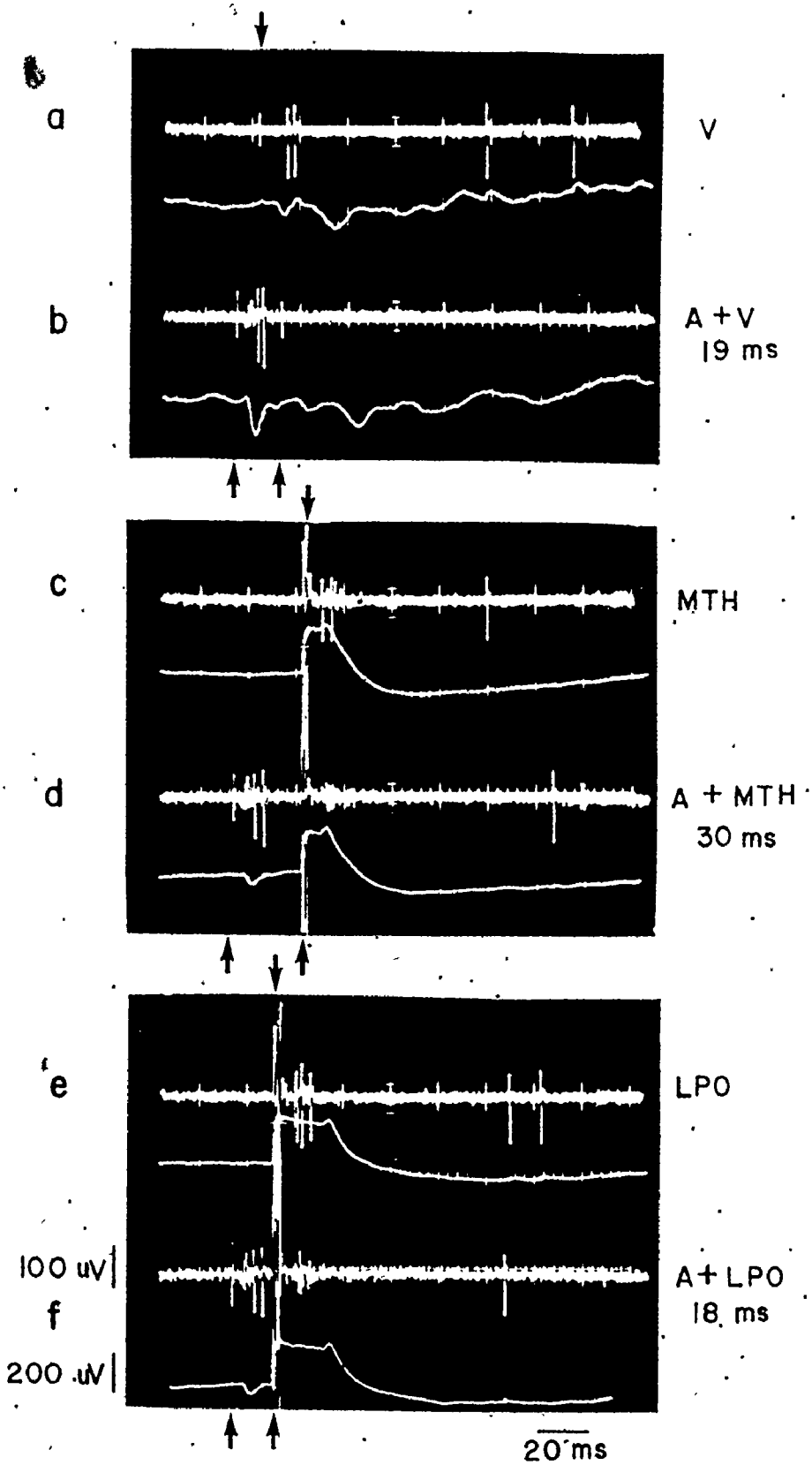



Figure 35

Figure 36

Blocking of LPO responses by V stimuli (cell #37). This interaction differs from the previous examples in that the cell does not exhibit action potentials in response to the conditioning stimulus (V), yet an LPO test response can still be blocked. a, absence of evoked and spontaneous spikes following V stimulus. b, unit and field potential response to LPO stimulus are intact when they follow V stimulus by 16 ms. c, the same LPO responses are blocked when they follow V by 20 ms. d, response to LPO stimulus applied alone. Note that in trace b, where LPO spikes are not yet into the blocking period following V, a second burst of spikes appears later in the record which does not occur to the LPO stimulus alone. Both facilitation of responding in trace b and blockade of responding in trace c, are evidence of subliminal excitatory and inhibitory influences from the visual system that are not manifest as spike activity. Stimuli at arrows. Positive deflections up, negative down.

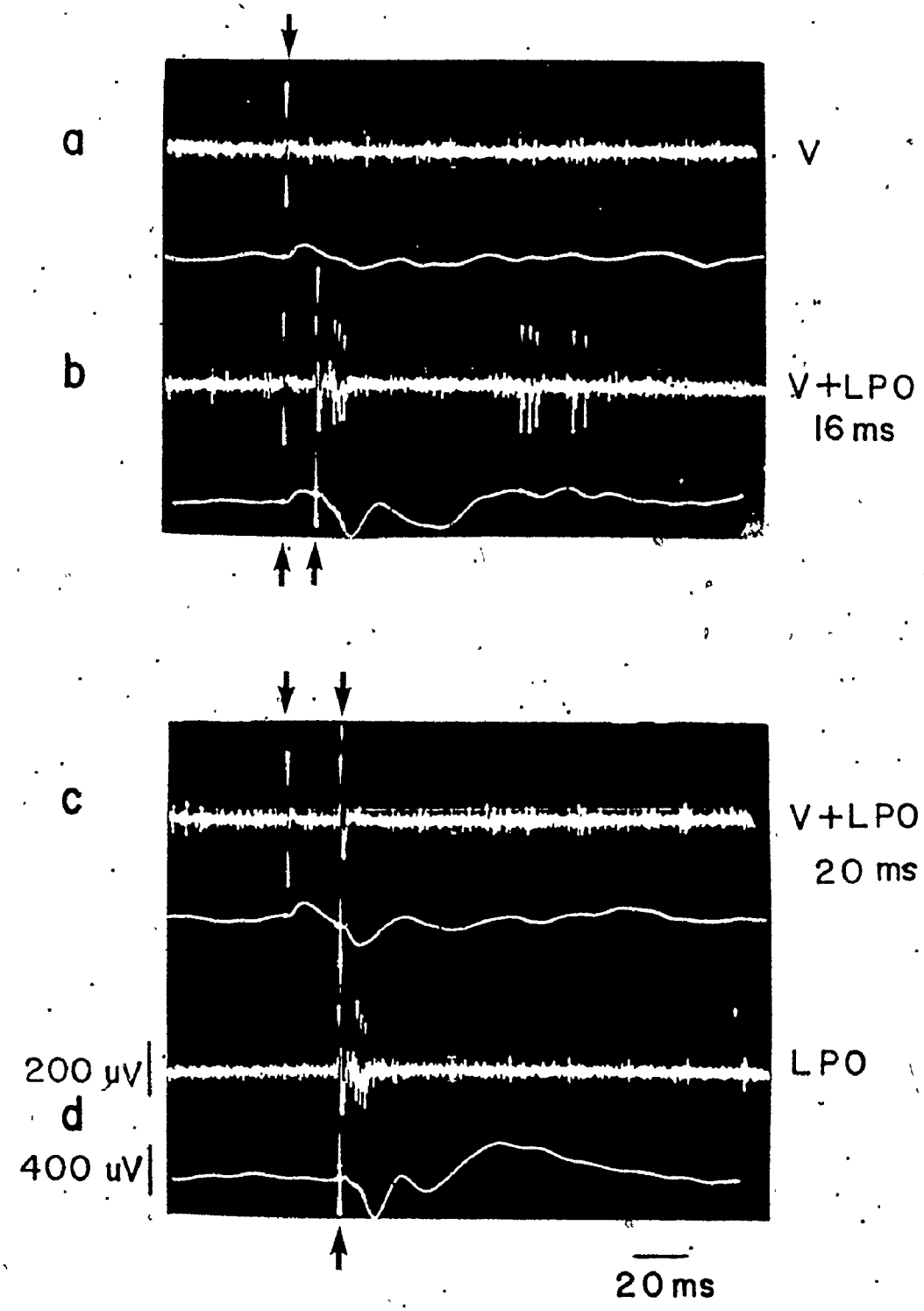



Figure 36

Figure 37

Blocking of LPO spindles by V stimuli (multiple unit recording #113). In each photograph, the top trace is the unit response and the middle trace is the field potential response. These two traces are synchronized and displayed during a slow sweep. The bottom trace is an expanded version of the first 100 ms of the unit activity displayed during a fast sweep so the spikes can be seen more clearly. The arrows refer to the occurrence of stimuli in the top two traces only. In the bottom trace, the stimulus occurs at the beginning of the sweep. a, response to V stimulus applied alone, bottom trace shows V spike response. b, response to LPO stimulus applied alone, the middle trace shows the evoked spindle and the bottom trace shows the LPO spike response, c, LPO is presented during spike response to V stimulus. LPO spindle (middle trace) and LPO spikes (bottom trace) are not yet blocked. d, LPO is presented after V spike response, i.e. during an apparent inhibitory period. The LPO spindle (middle trace) and spikes (bottom trace) are both completely blocked. e, at interstimulus delay of 320 ms, LPO spindle and spikes (bottom trace) are again evoked. f, in this photograph the order of stimuli is reversed. The V stimulus, presented 60 ms after the spindle has begun, is no longer effective in blocking it. Positive deflections up, negative down.

