# CENTRAL PHASIC ACTIVITY ASSOCIATED WITH REM SLEEP IN THE ALBINO RAT: THE HOMOLOGUE OF THE PGO SPIKE

by

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, the City University of New York.

1978

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

22 Sept '78
date

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Abstract

Central Phasic Activity Associated
with REM Sleep in the Albino Rat:
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by

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There has been controversy concerning the presence of a recordable event (PGO spike), reflecting an underlying REM sleep phasic event system in the laboratory rat. This controversy raises the question of whether the process responsible for the PGO wave is universal to mammalian REM sleep. This study reports on the identification of a waveform recorded from macroelectrode implanted in the dorsal pontine tegmentum of the albino laboratory rat that presents itself as a candidate for REM sleep - associated central phasic activity. The distribution of this monophasic wave, approximately 100 msec in duration with a 4:1 signal to background noise ratio, is fairly restricted to REM sleep, and to the two minutes of slow wave sleep preceding REM sleep onsets and spontaneous awakenings. Approximately 70 percent of the waves occur within REM sleep at a mean rate of 16 waves per minute. To test the similarities of the mechanisms involved in the control of this phenomenon between species, certain manipulations were performed in the rat and compared with similar studies

employing the cat: 1) para-chlorophenylalanine (PCPA) greatly increases the occurrence of the wave in waking and 2) a REM deprivation procedure increases the occurrence of the wave in the time period just preceding REM sleep onset. These results are comparable to the effects on PGO waves in the cat. A mapping study localizing the sites from which the waves can be recorded in the dorsal pontine tegmentum is also reported. These sites seem to be distributed in a discrete yet widespread manner. One discrete site is the area of the nucleus locus coeruleus and nucleustractus mesencephali. Other sites from which the wave can be recorded, including the cerebellum, are reported. The effects of PCPA on sleep are discussed. Based on the similarities in wave form, distribution with respect to sleep stage, and the effects of PCPA and REM deprivation between the waves reported here in the rat and PGO waves reported in the cat, it is concluded that this pontine wave recorded in the rat is the homologue of the PGO spike.

ACKNOWLEDGMENTS

To my Parents for their moral support

to the City University of New York for providing me with a quality education

to Prof. Soloman Steiner for his general support and assistance

to Mitchell Rubenstein and Essy Schneider for their dedicated technical assistance

to Prof. Howard P. Roffwarg who opened his laboratory to me with its facilities and lended great assistance in the writing of the manuscript

to Dr. Jorge Farber for giving me an opportunity to do the work, offering indispensable technical and scientific assistance and for being a friend

and most of all to my wife Nancy, for her great technical assistance and boundless moral support without which this work could not have been done.

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# I. Introduction

## Historical Background

Perhaps the best way to approach the investigative issues in respect to the unique system of radiating phasic discharge in rapid eye movement (REM) sleep is to first provide a brief history of the development of our knowledge of the REM state. It is important to note that even though the uncovering of a "new" sleep state now has occurred, earlier conceptions of sleep as a unitary state persisted for a time. Earlier, electroencephalographic (EEG) methods seemed to confirm the unitary view, given variations in the dimension of "depth of sleep" (Loomis et al, 1935). However, findings by Moruzzi and Magoun in 1949 started to shed light on an active neural mechanism in the brain underlying wakefulness, the reticular activating system (RAS). Wakefulness and sleep were seen as opposite poles in the activity level of the RAS. After 1953, Aserinsky, Dement and Kleitman, employing for the first time continuous all-night EEG recordings in man, discovered a phase of sleep associated with dreaming, characterized by rapid eye movements and a low voltage fast frequency EEG, which they labeled REM Sleep (See Dement and Kleitman, 1957). Initially, interpretations attempted to fit this finding into a theory of a continuum of RAS activity. Because of the associated electroencephalographic pattern resembling waking, REM sleep was interpreted as a "light" sleep stage in the continuum.

Nevertheless, a major inconsistency with a simple "continuum" (unitary) theory arose when Dement (1958) found in the cat that higher thresholds of stimulation of the mesencephalic reticular formation

were necessary to arouse the animal from REM sleep than from slow wave sleep (SWS). This meant that REM sleep could no longer simply be looked upon as "light" sleep. The work of Jouvet, Michel and others (Jouvet and Michel, 1958; Jouvet et al., 1959) in the cat, revealed that sleep does not result from merely a passive reduction of activity in the RAS, but rather is subserved by active brainstem mechanisms, and that different mechanisms are involved in the generation and maintenance of REM sleep and SWS. What emerged was the conceptualization of two separate "states" of sleep defined by their respective tonic aspects, as measured by the EEG, nuchal electromyograph (EMG) and electrooculograph (EOG). What followed in the chronology of the field, especially with respect to REM sleep, was an intensive set of attempts to discover the significance of these states for the functioning of the organism. One approach was that of Dement (1960), who developed a technique whereby a subject could be selectively deprived of REM sleep. Monitored by the polygraph, a subject could be awakened each time signs of REM sleep appeared. Since SWS typically precedes REM sleep, when the subject is allowed to resume sleep, entry is into SWS. This pattern of return to SWS continues for the duration of the experiment. Use of this method, allowed development of a REM deprived (in the sense of lack of REM sleep expression) experimental subject. SWS is not appreciably affected by the procedure if proper attention is paid to total sleep time.

The rationale for such experiments rested with the concept that REM sleep is an unidimensional state measurable in units of time; if the subject is not allowed to enter the state, the processes that are

normally discharged during it will not occur. This technique was based on standard neurophysiologic experimental logic, suitably qualified: Remove a structure and information will develop as to what value it had for the organism before removal, just as bilateral extirpation reveals that the eyes are organs of sight.

The results after twenty years of using this technique have not achieved the hoped for expectations (Vogel, 1975). Notwithstanding, two pronounced and widely accepted findings have emerged: 1) as REM deprivation proceeds, the subject attempts increasingly to enter REM sleep; 2) following REM deprivation, when the subject is allowed to sleep freely, there is a "rebound" effect; that is, REM sleep amounts (and percents of total sleep) are sharply increased over baseline amounts. This effect is observed in all mammals subjected to REM deprivation. To a point, the longer the deprivation the greater the "rebound", though ultimately a plateau is reached. In other words, after depriving cats for 30 days or rats for six days, additional deprivation does not produce a larger rebound (Morden et al., 1967).