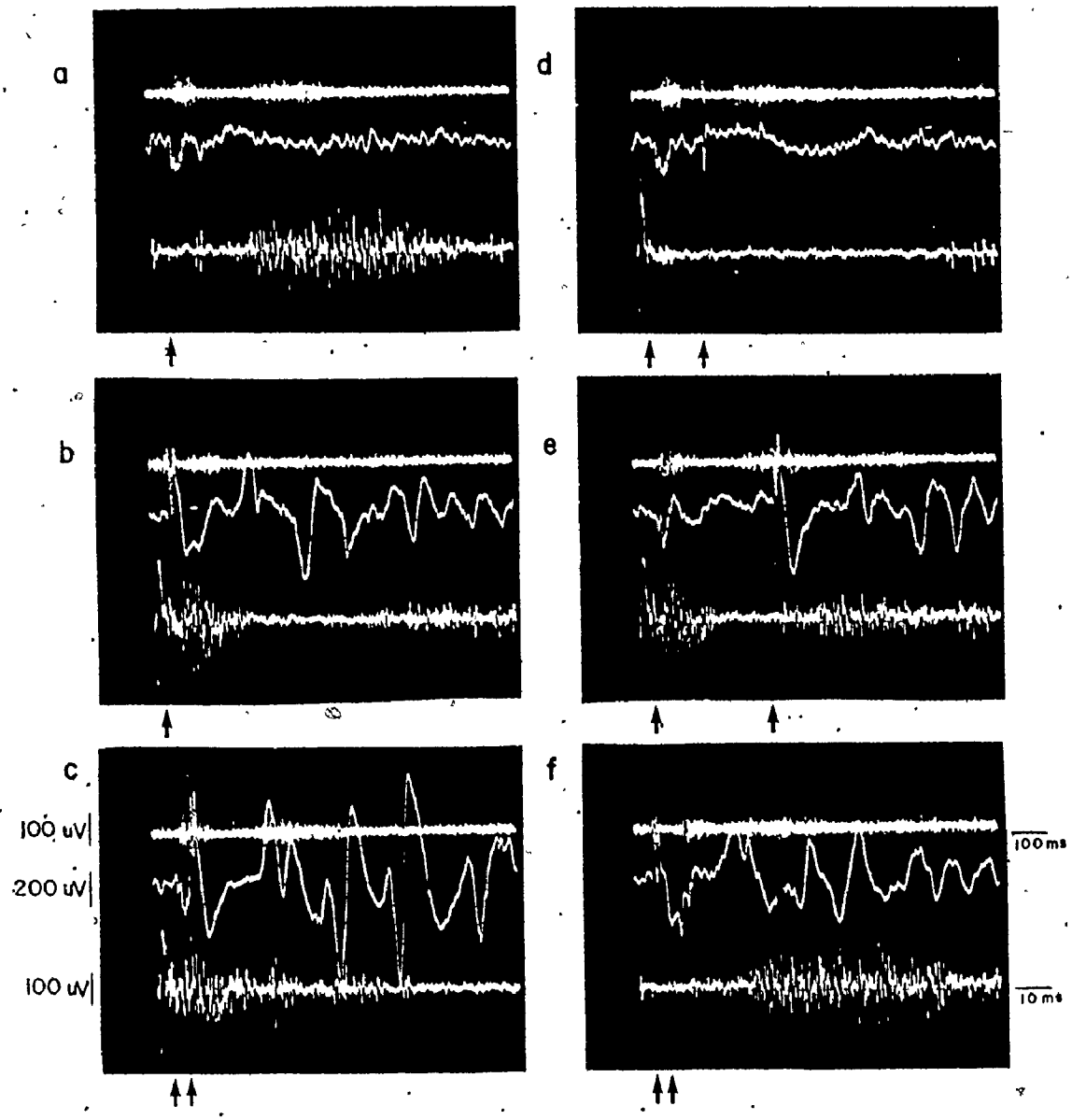



Figure 37

Figure 38

Summation of V and LPO inhibitory periods (cell #20). a, response to V stimulus. b, response to LPO stimulus. c, blocking of LPO unit response and delay of rebound excitation. (See text.) Stimuli at arrows. Positive deflections up, negative down.

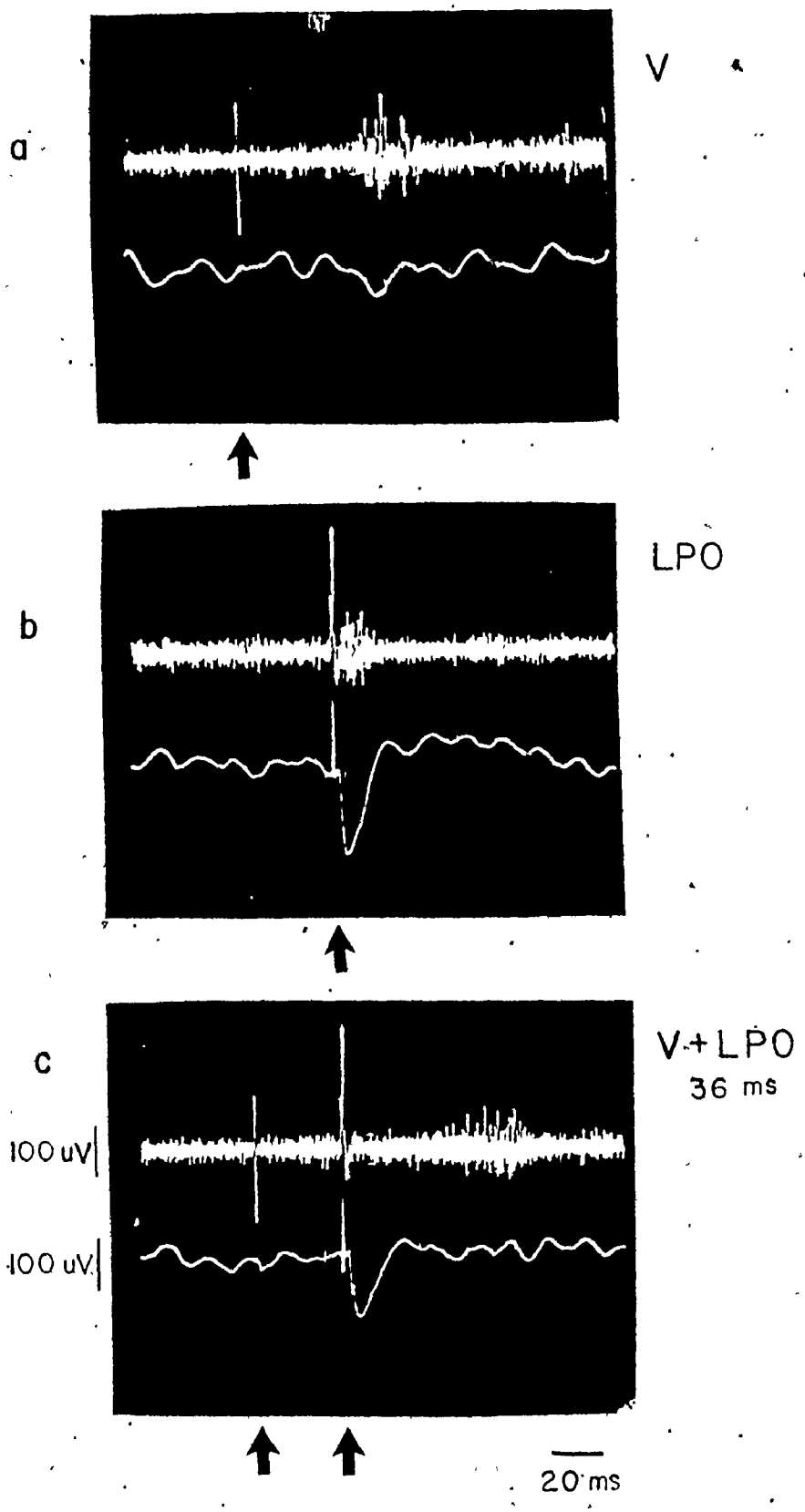



Figure 38

Figure 39

Reciprocal blocking interactions between V and MTH responses (cell #69). Response to V stimulus (a); response to MTH stimulus (b); decreased latency of MTH response and absence of second spike burst in V response at interstimulus interval of 12 ms (c); further decrease of MTH latency and continued absence of V response at 20 ms interval (d); inhibition of MTH response while V response is intact at 30 ms interval (e), coincidence of MTH and second V response at 40 ms interval (f); second period of inhibition when MTH stimulus follows second V response (g); continued inhibition of MTH response at 68 ms interval (h); partial recovery of MTH response at 98 ms interval (i); full recovery of MTH response at 112 ms interval (j). The arrow at bottom of page indicates the occurrence of the V stimulus in all traces. MTH stimuli are not marked, but can be identified by large stimulus artifact followed by a brief positive shift of the baseline at the delays indicated at the left side of the tracings.

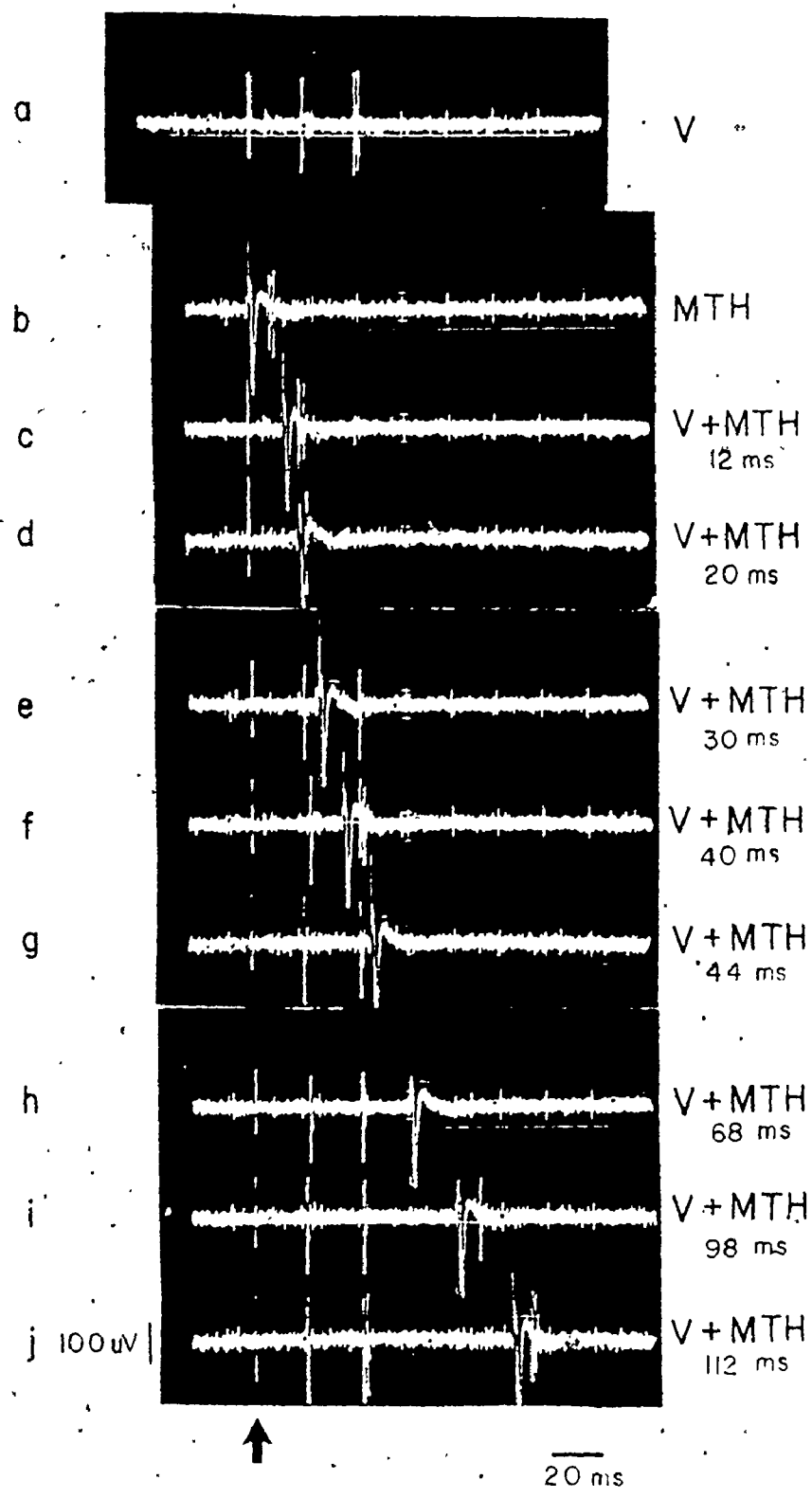

a
V
b
MTH
c
V+MTH
12 ms
d
V+MTH
20 ms
e
V+MTH
30 ms
f
V+MTH
40 ms
g
V+MTH
44 ms
h
V+MTH
68 ms
i
V+MTH
98 ms
j 100uV
V+MTH
112 ms
20 ms

Figure · 39

Figure 40

Poststimulus histograms (cell #43). a, response to V stimulus alone. b, response to LPO stimulus alone. c - k, responses to paired V and LPO stimuli. LPO stimulus always occurs at dotted line, location of V stimulus varies, and is indicated by arrow and circled "V". Note that spikes never occur between 20 and 30 ms after the LPO stimulus, i.e. just after the LPO spikes. Note also that the V response is greatly facilitated at longer latencies after the LPO spikes.

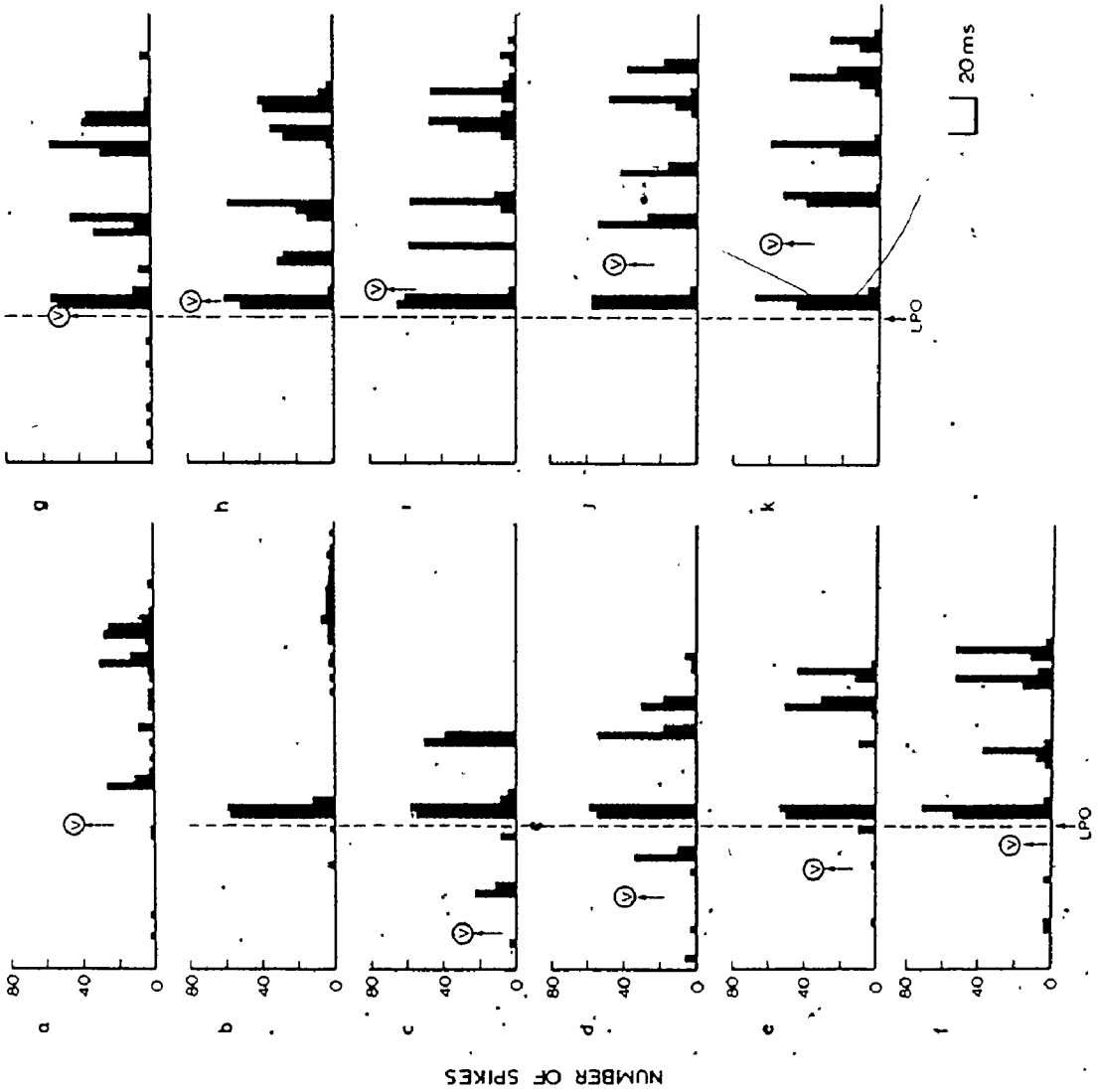



Figure 40

Figure 41

Changes in amplitude of V and LPO responses as a function of interstimulus interval (cell #43). a, each of the four peaks of the V response in Figure 40c - k was measured and expressed as a percentage of the corresponding control response in Figure 40a. The percentages are plotted here relative to the LPO stimulus and response displayed on the abscissa. It can be seen that each component of the V response is blocked as it falls during the brief inhibitory period following the LPO spike response, and facilitated at later delays. b, the same analysis of LPO response amplitude shows that it is slightly decreased when it occurs just after the first peak of the V response.

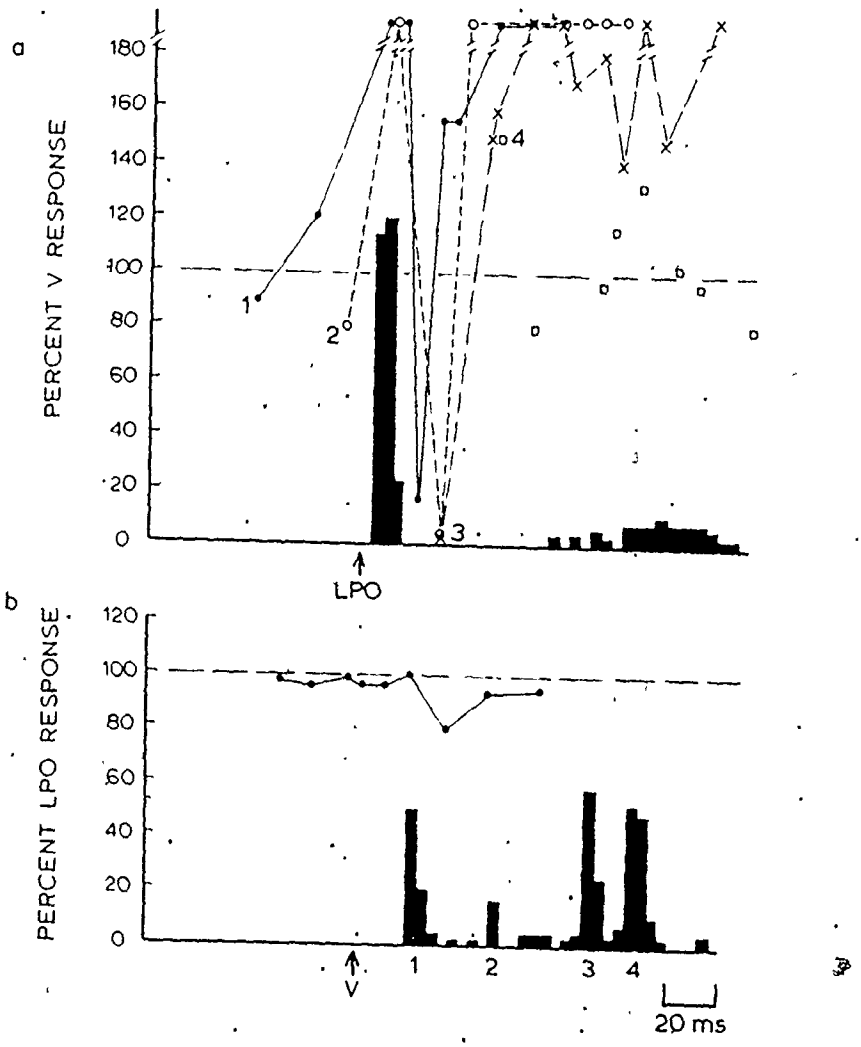

a
PERCENT V RESPONSE
180
160
140
120
100
80
60
40
20
0
1
2
3
4
LPO
b
PERCENT LPO RESPONSE
120
100
80
60
40
20
0
V
1
2
3
4
20 ms

Figure 41

Figure 42

Poststimulus histograms (cell #30). a, response to LPO stimulus. b, response to V stimulus. c - l, responses to paired V and LPO stimuli. LPO stimulus always occurs at dotted line, location of V stimulus indicated by arrow and circled "V". Note that no spikes occur for at least 15 ms following LPO spike response (except in part i).

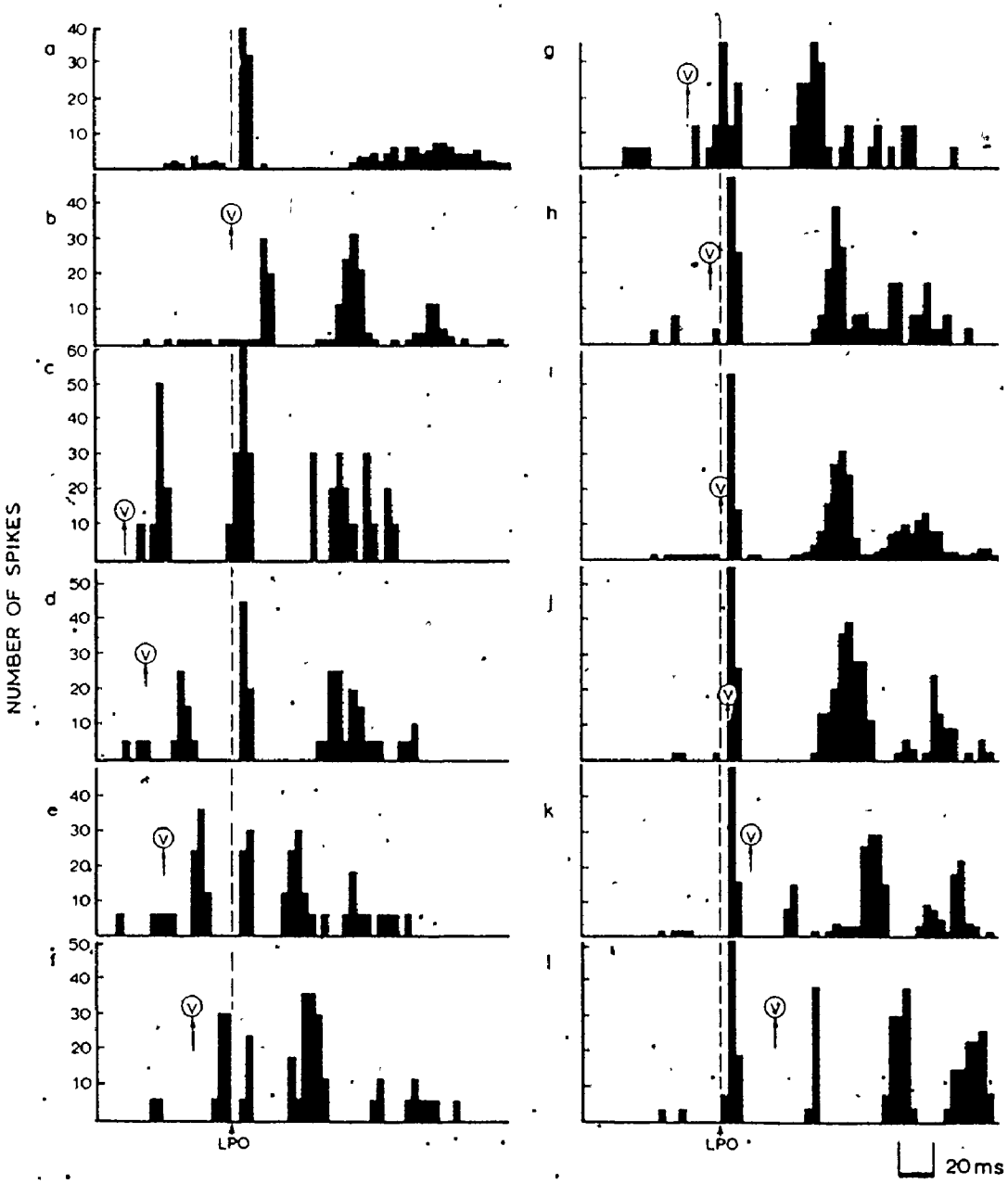



Figure 42

Figure 43

Changes in amplitude of V and LPO responses as a function of interstimulus interval (cell #30). a, V responses are blocked during a brief period immediately following LPO spike response. b, LPO response is blocked immediately following first peak of V response.