A "need" for REM sleep was postulated on the basis of the rebound effect (Dement, 1961). But, if there is indeed a "need" for REM sleep, why should it not keep increasing under the deprivation condition? In order to maintain that some "need" is fulfilled during REM sleep, and initially that the "need" is increasing during the deprivation procedure, one would have to hypothesize that when the plateau is reached, the "need" has somehow been fulfilled either outside of REM sleep during the deprivation or that the recovery ("rebound") sleep is somehow more "intense" and thus is needed less to make up for the accumulated loss. In either case, a REM stage dimension, in ad-

dition to time in stage had to exist, and the concept of a unitary process or state had to be questioned. The conclusion of an organismic "need" for REM sleep, established on the basis of the REM deprivation rebound phenomenon, is now generally accepted as tempting but not well founded. That REM sleep may have many vital functions is accepted. But its tendency to be "recovered" by the CNS, and the mechanisms subserving the "rebound", are quite likely independent of the routine functions and perhaps mechanisms of REM sleep. To honor the logic of the neurophysiological paradigm, that removal of a process will uncover its function, it was necessary to undertake a demonstration of functional deficits in REM deprived individuals during the deprived state. These demonstrations proved surprisingly difficult.

Many physiological phenomena are associated with REM sleep, but the defining tonic characteristics of the state are the low voltage, fast EEG and muscle atonia. Other characteristics are the abundant, almost explosive, phasic eruptions punctuating the tonic background. These include the rapid eye movements, and, in the cat and monkey, short duration phasic changes in the electrical activity in certain regions of the CNS. The occurrence of each of these phenomena is not, however, unique to REM sleep, and certain experimental manipulations can readily dissociate or differentially affect the tonic and phasic components of the state. For example, bilateral lesions in the dorsal pontine tegmentum can lead to a condition called REM sleep <u>without</u> muscle atonia (Henley and Morrison, 1974); also, serotonin depleting drugs can cause central phasic electrical activity to occur during the waking state (Delorme, 1966), and in light barbiturate anesthesia, phasic activity occurs periodically in the absence of the typical low voltage, fast electro-

corticogram of REM sleep (Cotte et al, 1974). In summary, the concept of REM sleep as a unidimensional entity has given way to the view that within the tonic condition, the frequency (density) of phasic activity can vary, and the latter must be considered in the quantification of the REM sleep condition. Dement (1973) now proposes that REM sleep is not a unitary state, but rather a confluence of many processes and that the heuristic value of the former concept is probably no longer useful. Dement suggests that at the present time we can identify at least three processes operating during REM sleep: 1) tonic inhibition, as exemplified in nuchal EMG suppression and inhibition in sensory systems (Pompeiano, 1967); 2) central nervous system arousal as seen in the low voltage, fast EEG and other measures of neural activity (Huttenlocher, 1961); 3) phasic activity, as seen peripherally in rapid eye movement, middle ear muscle activity (MEMA), paroxysmal muscular movement, cardiovascular irregularity, respiratory variability and other episodic phenomena, as well as centrally in the form of short duration changes in gross electrical activity in many areas along the neuraxis.

Ultimately, the understanding of the functional significance of REM sleep will have to be understood on the level of the functions of the component mechanisms. The value of this multi-process conception of REM sleep lies not in its immediate accuracy in identifying the totality of the basic processes, but in its ability to stimulate research in terms of more appropriate units of study. The aim of this thesis then is to study the latter processes mentioned above, specifically the central phasic electrical activity associated with REM sleep. What follows is a brief review of that area.

Orientation To The Research Problem

A. Ponto-Geniculo-Occipital Activity

Jouvet and Michel (1958) recorded with a macroelectrode 6 to 8 Hz spindle activity in the pontine reticular formation (PRF) of the cat that was specific to REM sleep. In 1963, Brooks and Bizzi implanted movable macroelectrodes into the PRF in order to investigate this spindling further. They found the spindles to consist of clusters of monophasic waves, which they could record in the pons and oculomotor nuclei, and whose occurrence was indeed associated with REM sleep. Mikiten et al (1961) had earlier reported a periodic "spike" discharge in the lateral geniculate nucleus (LGN) during sleep in the cat. This led Brooks and Bizzi to undertake an investigation of the sharp waves in the two structures which demonstrated their almost simultaneous discharge. When similar waves were found to be present in the visual cortex in REM sleep (Mouret et al 1963), Jeannerod et al (1965) coined the phrase ponto-geniculo-occipital activity, or PGO waves, identifying the then three major areas in which this activity could be recorded in the cat. In fact, PGO waves have now been located in many other places during REM sleep, including cranial nerve nuclei III, IV, VI (Costin and Hafeman, 1970), superior colliculus and parieto-occipital cortex (Calvet et al, 1964), oculomotor nuclei (Brooks and Bizzi, 1963) and cerebellum (Jeannerod, 1965). Based on the investigations of Brooks and Bizzi (1963), the wave has a typical form and distribution. An initial deflection is always observed in the same direction with a duration of approximately 90 msec to 120 msec. They found that a second deflection can follow in the opposite direction with lower amplitude and greater duration. Since the second component

parameters are much more variable, they are often not considered, and, conventionally, PGO waves were referred to as monophasic. The amplitude of the PGO wave is dependent upon the location of the electrode. In the abducens, at the level of the exiting facial nerve, high amplitude, 200-300 uV waves can be recorded. More lateral and dorsal, at the same anterior-posterior level, the amplitudes are 25-75 uV. A descending electrode in the pons shows changes in amplitude and polarity of the PGO wave at different stops. As mentioned, at any one site the parameters are fairly constant. Some investigators, however, report a variability in amplitude at the same site, approaching a 50% difference between the largest and smallest waves (Bowker and Morrison, 1976).

Some researchers believe that under normal conditions PGO waves never occur during waking (see Jouvet, 1972). There is some controversy on this point. Bowker and Morrison (1976) report that startling stimuli can elicit the waves in waking, but they habituate quickly. The waves can occur periodically during SWS (Thomas and Benoit, 1967). This is often referred to as an aborted REM period, but since no REM appears, this is a presumptive idea. Often these incidences of PGO activity precede spontaneous awakenings. It is characteristic that the PGO waves precede, by 30-90 sec, the onset of the cortical desynchronization of REM sleep and continue in the REM period. The waves can appear singly or in groups and parallel the single or burst characteristics of the eye movements. Under normal sleep-wake conditions in the laboratory the output of REM sleep PGO waves remains fairly consistent in the range of 13,000 spikes ($\pm$ 1500) per day (Jouvet, 1967). If REM time is

reduced for several days, the ratio of spikes to minutes of REM sleep will increase, though the absolute number of spikes does not achieve baseline levels. With compensation of lost REM time there is also a rebound of spikes. But the interaction of phasic and tonic attributes of REM sleep is attested to by the greater rebound of REM time when PGO spikes were not allowed to migrate into NREM sleep in deprivation and thus are maximally rebounding in recovery sleep. During the deprivation period an increase in the frequency of preREM waves is also seen (Vimount-Vicary, 1966).