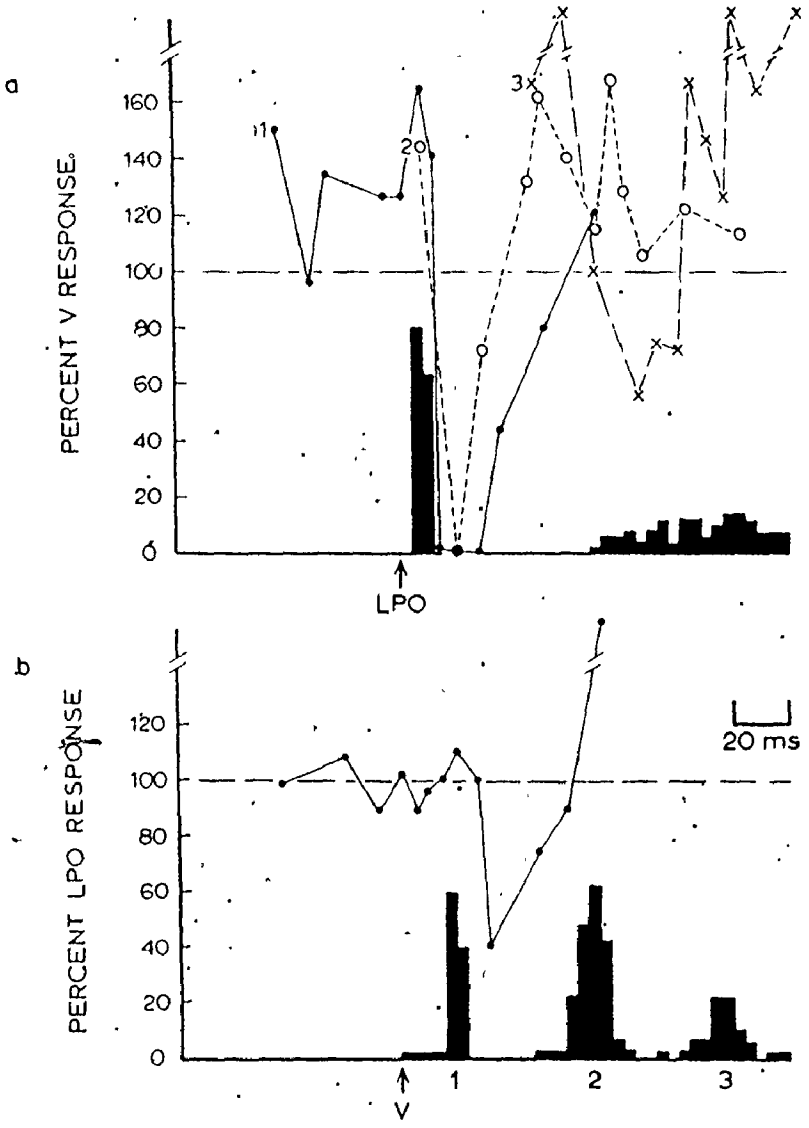

a
PERCENT V RESPONSE
160
140
120
100
80
60
40
20
0
1
2
3
LPO
b
PERCENT LPO RESPONSE
120
100
80
60
40
20
0
20 ms
V
1
2
3

Figure 43

Figure 44

Poststimulus histograms (cell #27). a, response to LPO stimulus. b, response to V stimulus. c - l, responses to paired V and LPO stimuli. LPO stimulus always occurs at dotted line, location of V stimulus indicated by arrow and circled "V". Blocking of V response by LPO stimulus is especially evident in parts e and f.

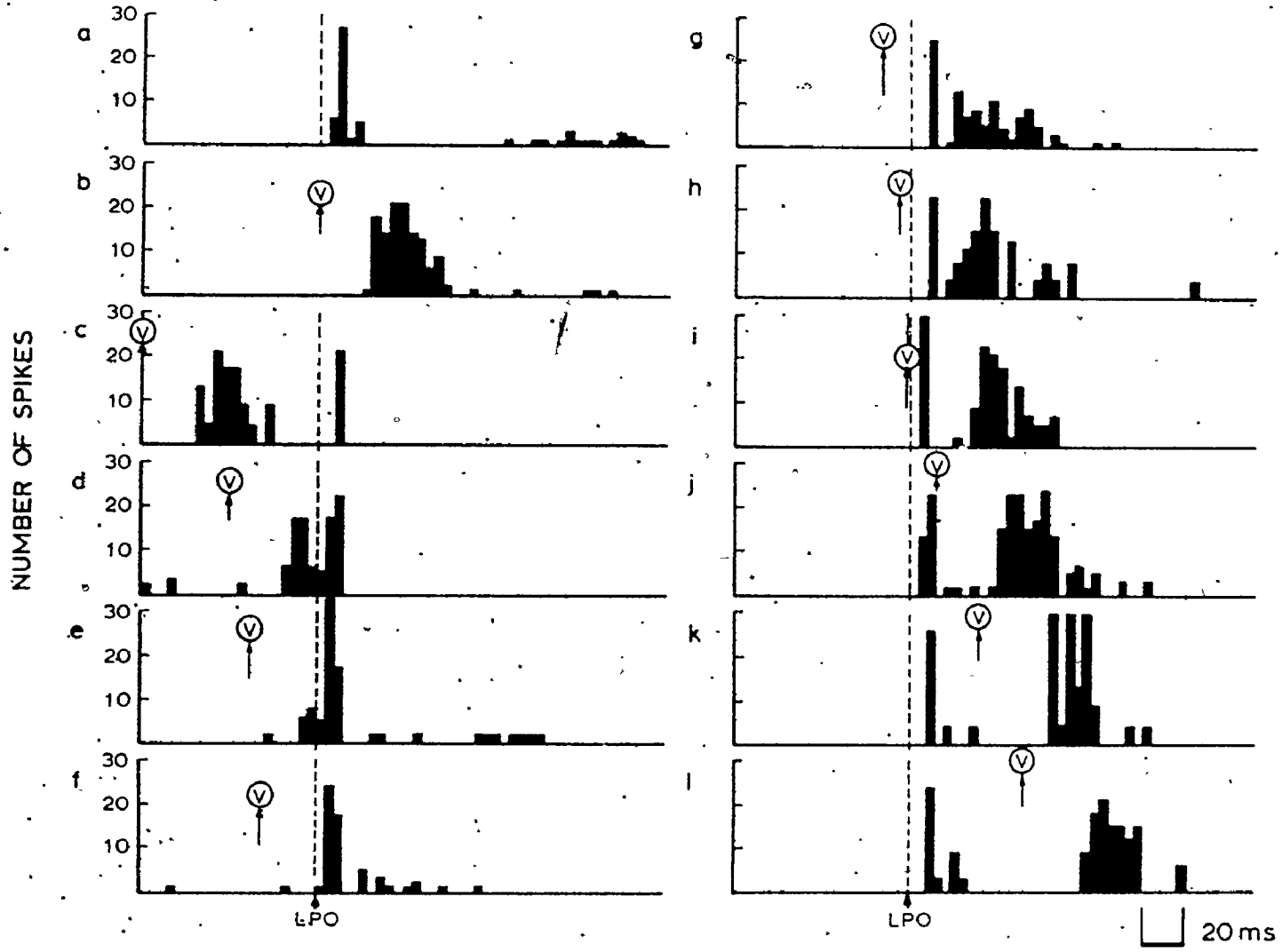



Figure 44

Figure 45

Changes in amplitude of V and LPO responses as a function of interstimulus interval (cell #27). As in previous two examples, V stimulus is reduced following LPO response (a) and LPO response is reduced following V response (b). In this cell, responses are less than 100% even before the second stimulus is applied; suggesting that the amplitude of the standard response decreased during the experiment.

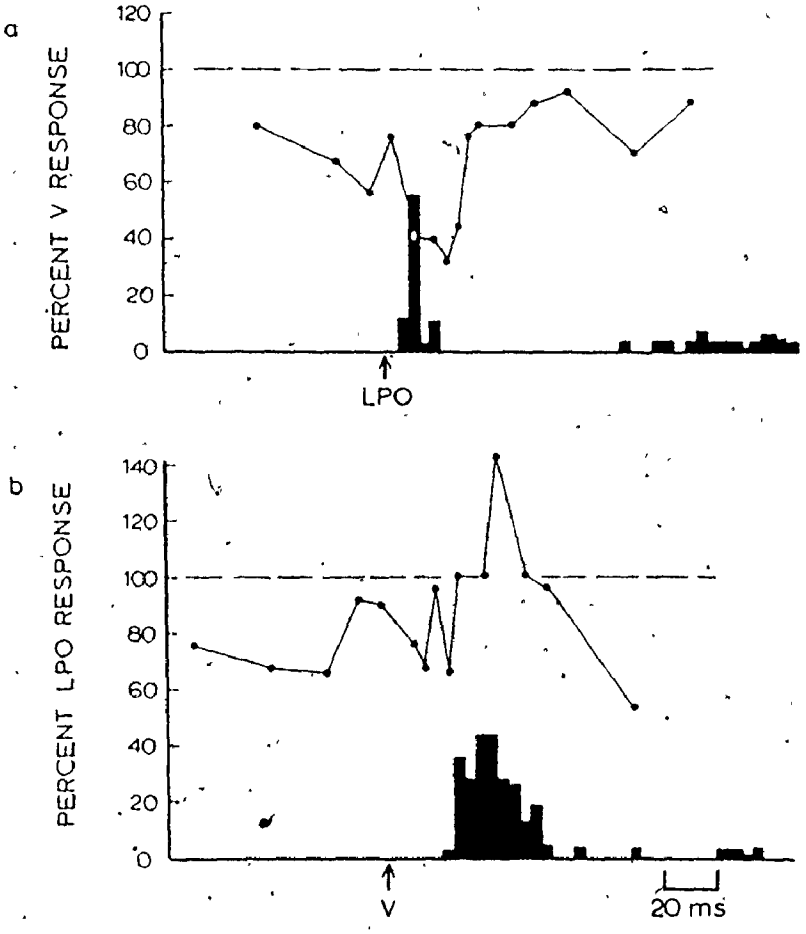

a
PERCENT V RESPONSE
120
100
80
60
40
20
0
LPO
b
PERCENT LPO RESPONSE
140
120
100
80
60
40
20
0
V
20 ms

Figure 45

Figure 46

Effects of repetitive LPO stimulation (cell #35). Unit and field potential responses at 5/sec (a); 6/sec (b); 8/sec (c); 10/sec (d); 13/sec (e); and 33/sec (f) stimulation. Note blockade of unit responses beginning in c, modifications of field potentials in each sequence, and constancy of the response to the first stimulus of each train regardless of the imposition of subsequent stimuli in f. Positive deflections up, negative down.

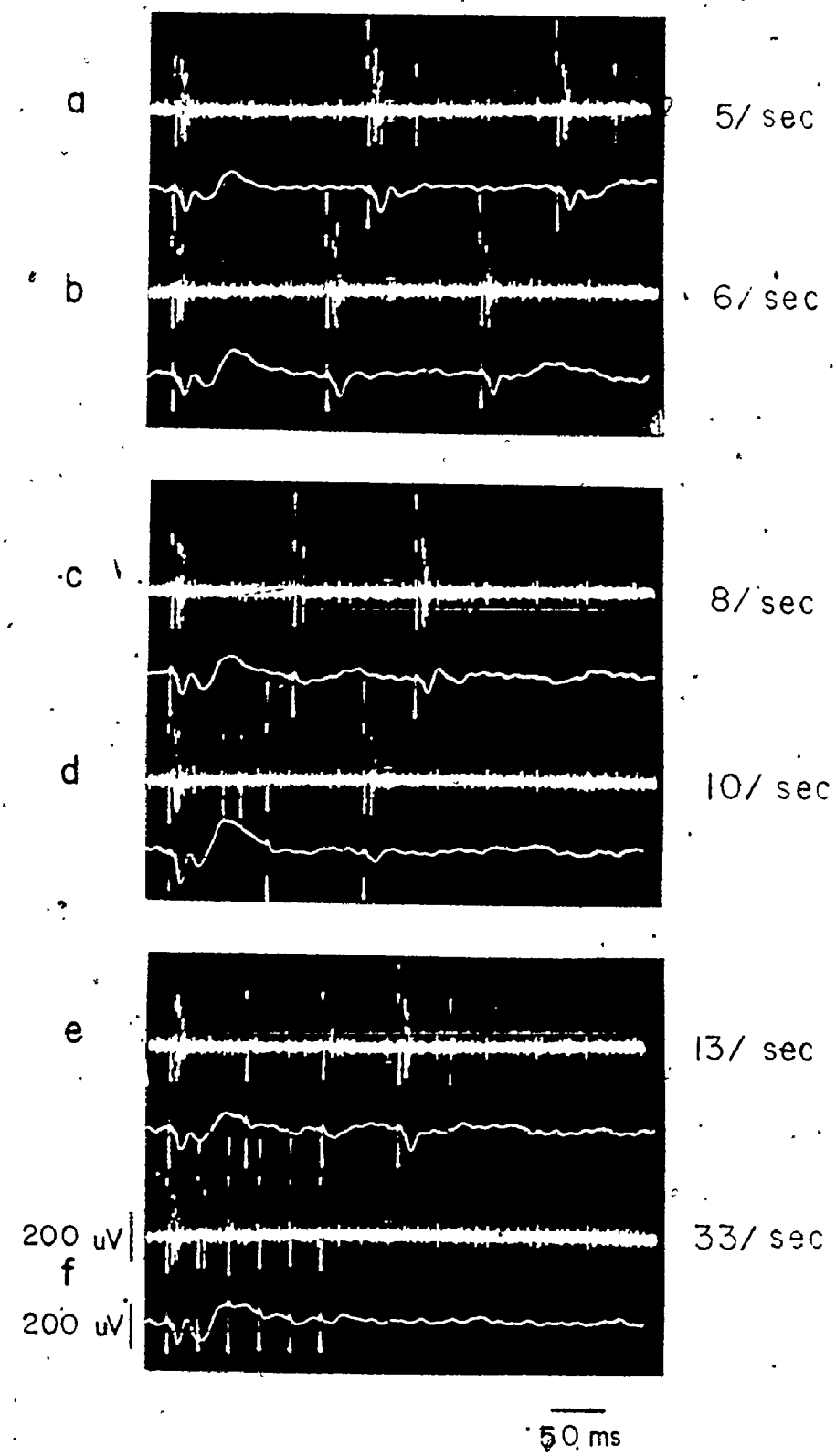



Figure 46

Figure 47

Schematic drawing of electronic circuitry for Experiment III.

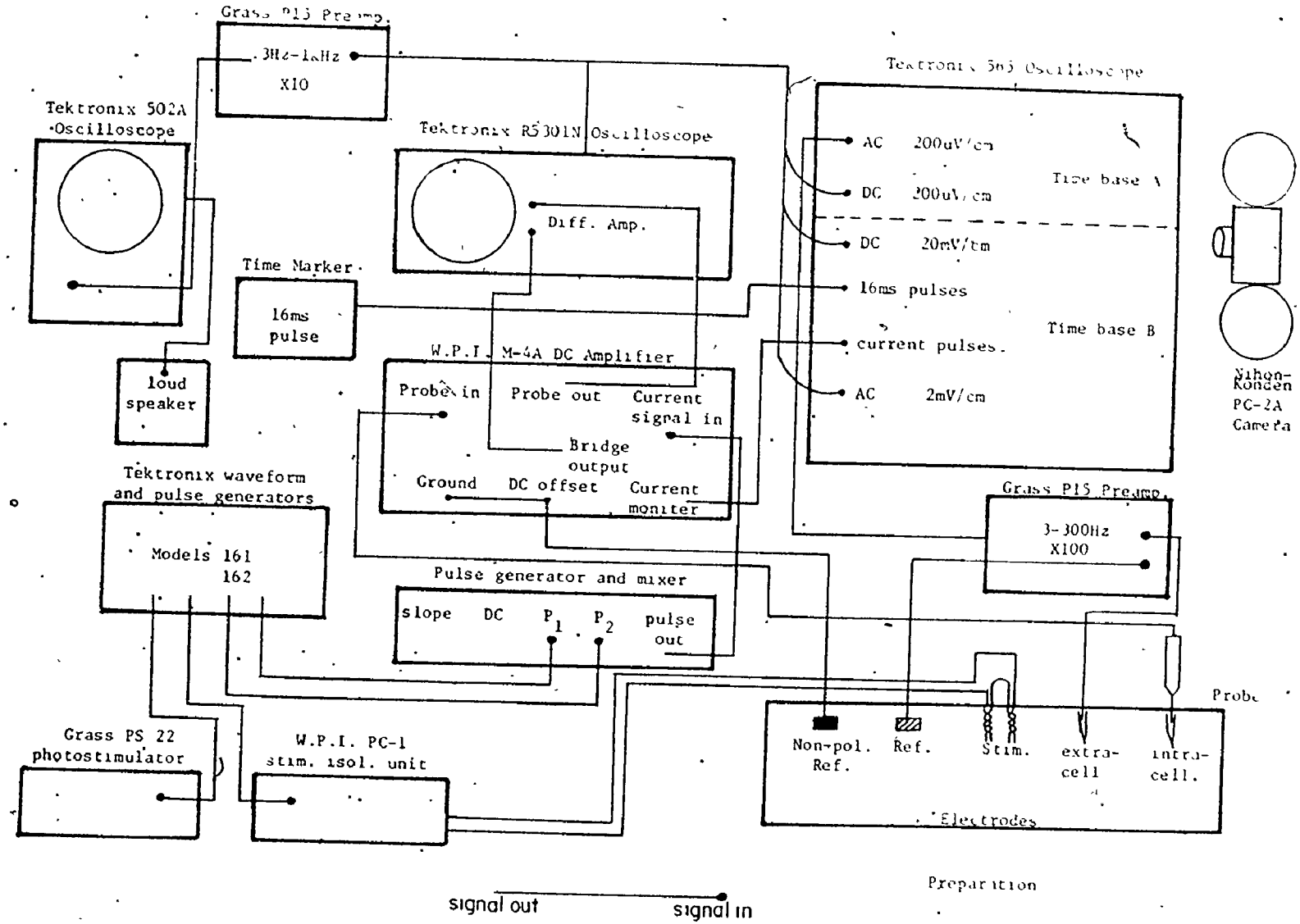

Grass P13 Preamp.
.3Hz-1kHz
X10
Tektronix 502A
Oscilloscope
Tektronix R5301N Oscilloscope
Diff. Amp.
Tektronix 565 Oscilloscope
AC 200uV/cm
DC 200uV/cm
Time base A
DC 20mV/cm
16ms pulses
Time base B
current pulses
AC 2mV/cm
Nihon-Konden
PC-2A
Camera
Time Marker
16ms
pulse
loud
speaker
W.P.I. M-4A DC Amplifier
Probe in
Probe out
Current
signal in
Bridge
output
Ground
DC offset
Current
monitor
Tektronix waveform
and pulse generators
Models 161
162
Grass P15 Preamp.
3-300Hz
X100
Pulse generator and mixer
slope
DC
P1
P2
pulse
out
Probe
Grass PS 22
photostimulator
W.P.I. PC-1
stim. isol. unit
Non-pol.
Ref.
Ref.
Stim.
extra-
cell
intra-
cell.
Electrodes
Preparation
signal out
signal in

Figure 47

Figure 48

Location of LPO stimulating electrodes. Frontal sections through the forebrain of the cat at A13, 15, and 16 (atlas of Jasper and Ajmone Marsan 1954). Placements indicated by black circles.

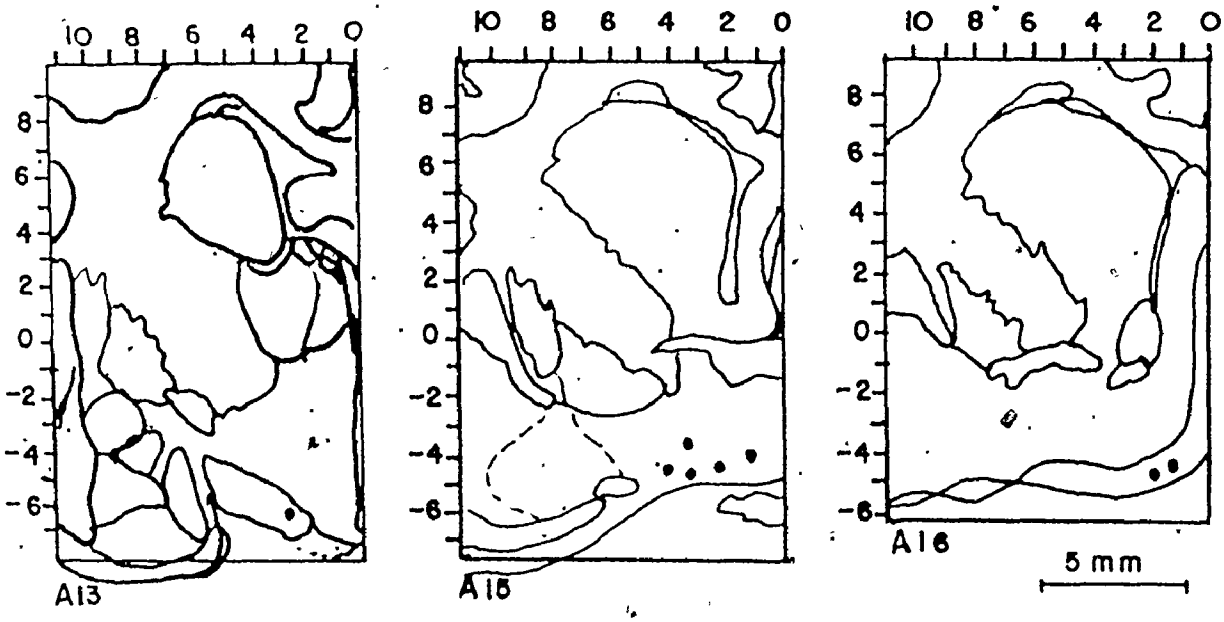



Figure 48

Figures 49 - 51

Figure 49a LPO response
Figure 49b LPO response
Figure 50a LPO response
Figure 50b LPO response
Figure 51 V response

Trace 1: reticular field potential
Trace 2: membrane potential and spikes at low gain
Trace 3: extracellular DC potential and time calibration pulse
Trace 4: membrane potential and spikes at high gain; the membrane potential is 20 mv/division more negative than extracellular potential (line 3)

Time scale for traces 1 and 2 is 100 ms/division. Time scale for traces 3 and 4 is given by 16 ms pulse in line 3.