B. PGO Activity Reflecting a REM Sleep Process

If we define the REM period by the tonic features of EEG activation and muscle atonia, then PGO activity always precedes REM onset (Dement, 1970). We identify the process reflected in the PGO wave as a REM sleep process because it seems to usher in the REM episodes and continues through them. Cats administered pharmacological agents that allow PGO waves to occur in waking will initially orient and react as if to have seen (hallucinated) objects when the waves appear on the polygraph record (Dement, 1970). There is a striking similarity between such treated animals and the bilateral pontine lesioned cats who lack muscle atonia in REM stages and act out prey catching and aggressive behavior in this state in conjunction with PGO bursts. As indicated, typically the occurrences of PGO waves are correlated with the timing of peripheral phasic activity such as eye movements, spasmodic muscular twitches, (Sakai and Jeannerod, 1970), middle ear muscle activity (Pessah and Roffwarg, 1972), blood pressure and heart rate changes, etc. Evidence that a central phasic process is responsible for peripheral phasic events was developed from intensive investigation of the visuo-

motor system during the REM phases (Cespuglio et al, 1975). This will be discussed later.

C. The Hypothesized Generator of PGO Activity

The concept has developed over the years that a single generator, or a fairly localized generator system is responsible for the concomitance of the diversely located potentials and the REM state. Jouvet and Michel (1959) performed midbrain transections that preserved the periodic appearance of the pontine sharp waves. Their appearances were coincident with loss of muscle tone. However, all signs of REM sleep were absent rostral to the transection. Conversely, a transection between the pons and the trapezoid bodies at a $60^{\circ}$ angle from the frontal plane, produced a preparation with no periodic variations in muscle tone, yet rostral to the transection alternating periods of slow waves and PGO waves were recorded. These data were compelling that some structure (s) located in the pons are necessary for the production of the signs of REM sleep.

Bizzi and Brooks (1963) investigated the relationship of the waves recorded in the LGN and pons. They found that the pontine waves were also bilateral. Usually, the pontine waves precede those in the LGN, suggesting an ascending circuit to thalamus and cortex. These workers found that stimulation of the pons during REM sleep yielded waves in the LGN with a latency of 25-35 msec that were identical to spontaneous waves. However, the LGN responses to stimulation could only be elicited at times when the spontaneous waves would normally occur, that is just preceding and during REM sleep. Stimulation of the LGN at <u>any</u> time could <u>not</u> elicit a wave in the PRF that resembled spontaneous waves.

Removal of the cortex does not abolish the LGN waves (Bizzi and Brooks, 1963); and bilateral destruction of the LGN does not abolish the cortical waves (Hobson et al, 1969). Evidence thus pointed to the pons as the center of control of the episodic discharges connected with the REM state. Indeed, it appeared that their antecedance of the conversion of tonic SWS to the tonic REM state suggests that they play a role in triggering the cortical desynchronization and EMG atonia.

In Jouvet's laboratory in Lyon, lesion and pharmacological work reported in a variety of studies seemed to implicate the area of the noradrenergic nucleus locus ceruleus (LC) in the dorsal pontine tegmentum as the REM generator structure in the sense that its integrity is necessary for all the signs of REM sleep, including PGO activity (see Jouvet, 1969). The morphology of the LC is very well suited for this function since it has been described as innervating all cortices of the brain (Ungerstedt, 1971). However, earlier lesion work has implicated the nuclei reticularis pontis caudalis and oralis (Jouvet and Mounier, 1960). Carli and Zanchetti (1965) performed similar lesions that were located even more rostral to the nucleus reticularis pontis oralis, and that they claimed eliminated all signs of REM sleep; Henley and Morrison (1974) also performed bilateral LC lesions and pointed out that only the muscle atonia of the REM state was obviated. More recently Jones et al (1975) have reported a 50% decrease in PGO activity with LC lesions without substantial change in the amount of EEG desynchronization of REM sleep.

Many other studies bear on the question of the site of REM state activation: Morrison and Pompeiano (1966), Hobson and McCarley (1975),

Siegel and McGinty (1975). The short length of post-lesion observation has often confounded some interpretations of results. Nevertheless, even if the LC does not turn out to be the site of REM activation or PGO generation, the fact that a generator or "pacemaker" system exists somewhere in the pons remains uncontradicted. Jouvet's theory, implicating the LC as a generator of PGO, has been suported by the findings of Maeda et al (1973) that in the cat, an intermediate catecholamine fiber system exists, originating in the nucleus subcoerulus, and innervating, among other places, the LGN. Laurent et al (1974) have used a local freezing technique to produce a reversible suppression of LGN PGO during REM sleep by cooling bilaterally, the pontomesencephalic isthmus, a site where the intermediate bundle is thought to pass. Sakai et al (1976) have replicated the PGO results with discrete electrolytic lesions to produce an irreversible suppression. Another piece of evidence comes from the histological technique using horseradish peroxidase (HRP) (Laveil and Laveil, 1972). Legar et al (1975) have traced afferents in the LGN to the LC and to the surrounding nuclei parabrachialis lateralis and medialis. This group also found that the III and VI cranial nerve nuclei, which innervate the extraocular muscles, are in turn innervated by the closely situated nucleus Kolliker Fuse, in the dorsal pontine tegmentum. It is intriguing that all these nuclei (Kolliker Fuse, parabrachialis lateralis and medialis, LC, and sub-LC) turn out to be part of a noradrenergic system (Jones and Moore, 1974), and are sometimes referred to as the ceruleus complex in the cat (Jouvet, 1972).

Of course, the core evidence for a generator system of PGO in the dorsal pontine tegmentum of the cat lies in Jouvet's lesion work, which as mentioned, is somewhat controversial. Investigations using single unit recording have not clarified the issue very much. Hobson and McCarley (1975) report that units of the LC decrease their discharge rate during REM sleep and are therefore not good candidates for "generator" cells. Chu and Bloom (1973, 1974) confirm this finding but have also recorded LC cells that do increase rates in REM sleep and active waking. Hobson and McCarley (1977) argue that certain giant cells in the giganto cellular tegmental field (FTG) are possible pacemaker cells because they are the first to fire, but Siegal (1975) claims that abducens units precede FTG unit firing.

One possible problem in localizing this elusive hypothesized generator may lie in the lack of its specificity to the REM sleep process only. If the generator of PGO activity also has other functions that have it operate at other times, it is possible to see how it may be overlooked if investigators invoke a PGO specificity requirement to all its activities. It is possible to have an alternative conception of the PGO generator that allows the activity of the generator to be reflected as PGO waves at one time and yet not at other times.

D. Evidence in Support of Gating Mechanisms

The administration of reserpine to cats causes a redistribution of the waves to all states, and pontine stimulation under reserpine is effective at eliciting LGN waves at any time. From these experiments came the concept of a gating mechanism or regulatory mechanism (Brooks and Gershon, 1976). It was further found that certain lesions of the LC, vestibular, and dorsal raphe nuclei all effect the distri-

bution of PGO waves (Jouvet, 1972, Morrison and Pompiano 1966, Simon et al 1973). All these structures and undoubtedly others are apparently involved in regulation of the generator or propagation of its influence. Simon et al (1973) unilaterally interfered only with the presumed connections between the midline raphe and the hypothesized pontine generator and found similar effects as with bilateral raphe lesions. To test the gating hypothesis stimulating electrodes were placed symetrically in the pons. Initially, when the waves would occur in all states stimulation of the ipsilateral pons could elicit LGN waves any time. Stimulation of the contralateral pons could only elicit LGN waves just prior to and during REM sleep. These and other results implicate the unilateral inhibitory effects of the raphe nuclei on the propagation of PGO waves, and an ascending crossed fiber system so that unilateral activity of the "generator" produced bilateral activity in the LGN. Further evidence of raphe involvement comes from studies in the cat of administration of parachlorophenylalanine (PCPA) which depletes raphe 5-hydroxytrytophan (5HT) (Koe and Weissman, 1970), and results in a redistribution of the waves, causing them to occur in waking (Delorme, 1966, Koella et al, 1968, Ferguson et al, 1969). Also single unit studies of the nucleus raphe dorsalis reveal a very strong relationship between a pause in discharge rate in that nucleus and the occurrence of LGN PGO waves (McGinty et al, 1973).

E. The Relationship of PGO and EMP Activity

Sakai et al (1976) have carried out a series of experiments comparing PGO waves and eye movement potentials (EMP). It has been

known that EMPs can also be recorded in the pons, LGN, and visual cortex (Brooks 1968, Jeannerod and Sakai 1970, Brooks and Gershon, 1971). There are many similarities between PGO and EMP waves yet several differences exist; cortical EMP are attenuated in the dark, abolished by bilateral optic nerve section and are generally of smaller amplitude during darkness (Brooks and Gershon, 1971); the amplitude of the EMP is related to the velocity of the eye movement and always follows the eye movement (Jeannerod and Sakai, 1970). Sakai and Cespuglio (1976) find that both the initiation of PGO and EMP in the VI nucleus precede potentials generated in the lateral rectus muscle with the same latency, however, the termination of the respective waves are such that the EMP is dependent on the duration of the eye movement and the PGO is not. Recording PGO and EMP activity at the levels of the LGN and cortex clearly distinguishes the two phenomena. The wave latency in these structures to VI nucleus PGO is on the order of 5-35 msec whereas with the EMP it is greater than 60 msec (Sakai and Cespuglio, 1976). The Sakai group hypothesizes that both PGO and an endogenous component of EMPs may be a response to the same pontine generator, and that the differences between them may be accounted for by selective activity of the various regulatory influences.

The association between PGO and the visual system goes beyond the similarities between PGO and EMP. As mentioned previously, a gross association had been shown between eye movements and PGO waves, but Cespuglio et al (1974) have used a more sensitive measure than EOG to establish visuomotor outflow during REM sleep by recording

directly from the lateral rectus muscle itself. They find a direct albeit complex relationship between PGO waves in the VI nucleus, LGN, and visual cortex and the lateral rectus muscle potentials. This partial elucidation of such a system is evidence that central phasic activity, at least in the oculomotor system, is the direct cause of the peripheral phasic activity observed in REM sleep, i.e., the eye movements.

F. PGO Activity is Not Unique to the Visuomotor System

Thus far, it has been quite compelling to think of PGO activity as a visual phenomenon. Ten percent of the PGO waves recorded in the VI nucleus, however, do not lead to any visual activity (Sakai and Cespuglio, 1976). Just as PGO are grossly associated with eye movements in REM sleep as measured by the EOG, they are associated with muscle twitching in the extremities, facial muscles, vibrissa, the muscles of the middle ear, phasic respiratory changes and cardiovascular changes, and other peripheral phasic events. It is possible that many if not all sensory and motor systems are involved. In the pons, the waves are not localized to a single nucleus (Brooks and Bizzi, 1963), and may span areas involved in different functions; in the thalamic region the waves are not restricted to the visual LGN, but can be recorded in areas including the pulvinar, the central lateral nucleus, the ventral posterior medial nucleus, the lateral posterior nucleus, and the habenula (Hobson et al, 1969); in the cortex, if transcortical electrodes are used, the waves are not found to be confined to the primary visual receiving area (Hobson, 1964). It may be that all of these phenomena, and more, may be linked to a single

generator system or a system of linked generators. We should not be misled by the fact that most of our discrete neurophysiological information comes from studies on the visual system. To use Roffwarg's term, it may be that sleep neurophysiologists have suffered from a visual "myopia" with respect to the PGO system despite continual leads that PGO is propagated into other systems as well. One thing is clear, that there are mechanisms operating just before the onset of REM sleep and during REM sleep that justify labeling them a REM sleep process with a wide ranging group of attendant phenomena. The gating mechanism bearing on the generator may account for absence of firing at times or different firing pattern in certain structures that are part of the radiating PGO network.

G. PGO Activity in the Acoustomotor System

Substantial evidence is beginning to be accumulated about central phasic activity in other systems (Roffwarg, 1975). The auditory system is a very promising area of investigation. The stapedius muscle of the middle ear undergoes phasic contractions during REM sleep (Dewson and Dement, 1965). Roffwarg (Pessah and Roffwarg, 1972) has been studying this phasic event both in cat and in man in whom it can be measured discretely by changes in tympanic membrane tension, and confirms that this phasic activity is present in every REM period and that it may be even a more sensitive measure of the underlying excititory process than eye activity. Middle ear muscle activity can be recorded in man before the onset of the ocular movements at the beginning of REM periods and this activity continues in REM sleep even when eye movements are reduced by REM stage inhibitory influences. (See Figure 1).

There are many parallels between the visual and auditory systems such as VI nerve innervation of the lateral rectus and VII nerve innervation of the stapedius muscle; the thalamic relay stations of the LGN and the medial geniculate nucleus (MGN), and the primary cortical visual and auditory receiving areas. If the organization of phasic activity in the auditory system parallels that of the visual system, we may be able to assess some general principles of phasic event systems in our work. If it can be shown, by using a technique such as HRP, (Lavail and Lavail, 1972) that the auditory and visual system share the same hypothesized generator, we will be that much closer to the concept of a single generator subserving both central and peripheral phasic phenomena in REM sleep.

Roffwarg's laboratory has launched a preliminary investigation into this problem with a macroelectrode investigation of the two middle ear muscles (t. tympani and stapedius) the Vand VII n. nuclei that innervate them, MGN and auditory cortex. PGO waves have been identified in the V and VII nerve nuclei and in the auditory cortex. They occur usually locked to the discharges of MEMA (Roffwarg, 1975). (See Figure 2).