Positive deflections up, negative down. Arrow above photograph indicates occurrence of stimulus in traces 1 and 2, arrow below indicates occurrence of same stimulus in traces 3 and 4.

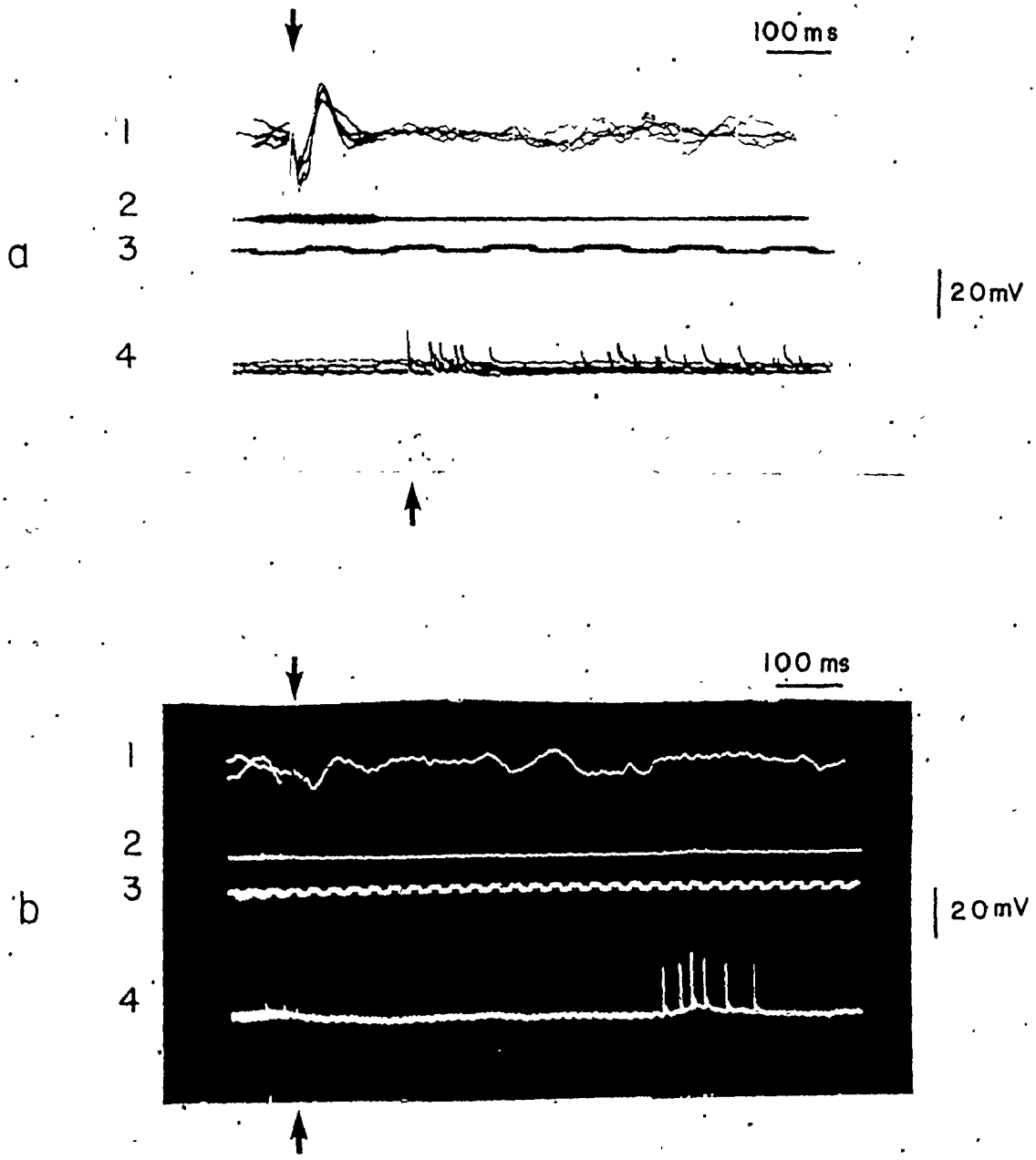



Figure 49

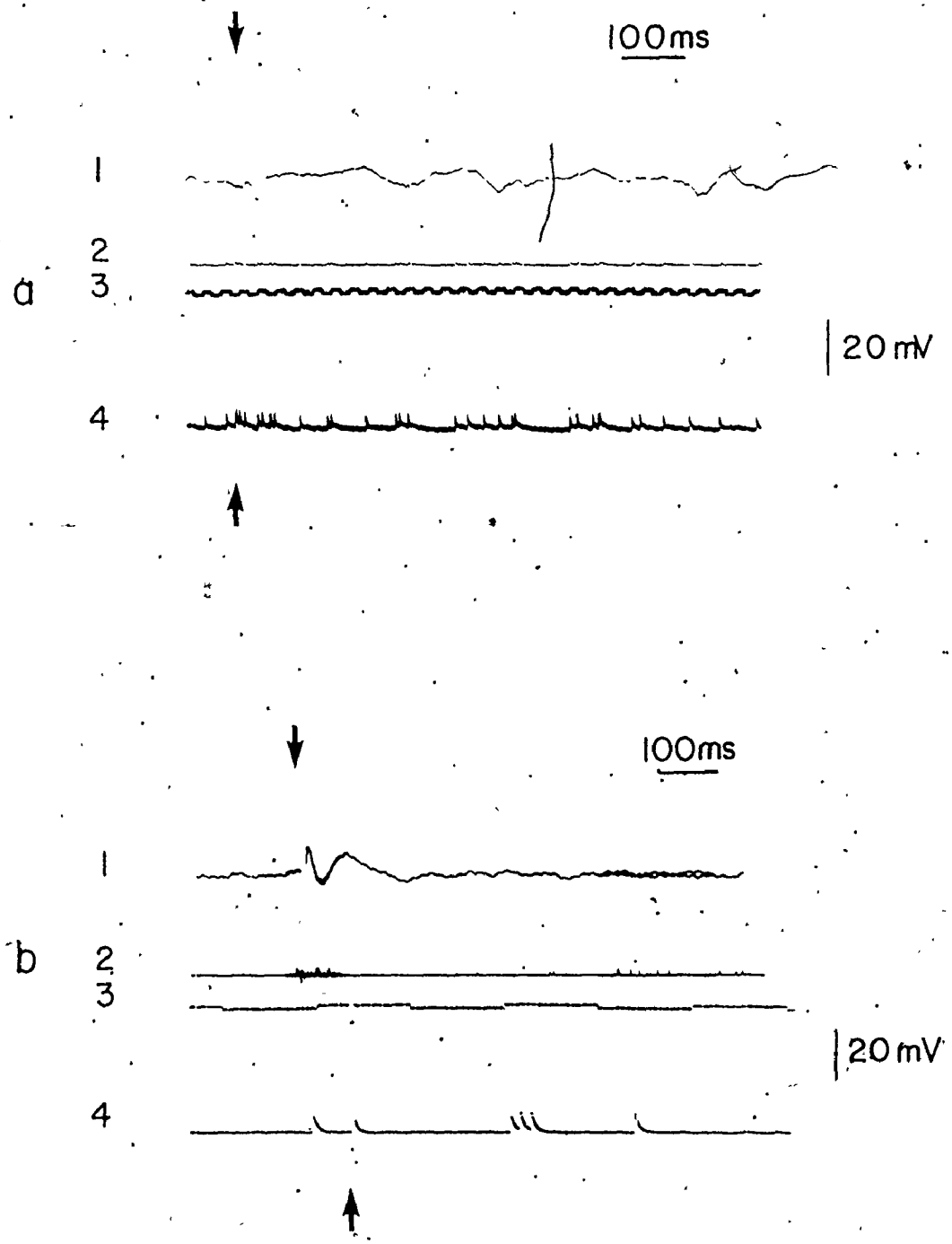



Figure 50

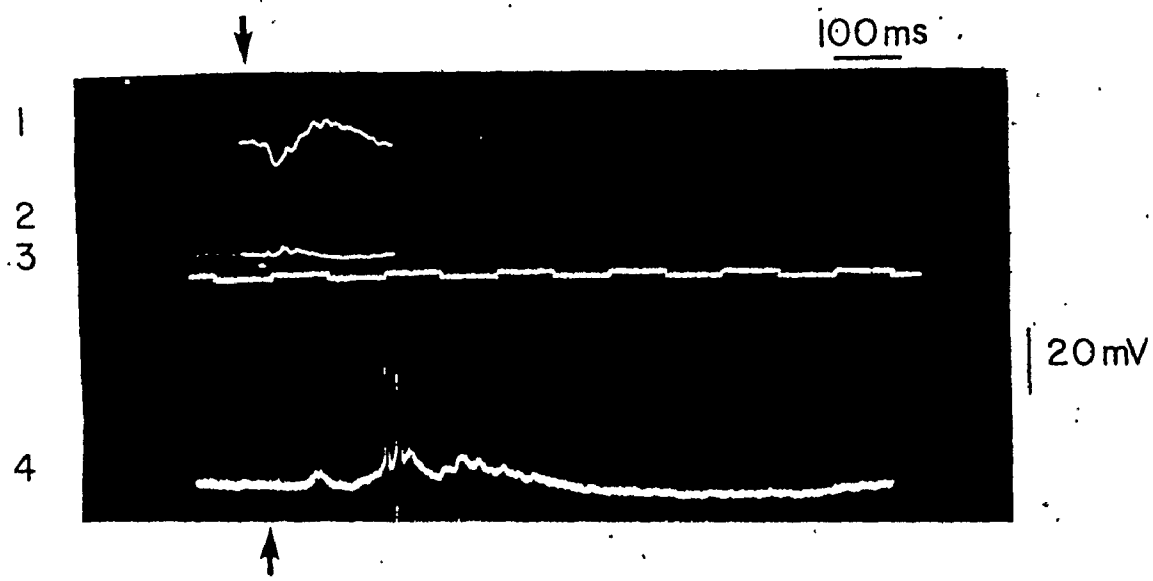



Figure 51

Figure 52

Spike responses to current pulses. a, percentage of current pulses that evoked spikes at each of several delays following a V stimulus. The V stimulus occurs at time 0, and the delay is relative to the onset of the current pulse. Also plotted are the latencies of the spikes relative to the onset of the current pulse. b, in another cell, the effectiveness of current pulses at different delays following an LPO stimulus. Each graph shows increases and decreases of membrane excitability seen in terms of likelihood and latency of evoked spikes (see text for details).