Gross potential changes at these times from the MGN electrodes is much less consistent. This finding has not been additive to our overall concept of a pattern of phasic event stimulation in REM sleep, not restricted to the visuomotor system and constituting a more generalized phenomenon, discharging into multiple, functionally linked components. Nevertheless, this lack of completeness does not detract very much from the fundamental discovery of REM sleep phasic processes

being discharged into yet another major perception related system, the acoustomotor system. It is possible that generator-induced stimulation may be directed at the cat MGN, but the resultant gross potential changes are too weak for current gross recording techniques. It may be relevant that Adrian et al (1966) found a high degree of asynchrony and variability in discharge rates in single cells of the cat MGN, because macroelectrode recording is dependent on the simultaneous behavior of large populations of cells, it might be conjectured in the MGN that a PGO wave is masked by more heterogeneous cellular activity unrelated to the underlying REM process. Perhaps more sensitive recording techniques will reflect this.

H. A Question of the Basic and Universal Nature of the REM Sleep Phasic Event System

I have presented evidence that a phasic event system exists in the cat and that PGO waves are a measure of its activity. With others, I conclude that the operation of this system is a basic and universal process of mammalian REM sleep. If this conclusion is correct then the study of the central phasic event system is central to the understanding of the phenomenon of REM sleep, and to the extent that this system is the substrate of endogenous activity, it may be the key to uncovering the functional significance of REM sleep.

This view is not held by every researcher in the field. Most of the skepticism centers around the belief that either central phasic activity is not a universal factor of REM sleep, or that the PGO wave is not a measure of a basic REM process.

Morrison and colleagues (Bowker and Morrison, 1976; Bowker and Morrison, 1975) hold the last of these alternatives. They claim that stimuli that startle the cat can elicit typical PGO waves at any time. Their appearance preceding, and during REM sleep, then is an aspect of a startle mechanism triggered off by the increased neural activity of REM sleep. Therefore, they conclude, PGO waves are an epiphenomenon of the startle mechanism and do not have a function. There are several things wrong with this logic. First, I don't think anyone has proposed a function for PGO waves. They are but a reflection of an underlying process so that they are indeed an epiphenomenon. Applying this term in no way decreases the wave's ability to reflect the underlying process that generates them. Second, these authors feel that the underlying mechanism is at least in part shared by a hypothetical startle mechanism. Even if the waves are part of a startle mechanism it does not reduce their appearance to a mere inconsequence. There are certain facts not brought to bear in Morrison's arguments that are quite intriguing. Following the presentation of startling stimuli, such as a very loud and sudden sound, muscles of the inner ear contract, and a rapid eye movement may appear coupled with a paroxysmal contraction of skeletal muscles, a flinch. These events are very reminiscent of the peripheral phasic events of REM sleep. It is very possible that something akin to the startle mechanism is involved. The stimuli that can elicit startle also have a high probability of waking a sleeping animal up. PGO wave frequencies increase preceding many spontaneous awakenings. Might then part of the mechanism generating PGO waves

be involved in the startle response, and also in producing spontaneous awakenings? The main and significant difference between the mechanism that produces startle and the mechanism underlying PGO waves is the source of eliciting stimuli. One is produced external to the animal and the other, as all REM sleep phenomena, is produced internally. Morrison's arguments, rather than bringing forth evidence to demonstrate PGO activity as an area that is not fruitful to investigate, has possibly presented information leading to yet another function of REM sleep processes.

Another objection to placing the mechanisms responsible for PGO in a central position in REM function is a question of its universality. Of about 5,000 extant mammalian species, only approximately 53 have been studied. Of the 53, PGO activity has reportedly been investigated in four types: man, monkey, cat and rat. With such a small sample it would be impossible to say that any sleep parameter was universal to mammals. Even with the parameter of PGO activity, if it cannot be detected in a particular species, it does not necessarily follow that the underlying process is not present. There is a region in the cat brain in which macroelectrodes fail to record a PGO wave, and yet single unit investigations reveal activity associated with the wave's occurrence elsewhere (Brooks, 1973). On the other hand, the absence of the ability to record PGO waves does shed some doubt as to the universality of the process. In man, where neurophysiological investigations are hampered in ways that animal research is not, preliminary evidence indicates that PGO waves are present (Salzarulo et al, 1973). They certainly are present in the cat, which is

the prototype of this activity. It has also been detected in the monkey (Perachio, 1973; Balzamo, 1975). The presence of central phasic electrical activity reflecting a REM sleep process in the rat has been met with controversy.

Stern et al (1974) could not find PGO type waves in the LGN or visual cortex of the albino rat. They concluded that this activity is not a primary process of REM sleep. They also offered an explanation involving the poor vision of albino rats explaining why this essentially visual PGO phenomenon is absent. First it has been adequately discussed here that PGO activity in the cat is not restricted to the visual system, and therefore should not be thought of as solely visual phenomena. Although, since these authors only investigated the visual system an explanation for their negative findings may lie in the visual system of the rat. Secondly, these author's investigations do not shed any light on whether the underlying process of this phasic activity is present in the rat. It would however, be of great convenience to have an easily recordable event reflecting this underlying process. Stern et al's (1974) report was very influential in the field in spite of the presence of peripheral phasic activity such as rapid eye movements and muscular twitches, and an earlier report that claimed to have found REM related waves in the rat.

Gottesman (1969) reported PGO type wave forms from the oculomotor nuclei and parasaggital pons in the albino rat during REM sleep. The report itself, however, was not clear. It included no histology and his descriptions were laden with metaphors. Reserpine did not

seem to alter the distribution of these waves. Coupled with the problems of the report, the effect of reserpine, and Stern et al's report, it is not difficult to see why the general attitudes of workers in the field were in favor of an absence of PGO interpretation. If one is to objectively evaluate the evidence, however, one does not necessarily have to arrive at this conclusion. If we discount Gottesman's writing style, there is a report of the presence of these waves and a report of their absence. These investigators, however, were looking at different loci. It is possible that REM related central phasic waves exist in the rat but not in the LGN or visual cortex. It has been shown that the cytoarchitectonics of the LGN and visual cortex of the albino rat are aberrant (Guillery, 1974). Electrical potentials recorded by a macroelectrode are dependent on the simultaneous behavior of a large population of cells. It is not unreasonable to assume that if connections between the cells in a population are altered then simultaneous activity will be altered as well. The visual-sensory system is a bad place to look for universality in the laboratory rat when it is known to possess an aberrance unique to albinism. The fact that reserpine seemed not to alter the distribution of the waves is not that disturbing either. Reserpine has very different effects on the rat than on the cat (Contrast, Delorme et al, 1965 with Gottesman, 1966). Any comparison of results using this drug, between these species, would be tenuous to say the least.

Cespuglio et al (1975a), in a preliminary report, has confirmed PGO type waves during REM sleep in the VI and III cranial nerve

nuclei in the agouti rat. These motor nuclei innervate the extra oculomotor muscles and may be a central representation of the rapid eye movements seen during REM sleep in this species. Before investigating the possibility of a central generator, as research supports in the cat, it is important to demonstrate not only that the central phasic waves of REM sleep are not confined solely to visual motor nuclei, but that the general topography of the response to manipulations hints at a similar mechanism operating in the rat as it does in the cat. In this way we can get closer to demonstrating the universality of this process that may be a primary one in mammalian REM sleep phenomena.

## II. Overview of the Experimental Procedures and Rationale

### Purpose of the Research

The major task of the proposed experiments is to gain evidence for the existance of a REM sleep phasic event system in the rat. Eventually I would like to demonstrate a generator system in the pons, modified by gating mechanisms influencing neuronal activity all along the neuraxis. As the first step, a discrete event that is a candidate for being a reflection of the underlying REM sleep phasic event system - the homologue of the cat PGO spike must be identified in the rat. Once the electrophysiological event has been identified, certain experimental manipulations will further reveal the nature of the underlying mechanisms. Future research will attempt to determine the extent of this radiating system and ultimately the uncovering of its hub, the generating and gating mechanisms.

I have one additional aim in a set of proposed experiments, that of shedding more light on the 5-HT sleep theory proposed by Jouvet (1972). The results of experiments with rats using the 5-HT depleting drug PCPA have been very inconsistent (Mouret et al, 1968, Rechtschaffen et al, 1973). These experiments are in great need of replication. If I am successful at identifying the homologue of the PGO wave in the rat, then I have something more to offer than mere replication. With the addition of a new dependent variable in the rat, new insights into past results may be obtained.

### General Procedures

A. Obtaining the animal preparation.

Laboratory albino rats will be used as subjects. The choice

of this animal versus a pigmented strain of rat is dictated by their docile demeanor and our proven ability to accurately implant electrodes into sites deep in the brain. Due to the known aberrancy in the visual system of these albinos, loci will be chosen to avoid such areas.

I have chosen to look for an electrophysiological event in the dorsal pontine tegmentum of the rat reflecting the activity of a hypothesized generator of REM sleep phasic events. All of the hypotheses with regard to the REM sleep phasic event system are derived from experiments on cats. I hypothesize that PGO activity in this animal is a reflection of the underlying REM sleep process. Therefore, I am looking for a similar electrophysiological event in the rat that may be a reflection of the same process. The dorsal pontine tegmentum is not associated with the visual aberrancy of albinos. PGO waves can be recorded there in the cat, and it is the hypothesized site of the PGO generator system. Although it does not necessarily logically follow that if a PGO type wave form can not be isolated at a particular locus the underlying REM sleep process is not operating at that site. It would, however, be a great convenience. My first approach is to examine the area of the dorsal pontine tegmentum of the rat where I have already isolated a candidate for the PGO type wave (Farber et al 1976).

Electrophysiological recording of the brain of animals is achieved by surgical implantation of electrodes. Bipolar electrodes will be used and gross electrical potential changes between the poles will be displayed on an ink writing polygraph. In each subject

either one or two electrodes, each pole diameter 0.01 inches, will be stereotaxicly implanted into a deep structure of the brain. Similar diameter electrodes have been used in the cat to record PGO waves in a variety of brain loci. In addition, an array of electrodes will be implanted for standard chronic sleep recording. Since the most important criteria for a PGO wave is its distribution with respect to sleep phases, the state of the subject; awake (AW), slow wave (SW) sleep, REM, using standard criteria, will be determined for each 15 or 30 second epoch by an examination of activity recorded from EEG, EMG, and EOGelectrodes.

It is not necessary to have information from the EOG in order to score the sleep recordings of rats. Having this information could only increase the reliability to identify states, and with the administration of pharmacological agents that may distort the usual configuration of indicators, this information may be useful. In addition, PGO activity in the cat is grossly associated with eye movements. I hypothesize that a similar relationship will exist in the rat. Obtaining information concerning this peripheral phasic event will allow this to be tested.

Since stress has been shown to be a powerful REM sleep depriving condition, efforts will be made to keep it at a minimum. Animals will be allowed to fully recover from surgical trauma for at least one week. An adaptation to the recording situation (at least two days) will precede all experiments. During the experiments the animals will be housed in glass aquarium tanks with saw dust bedding, food, and water inside a sound attenuated, ventilated running

box. The cable connecting the electrodes to the polygraph will pass through a commutator to allow for rotational movement, and a spring will hold a loop in the cable for translational movement.

B. Identification and evaluation of the phenomenon.

I hypothesize that a wave form recorded in the rat reflecting the underlying REM sleep process will be similar in its characteristics and distribution to the PGO wave of the cat. The first step is to identify a candidate wave form. Since the amplitude parameters of the PGO wave are quite variable from site to site no strict criteria for amplitude can be preset. However, the waves must be identifiable with a certain degree of reliability. We arbitrarily set a signal to background noise ratio of equal to or greater than two. Based on the general parameters of PGO waves I expect: 1) the duration of the wave to be between 90-120 msec., 2) its polarity to always be in the same direction, 3) the distribution of its occurrence to be such that the great majority of the waves (80%) occur in REM sleep, in SWS two minutes before REM sleep onset and spontaneous awakenings, and 4) their presence in every REM period.

If such a wave form appears on the polygraph record, then a criteria can be developed in terms of its amplitude, duration and polarity that will yield a wave with the hypothesized distribution. If no such wave exists then no constant criteria could possibly be derived to produce a wave that has this distribution.

Gross potential AC polygraphic recordings from the brain reveals a series of waves of alternating polarity and variable fre-

quency and amplitude. The procedure for identifying a PGO type wave in a recording is to look at the first three REM periods. If a waveform meeting the general requirements can be found it will be additionally defined by a minimal amplitude criteria (MAC). All of the aforementioned criteria are then used to identify each wave on the record up until the end of the third REM period. The distribution of the waves will then be examined with respect to sleep stage. Initially the MAC is set liberally (low). I then will try to improve the distribution by increasing the MAC. I use this method for I would rather not identify a wave that is dependent on the underlying REM process than make the error of identifying a wave, which is not dependent on the process. If a criteria cannot be developed so that 80% of the waves are distributed as hypothesized, then, I conclude that I can not record a REM sleep central phasic event from this electrode. If a criteria can be developed, then the criteria will define the PGO type wave for that site in that animal for every subsequent analysis.

In order to determine the distribution of the waves I must know in which state they occur. Since sleep staging is dependent on the amplitude and frequency of the EEG, and frequency can only be measured over time, sleep stage can not be determined for each instant but rather over intervals. Therefore I must artificially quantify the continuous variable of stage into discrete time intervals called epochs. Epoch length should not be so small as to make it impossible to determine the trend of the EEG frequency or to produce an inordinate burden upon the scorer. Epoch length

also should not be so large as to produce gross inaccuracies as to each epoch's characterization. The method requires that to determine the state in which a single wave occurs is to determine the state characterization of the epoch in which the wave occurs. This will introduce some degree of inaccuracy.