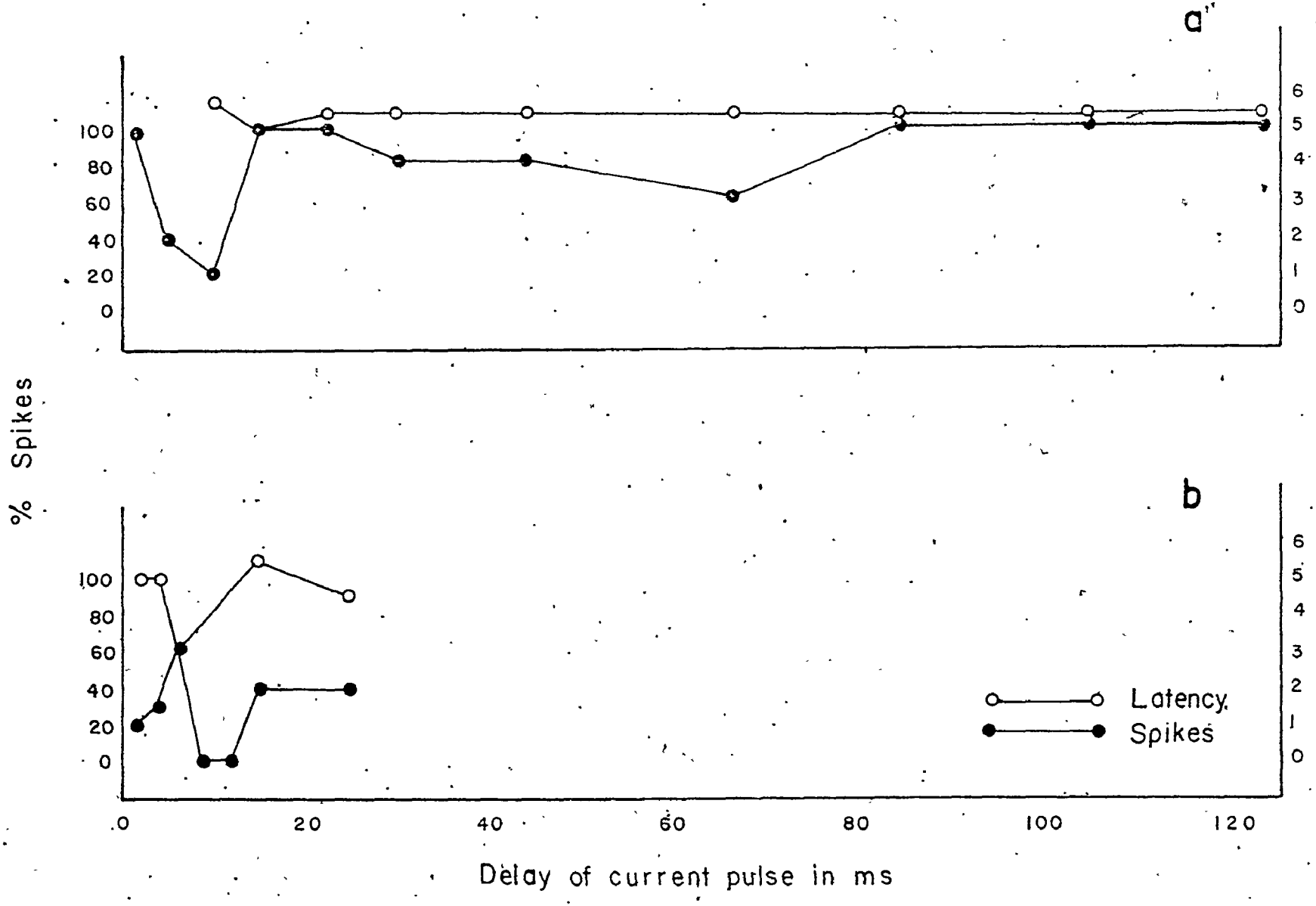

a
b
% Spikes
Latency of spikes in ms
100
80
60
40
20
0
6
5
4
3
2
1
0
.0
20
40
60
80
100
120
Latency
Spikes
Delay of current pulse in ms

Figure 52

Table 1

Schedule of stimulation and control trials. MTH, medial thalamic stimulation; LPO, lateral preoptic stimulation; Hz, Hertz; mA, milliamperes; v, volts; C, control trials. For each subject, the type and number of trials presented each day of the experiment are given. Trials in session 1 (morning) were separated by 10 minutes, and trials in session 2 (afternoon) were separated by 10 minutes. Session 1 and session 2 were separated by one hour. Usually stimulation and control conditions were counterbalanced on two consecutive days, and the number of stimulation and control trials per day were equal.

Table 1

Schedule of Stimulation and Control Trials

| Subject | Day | Session 1 Condition | Session 1 Number of Trials | Session 2 Condition | Session 2 Number of Trials |
|---|---|---|---|---|---|
| M14 | 1 | C | (4) | | |
| | 2 | MTH, 6Hz, .2mA, 6v | (4) | | |
| | 3 | C | (4) | | |
| | 4 | MTH, 6Hz, .2mA, 6v | (4) | | |
| | 5 | C | (4) | | |
| | 6 | MTH, 6Hz, .4mA, 8v | (4) | | |
| | 7 | C | (4) | | |
| | 8 | MTH, 6Hz, .5mA, 8v | (4) | | |
| | 9 | LPO, 150Hz, .05mA, 1v | (4) | C | (4) |
| | 10 | C | (4) | LPO, 150Hz, .05mA, 1v | (4) |
| | 11 | LPO, 6Hz, .05mA, 1v | (4) | C | (4) |
| | 12 | C | (4) | LPO, 6Hz, .06mA, 1v | (4) |
| | 13 | LPO, 6Hz, .15mA, 2.5v | (4) | C | (4) |
| | 14 | C | (4) | LPO, 6Hz, .15mA, 2.5v | (4) |
| | 15 | C | (3) | | |
| | 16 | LPO, 150Hz, .2mA, 2.5v | (4) | C | (1) |
| | 17 | C | (2) | LPO, 150Hz, .2mA, 2.5v | (2) |
| M15 | 1 | C | (4) | MTH, 6Hz, .2mA, 3v | (4) |
| | 2 | MTH, 6Hz, .2mA, 4v | (4) | C | |
| | 3 | C | | MTH, 6Hz, .5mA, 6v | (4) |
| | 4 | MTH, 6Hz, .5mA, 1v | (4) | C | (4) |
| | 5 | C | (4) | MTH, 6Hz, .4mA, 6v | (4) |
| | 6 | MTH, 6Hz, .4mA, 6v | (4) | C | (4) |
| | 7 | LPO, 6Hz, .05mA, 3v | (4) | C | (4) |
| | 8 | C | | LPO, 6HZ, .05mA, 3v | (4) |
| | 9 | LPO, 6Hz, .2mA, 7v | (2) | C | (2) |
| | 10 | C | (4) | LPO, 6Hz, .2mA, 7v | (4) |
| | 11 | LPO, 6Hz, .2mA, 7v | (2) | C | (2) |
| | 12 | LPO, 150Hz, .05mA, 2.5v | (3) | C | (3) |
| | 13 | C | (3) | LPO, 150Hz, .2mA, 6v | (3) |
| M16 | 1 | C | (3) | MTH, 6Hz, .05mA, 4v | (3) |
| | 2 | MTH, 6Hz, .05mA, 3v | (3) | C | (3) |
| | 3 | C | | MTH, 6Hz, .1mA, 5v | (3) |
| | 4 | MTH, 6HZ, .1mA, 5v | (3) | C | (3) |
| | 5 | C | | MTH, 6Hz, .2mA, 8v | (3) |
| | 6 | MTH, 6Hz, .2mA, 8v | (3) | C | (3) |
| | 7 | C | (3) | LPO, 6Hz, .05mA, 4v | (3) |
| | 8 | LPO, 6Hz, .05mA, 4v | (3) | C | (3) |
| | 9 | C | (3) | LPO, 6Hz, .2mA, 8v | (3) |
| | 10 | LPO, 6Hz, .4mA, 16v | (3) | C | (3) |
| | 11 | C | (3) | LPO, 6Hz, .4mA, 16v | (3) |
| | 12 | LPO, 150Hz, .05mA, 2v | (3) | C | (3) |
| | 13 | C | (3) | LPO, 150Hz, .05mA, 2v | (3) |
| | 14 | LPO, 150Hz, .2mA, 8v | (3) | C | (3) |
| | 15 | C | (3) | LPO, 150Hz, .2mA, 8v | (3) |
| | 16 | LPO, 6Hz, .2mA, 10v | (3) | C | (3) |

| Subject | Day | Session 1 | | Session 2 | |
|---|---|---|---|---|---|
| M17 | 1 | MTH, 6Hz, .05mA, 1.5v | (3) | C | (3) |
| | 2 | C | (3) | MTH, 6Hz, .05mA, 2v | (3) |
| | 3 | MTH, 6Hz, .1mA, 5v | (3) | C | (3) |
| | 4 | C | (3) | MTH, 6Hz, .1mA, 3v | (3) |
| | 5 | MTH, 6Hz, .2mA, 5v | (3) | C | (3) |
| | 6 | C | (3) | MTH, 6Hz, .2mA, 5v | (3) |
| | 7 | LPO, 6Hz, .05mA, 2v | (3) | C | (3) |
| | 8 | C | (3) | LPO, 6Hz, .05mA, 1.5v | (3) |
| | 9 | LPO, 6Hz, .2mA, 3v | (3) | C | (3) |
| | 10 | C | (3) | LPO, 6Hz, .4mA, 8v | (3) |
| | 11 | LPO, 6Hz, .4mA, 8v | (3) | C | (3) |
| | 12 | C | (3) | LPO, 150Hz, .05mA, 2v | (3) |
| | 13 | LPO, 150Hz, .05mA, 2v | (3) | C | (3) |
| | 14 | C | (3) | LPO, 150Hz, .2mA, 3v | (3) |
| | 15 | LPO, 150Hz, .2mA, 3v | (3) | C | (3) |
| | 16 | LPO, 6Hz, .2mA, 4v | (3) | C | (3) |
| M18 | 1 | LPO, 6Hz, .09mA, .9v | (3) | C | (3) |
| | 2 | C | (3) | LPO, 6Hz, .09mA, .9v | (3) |
| | 3 | LPO, 6Hz, .09mA, .9v | (3) | C | (3) |
| | 4 | C | (3) | LPO, 6Hz, .09mA, .9v | (3) |
| | 5 | LPO, 6Hz, .09mA, .9v | (3) | C | (3) |
| | 6 | C | (3) | MTH, 6Hz, .1mA, 1.4v | (3) |
| | 7 | MTH, 6Hz, .1mA, 1.4v | (2) | C | (2) |
| | 8 | C | (4) | MTH, 6Hz, .1mA, 1.3v | (4) |
| | 9 | MTH, 6Hz, .1mA, 1.4v | (3) | C | (3) |
| | 10 | C | (3) | MTH, 6Hz, .1mA, 1.4v | (3) |
| | 11 | LPO, 150Hz, .09mA, .8v | (4) | C | (4) |
| | 12 | C | (4) | LPO, 150Hz, .09mA, .8v | (4) |
| | 13 | LPO, 150Hz, .09mA, .8v | (4) | C | (4) |
| | 14 | C | (2) | | |
| | 15 | MTH, 6Hz, .1mA, 7v | (3) | | |
| | 16 | C | (3) | | |
| | 17 | C | (3) | MTH, 6Hz, .1mA, .8v | (4) |
| M19 | 1 | LPO, 6Hz, .09mA, .9v | (3) | C | (3) |
| | 2 | C | (3) | LPO, 6Hz, .09mA, .9v | (3) |
| | 3 | LPO, 6Hz, .09mA, .9v | (3) | C | (3) |
| | 4 | C | (3) | LPO, 6Hz, .09mA, .9v | (3) |
| | 5 | LPO, 6Hz, .09mA, .9v | (3) | C | (3) |
| | 6 | C | (2) | MTH, 6Hz, .1mA, 1.2v | (2) |
| | 7 | MTH, 6Hz, .1mA, 1.2v | (4) | C | (4) |
| | 8 | C | (3) | MTH, 6Hz, .1mA, 1.2v | (3) |
| | 9 | MTH, 6Hz, .1mA, 1.2v | (3) | C | (3) |
| | 10 | C | (3) | MTH, 6Hz, .1mA, 1.2v | (3) |
| | 11 | LPO, 150Hz, .09mA, .6v | (4) | C | (4) |
| | 12 | C | (4) | LPO, 150Hz, .09mA, .6v | (4) |
| | 13 | LPO, 150Hz, .09mA, .6v | (2) | | |
| | 14 | C | (3) | MTH, 6Hz, .1mA, 1v | (3) |
| | 15 | MTH, 6Hz, .1mA, 1v | (3) | C | (3) |
| | 16 | C | (4) | MTH, 6Hz, .1mA, 1v | (3) |
| M36 | 1 | MTH, 6Hz, .2mA, 6v | (2) | C | (1) |
| | 2 | C | (4) | MTH, 6Hz, .2mA, 6v | (5) |
| | 3 | MTH, 6Hz, .2mA, 10v | (2) | C | (1) |

Table 2

Friedman two-way analyses of variance. There were no significant differences ($\alpha$ = .05) in the mean latency or mean duration of SWS sleep under six stimulation conditions (Fr = 4.43 and Fr = 9.0), under six control conditions (Fr = 6.57 and Fr = 5.6), or under all 12 stimulation and control conditions (Fr = 13.1 and 13.0).