Each scorable epoch will be assigned one of six possible scores. If REM sleep is absent and the majority of an epoch is awake it receives a score of awake (A), or if the majority is SWS it receives a score of slow wave (S). The rare occurrences of equal amounts of these two stages will be scored S. If the majority of an epoch is REM sleep then it receives a score of REM (R). Since I hypothesize that the waves will be associated with REM sleep it is important that the scoring system be sensitive to the occurrence of this phase of sleep. Each epoch will be divided into 12 equal periods. If less than half of an epoch contains REM sleep the number of periods containing a majority of REM sleep are counted. If the marjority of an epoch containing REM is awake then it receives a score of awake-REM (AR) with the number of periods of REM sleep recorded. If the majority is SWS then it receives a score of sleep-REM (SR) with periods of REM recorded. The last possible score an epoch can receive is mixed (M). This denotes that no state occupies a majority of the epoch and a number of periods of REM sleep are present. Scoring sheets will be prepared such that seven categories will be present for each epoch. An A, S or R will receive an "X", or an AR, SR, or M will receive a number in a category for the epoch representing the number of periods of REM sleep. The last

category will receive the number of central phasic waves that appear in the epoch.

A 30 sec epoch length will be used, this is mainly dictated by convention. One animal will be scored in 15 sec epoch lengths to determine whether this variable produces a significant difference in results.

Inaccuracy is always present in the quantification of a continuous variable. I believe that the error I introduce by my method is a tolerable one. Even more important than random error is systematic error or bias. The method used to quantify the distribution of the waves is to measure the frequency of their occurrance in five mutually exclusive categories: 1) awake, 2) REM, 3) SWS, 4) SWS within 2 minutes of REM onset (R-2") and 5) SWS within 2 minutes of a spontaneous awakening (AW-2"). Categories 2, 4 and 5 are where I hypothesize the waves to occur, and categories 1 and 3 where they will not occur.

It is possible to have a wave appear in an epoch scored A that actually occurs during SWS. This could happen if the animal entered sleep at the end of an awake epoch. It is highly unlikely that this interval of SWS is within 2 minutes of a REM period since rats will have several minutes of SWS before entering REM sleep from awake. The error does not produce bias in the direction of my hypothesis since both AW and SWS are categories in which I predict no spikes. Another possibility is that the subject awakens from SWS at the beginning of the epoch. Such a case would produce bias, however, it is against the hypothesis since the wave occurred

in SWS within 2 minutes of a spontaneous awakening but is counted as awake. Another set of possible errors can occur in epochs scored SWS. The animal could enter SWS near the beginning of the epoch, and the wave could occur during the AW part. Again, it is highly unlikely that it is within 2 minutes of a REM period, and no bias with respect to the hypothesis is introduced. If the animal awakens near the end of the epoch, however, the awake wave would be scored in SWS within 2 minutes of a spontaneous awakening. This does introduce bias. I will sample the records and empirically make any corrections in the scoring system as necessary. For example, if I find that one out of 20 epochs of S within 2 minutes of a spontaneous awakening contains an awake wave I can take one out of 20 waves scored in this category and put it in the awake category. To elaborate on the remaining contributions of R, AR, SR and M with all their permutations would take several pages so suffice it to say that I am aware of the problem and will empirically determine any adjustments that will have to be made in the scoring procedure.

The Experiments

A. The Normative Study

I hypothesize that the normative characteristics of the central phasic event in the rat will be similar to that of the PGO wave recorded in the cat. Specifically, that the distribution of the waves will not be random through the day but related to REM sleep reflecting the operation of the underlying REM sleep process.

Electrophysiological data will be recorded continuously for a 24 hour period in six rats in which a candidate for a REM related wave has been tentatively identified. By analyzing this data I can determine the distribution of the waves with respect to my predictions. Randomly selected portions of this material will be recorded on magnetic tape for a subsequent analysis of the waveforms on an oscilloscope, and a determination of the relationship between the wave and eye movements will be made.

B. The Mapping Study

I hypothesize that the loci in the brain from which REM sleep central phasic activity can be recorded will be supportive of the concept that this activity is dependent on a central multi-directed brainstem outflow, I expect to find relatively discrete, however widespread areas from which central phasic waves can be recorded.

Bipolar macroelectrodes will be stereotaxically aimed at the pons of 40 rats. Analysis of the electrical activity obtained from these electrodes will be based on recordings including at least three REM periods. The animals will be sacrificed and the locus at the tip of the electrode will be determined from the appropriate histological material. Each site and a code representing the nature of the electrical activity obtained will be placed on a series of coronal sections representing the pons. Relationships of the sites and activity will be determined from these maps.

C. Manipulations to Effect the Distribution of Central Phasic Waves

1. The PCPA study

I hypothesize that a similar 5-HT dependent gating mechanism

that controls the distribution of PGO waves in the cat also influences the distribution of central phasic waves in the rat. Therefore, by administering the 5-HT depleting drug PCPA I should cause a significant increase in the frequency of waves that appear in waking. If I am successful at demonstrating this effect not only has the existance of a REM related wave in the rat been demonstrated but evidence has been gathered to support the fact that mechanisms subserving it are similar to those subserving PGO waves in the cat, further evidencing a universal mammalian REM sleep process. 300 mg/kg dl PCPA methyl ester will be used. This form is preferred because of its water solubility. In the rat, 316 mg/kg has been shown to reduce brain 5-HT levels to 18% $\pm$ 2% of control in 24 hours (Koe and Weissman, 1970). The effect of the drug will be measured in four rats in which I have identified central phasic waves. The distribution of the waves will be examined for a 24 hour period following 24 hours after drug administration. Maximal depletion of 5-HT is achieved during this period (Koe and Weissman, 1970). The results will be compared to a 24 hour period following 24 hours after the administration of an equal volume of sterile normal saline. Each animal will serve as its own control.

The literature dealing with rats and PCPA on sleep amounts is inconsistant (Rechtschaffen et al, 1973). I offer a tentative hypothesis that PCPA, 300 mg/kg, will reduce SW sleep and REM sleep while increasing waking. This will be tested in five rats using PCPA methyl ester HCl in the same paradigm just described.

I am also interested in the relationship between PCPA induced changes in sleep stage amounts and central phasic wave distribution. It is possible that PCPA has a direct effect on only one of these variables which is then causative to any changes in the other variable.