Table 2

Analyses of Variance

| | Latency | | Duration | |
|---|---|---|---|---|
| | df | Fr | df | Fr |
| Stimulation Condition | 5 | 4.43* | 5 | 9.0 * |
| Control Condition | 5 | 6.57* | 5 | 5.6 * |
| Stimulation and Control Conditions | 11 | 13.1 * | 11 | 13.0 * |

* P > .05

Table 3

Wilcoxon matched pairs tests. MTH, medial thalamus; LPO, lateral preoptic area; Hz, hertz; mA, milliamperes, N, number of trials, T, value of Wilcoxen matched pairs test; S, sign of difference between mean stimulation latency (or duration) and mean control latency (or duration): + , mean control latency (duration) is longer; -, mean stimulation latency (duration) is longer.

Four tests produced T values having a probability $\leq .05$ (*). In one case, the animal fell asleep significantly sooner on stimulation trials; in the other three, the animals fell asleep significantly sooner on control trials.

Table 3

Wilcoxon Matched Pairs Tests

| Subject | Stimulus | | | | Latency | | | Duration | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Site | F(Hz) | I(mA) | V(volts) | N | T | S | N | T | S |
| M14 | MTH | 6 | .20 | 4 | 8 | 1.5* | + | 8 | 13 | - |
| | MTH | 6 | .40 | 6 | 8 | 17.5 | - | 8 | 6 | + |
| | LPO | 6 | .05 | 1 | 7 | 6 | + | 7 | 12.5 | - |
| | LPO | 6 | .15 | 2.5 | 8 | 6.5 | + | 7 | 12 | + |
| | LPO | 150 | .05 | 1 | 7 | 6 | + | 7 | 11.5 | + |
| | LPO | 150 | .20 | 3 | - | ---- | - | - | ---- | + |
| M15 | MTH | 6 | .05 | 11 | 8 | 13 | - | 7 | 7 | - |
| | MTH | 6 | .20 | 4 | 8 | 12 | + | 8 | 10 | + |
| | MTH | 6 | .40 | 6 | 8 | 12 | - | 6 | 4 | + |
| | LPO | 6 | .05 | 3 | 7 | 0* | - | 8 | 15 | - |
| | LPO | 6 | .20 | 7 | 7 | 6 | - | 6 | 10.5 | - |
| | LPO | 150 | .05 | 2 | 6 | 5 | - | 6 | 7 | - |
| | LPO | 150 | .20 | 6 | - | ---- | - | - | ---- | - |
| M16 | MTH | 6 | .05 | 3 | 6 | 3 | - | 4 | 1 | + |
| | MTH | 6 | .10 | 5 | 6 | 6.5 | - | 5 | 5 | + |
| | MTH | 6 | .20 | 7 | 6 | 8 | - | 6 | 1.5 | - |
| | LPO | 6 | .05 | 4 | 6 | 10 | + | 6 | 4 | + |
| | LPO | 6 | .20 | 8 | 6 | 0* | - | 6 | 9 | - |
| | LPO | 6 | .40 | 16 | 6 | 10 | - | 5 | 3 | - |
| | LPO | 150 | .05 | 2 | 6 | 6 | - | - | ---- | + |
| | LPO | 150 | .20 | 8 | 6 | 6 | + | 5 | 1 | - |
| M17 | MTH | 6 | .05 | 1 | 6 | 9 | + | 5 | 7 | - |
| | MTH | 6 | .10 | 4 | 5 | 0 | - | 5 | 6 | + |
| | MTH | 6 | .20 | 5 | 6 | 8 | - | 4 | 4.5 | - |
| | LPO | 6 | .05 | 2 | 6 | 5 | - | 4 | 1 | + |
| | LPO | 6 | .20 | 3 | 6 | 6.5 | - | 5 | 6 | + |
| | LPO | 6 | .40 | 5 | 5 | 4 | - | 6 | 7 | + |
| | LPO | 150 | .05 | 2 | - | ---- | - | 5 | 5 | + |
| | LPO | 150 | .20 | 4 | 6 | 9 | + | - | ---- | + |
| M18 | MTH | 6 | .10 | 1.2 | 12 | 25 | - | 12 | 23.5 | + |
| | MTH | 6 | .10 | .7 | 5 | 6 | + | 5 | 5 | - |
| | LPO | 6 | .09 | .9 | 12 | 27.5 | - | 12 | 34 | - |
| | LPO | 150 | .09 | .8 | 8 | 10 | - | 8 | 18 | - |
| M19 | MTH | 6 | .10 | 1.2 | 12 | 37 | - | 12 | 17 | + |
| | MTH | 6 | .10 | 1.2 | 5 | 6 | - | 6 | 7 | - |
| | LPO | 6 | .09 | .9 | 12 | 31.5 | + | 12 | 31 | + |
| | LPO | 150 | .09 | .7 | 8 | 1* | - | 7 | 11 | - |
| M36 | MTH | 6 | .20 | 6 | - | ---- | + | - | ---- | - |

Table 4

Number and percentage of convergent responses. V, visual; A, auditory; M, medial thalamic; L, lateral preoptic.

Table 4

Number and Percentage of Convergent Responses

| Stimuli | Number of Cells Tested | Number of Cells Responding | % Responding |
|---|---|---|---|
| V and A | 67 | 10 | 15% |
| V and M | 55 | 15 | 27% |
| V and L | 106 | 42 | 40% |
| A and M | 55 | 10 | 18% |
| A and L | 59 | 12 | 20% |
| L and M | 51 | 22 | 43% |
| V, A and M | 55 | 7 | 13% |
| V, A and L | 59 | 8 | 14% |
| V, M and L | 51 | 13 | 25% |
| L, M and A | 51 | 9 | 18% |
| V, A, L and M | 51 | 6 | 12% |

Table 5

Number, percentage, and mean latency of unit responses.

Table 5

Number, Percentage and Mean Latency of Unit Responses

| Stimulus | Number of Cells Tested | Number of Inhibitory Responses | % Inhibitory Responses | Number of Excitatory Responses | % Excitatory Responses | Mean Latency of Excitatory Responses in ms. |
|---|---|---|---|---|---|---|
| V | 116 | 1 | 0.8% | 54 | 47% | 25 |
| A | 67 | 2 | 3% | 22 | 33% | 13 |
| LPO | 106 | 0 | 0 | 75 | 71% | 9 |
| MTH | 55 | 1 | 2% | 24 | 44% | 10 |

Table 6

Blocking Interactions

$S_1$: conditioning stimulus

$S_2$: test stimulus

N: number of cells tested with that pairing of $S_1$ and $S_2$

N (%)$S_1$ response: number and percentage of cells in which the response to $S_2$ was blocked after the cell gave a spike response to $S_1$.

N(%) no $S_1$ response: number and percentage of cells in which the response to $S_2$ was blocked, but the cell did not give a spike response to $S_1$

$L_{ms}$: mean latency in milliseconds of blocking interactions

$D_{ms}$: mean duration in milliseconds of blocking interactions.

Table 6

Blocking Interactions

| $S_1$ | $S_2$ | N | N(%) $S_1$ response | $L_{ms}$ | $D_{ms}$ | N(%) no $S_1$ response | $L_{ms}$ | $D_{ms}$ |
|---|---|---|---|---|---|---|---|---|
| V | A | 13 | 0 | -- | -- | 4(31%) | 50 | 58 |
| V | LPO | 61 | 18(30%) | 62 | 125 | 19(31%) | 26 | 197 |
| V | MTH | 18 | 10(56%) | 30 | 66 | 5(28%) | 24 | 64 |
| A | V | 19 | 2(11%) | 9 | 45 | 0 | -- | -- |
| A | LPO | 24 | 1(4%) | 20 | 10 | 1(4%) | 13 | 17 |
| A | MTH | 9 | 2(22%) | 16 | 22 | 1(11%) | 38 | 25 |
| LPO | V | 41 | 20(49%) | 20 | 46 | 1(2%) | 20 | 40 |
| LPO | A | 10 | 4(40%) | 14 | 25 | 0 | -- | -- |
| MTH | V | 16 | 7(44%) | 16 | 26 | 0 | -- | -- |
| MTH | A | 9 | 4(44%) | 12 | 19 | 0 | -- | -- |

Table 7

Comparisons of V and Á responses of mesencephalic reticular neurons.

Table 7

Comparisons of V and A responses

| | Total number of cells tested | % V responses | Latency in ms mean (range) | % A responses | Latency in ms mean (range) | % convergence |
|---|---|---|---|---|---|---|
| Present experiment | 116 | 47% | 25 (10-60) | 33% | 13 (3-40) | 15% |
| Scheibel et al. 1955 | - | - | - | - | (4-20) | - |
| Bell et al. 1963 | 110 | 46% | (15-60)[1] | 39% | (15-60)[2] | 14%-21%[3] |
| Groves et al. 1973 | 304 | 11% | 46 (15-110) | 16% | 13 (5-35) | 6% |

[1,2,3] includes V,A and somatic responses

Table 8

Comparisons of MTH and LPO responses of mesencephalic reticular neurons.

Table 8

Comparisons of MTH and LPO responses

| | Total number of cells tested | % MTH responses | Latency in ms mean (range) | % LPO responses | Latency in ms mean (range) | % convergence |
|---|---|---|---|---|---|---|
| Present experiment | 116 | 44% | 10 (6-20) | 71% | 9 (8-12) | 43% |
| Bremer 1970 | - | - | - | - | 10[1] | - |
| Lineberry and Siegel 1971 | 136 | - | - | 54% | 7 (0-42)[2] | - |
| Mancia et al. 1974 | 138 | 37% | (1-25)[3] | - | - | - |
| Mancia et al. 1976 | 47 | - | - | 11%-13% | (7-10)[4] | - |
| Grantyn et al. 1971, 1973 | 65 | 30% | (1-4) | 89%[6] | 12[5] | - |

[1] field potential responses

[2,6] includes initially inhibitory responses

[3,4,5] latency of EPSPs

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