I hypothesize that the effects of 5-HT depletion on sleep stage amounts are mediated through the effects on the phasic event system of REM sleep. Dement (1970) has hypothesized that after PCPA the appearance of PGO waves in SWS at times when they would not normally occur, awaken the animal thus accounting for the reduced amount of SWS. REM sleep amounts may be reduced, first, by its natural dependence on preceeding amount of SWS to occur before its appearance, and second, due to a diminution in the need for REM sleep since a process that normally occurs during this period is now occurring at other times. The evidence for such a view consists of: 1) the alteration of the distribution of PGO waves is more highly correlated to brain 5-HT levels after PCPA than is the induced insomnia, 2) the occurrence of PGO waves during SWS is associated with spontaneous awakenings, and 3) during PCPA treatment when PGO waves are not tied to the occurrance of REM sleep, a REM deprivation procedure does not lead to a rebound, possibly indicating a reduced need for REM sleep.

These hypotheses lead to specific predictions about the relationships between changes in stage amounts and the distribution of central phasic waves under PCPA and will be tested on the four subjects used in the PCPA on waves paradigm.

Further, if PCPA on sleep amounts leads to inconsistant results across subjects it may be possible to account for this variability by using the distribution of waves variable. I hypothesize that a rat which does not show reduced SWS after PCPA either does not re-distribute the waves into SWS, or it can be shown for this particular case that the waves are not normally associated with spontaneous awakenings. I also hypothesize that if PCPA causes waves to appear in waking and SWS, then reductions in REM sleep will also be seen regardless of a reduction of SWS amounts.

2. The REM deprivation study

I hypothesize that by selectively depriving rats of REM sleep the occurrence of central phasic waves will shift so that the number of waves occurring in the category of SWS, 2 minutes before REM onset will be increased. This effect has been observed in the cat (Vimont-Vicary, 1966) and has been used as evidence of the primacy of the central phasic event process in that deprivation of the tonic aspects of REM sleep does not stop the occurrence of the phasic events but increases the frequency of PGO waves just before the REM period. The significance of the REM deprivation phenomenon to my work is that it gives me yet another opportunity to perform a manipulation to test the congruence in mechanisms between the process that generates PGO waves in the cat and that which generates REM sleep central phasic waves in the rat.

The REM deprivation will be achieved through hand awakenings. The polygraph will be monitored continuously. When a subject is observed to have entered into REM sleep the experimenter will run

into the next room and shake the running box to awaken the animal. This procedure will be repeated every time the tonic signs (EEG, EMG) of REM sleep appear. In a 24 hour period more than 200 awakenings may occur.

There are more convenient ways to REM deprive small animals. One such method is the island technique. Such a method is not adequate for the purposes here. The scoring system necessitates knowing whether an awakening is spontaneous or induced by the experimental manipulation, (Category; SWS within 2 minutes of a spontaneous awakening). Therefore only with the method of hand awakenings can it be known if an awakening was spontaneous or experimenter induced.

The experimental design will incorporate 72 hours of continuous polygraphic recording representing three 24 hour conditions. The first will be a baseline control condition, the second, the REM sleep deprivation condition and the third, a recovery from the deprivation.

## III. Details of Experimental Methods

### Subjects

Sprague-Dawly, albino rats (Holtzman) weighing between 295 and 585 gms at the time of operation were used. Sixty-four rats survived the surgical procedure.

### Surgery

All injections were administered intraperitoneally (IP) unless otherwise stated. Subjects were pretreated with 0.3 ml of 0.43 mg/ml atropine sulfate (Vitarine). Initial anesthesia, 40mg/kg pentobarbitol (Abbott), was followed by 150mg/kg chloral hydrate (Ruger). If a surgical depth of anesthesia was not reached after 15-20 minutes ether was administered. The subject's head was shaved, washed and placed in the ear bars of a stereotaxic apparatus (Kopf). A midline incision was made through the skin of the scalp and neck. The skin was reflected and the skull exposed. The incisor bar was adjusted to achieve a flat skull so the lambda ($\lambda$) and bregma ($\beta$) sutures were at the same stereotaxic height. A deep level of anesthesia was maintained through supplementary doses of chloral hydrate (approximately 30mg/hour), lidocaine hydrochloride (2%w/v) Vitarine) at pressure points, and ether when necessary.

All subjects were prepared for chronic sleep recording using prefabricated bipolar stainless steel electrodes and connector assemblies (Plastic Products): 1) EEG electrodes consisted of two stainless stell screws tapped into the skull: (a) placed between the $\lambda$ and $\beta$ sutures, 2.0 mm lateral left from the midline, and (b) placed 1.5 mm anterior to $\beta$, 1.5 mm lateral right from the

midline. Each pole (0.01 inch in diameter) of an insulation free electrode assembly was wrapped around each screw. 2) EOG electrodes consisted of the same type of electrode used for the EEG. Removing 1.0mm of insulation from the tips, each pole was placed subcutaneously near the inner and outer canthi of one eye. 3) EMG electrodes consisted of a bipolar spring electrode. Teflon tubing insulated each pole except for 2cm from the tip. After incising the acromiotrapezius muscle bilaterally the levator scapular dorsalis muscles of the neck were exposed. Suture material was then led around each of these longitudinal muscles in two places. One pole of the electrode was placed on top of each muscle. The suture was then slipped between the spring elements and tied holding each pole in place. The trapezius muscle was then closed with dissolvable suture and the skin closed with silk. All electrode assembly connectors were fastened to the skull with a quick drying dental acrylic cement (Yates).

The recording of gross electrode potential changes from deep structures in the brain was achieved by stereotaxically implanting either one or two twisted bipolar electrodes (0.01 inch diameter) insulated, except at the cross-section of the tips. A coordinate system based on skull landmarks was employed. Anterior-posterior coordinates are based on a hypothetical line in the frontal plane passing through the $\lambda$ suture 2.0mm lateral to the midline ($\lambda\lambda$). Lateral measurements are taken from the midsagittal suture and depth measurements are taken from the surface of the skull. The majority of placements were aimed at the nucleus locus ceruleus (LC), with

a vertical entry 0.3mm posterior to λλ , 1.0mm lateral from the mid-line and 7.0mm from the surface of the skull, (λλ -0.3/L-1.0/7.0 fs). These coordinates served as the center of the investigation of the pons. Each dimension varied up to ± 1.0mm. The anterior lobe of the cerebellum was also included.

The depth electrode assemblies were fastened to the skull with acrylic cement and the scalp sutured closed. Post-operative treatment consisted of applying a topical antibiotic (Bacitracin) to the wound and placing the animal in a clean cage lined with linen. Water and wet mash food was available. After two days animals were moved to standard rack mounted shoe box cages lined with saw dust bedding on an ad libitum diet of rat pellets (Purina or Wayne) and water on a 12 hour/12 hour light-dark cycle. At least seven days elapsed before any further procedures were carried out.

Recording situation

The recording situation consisted of four sound attenuated, light tight running boxes (Scientific Prototype) with impedence protected fans for ventilation. These boxes were supported by metal shelving in a wooden enclosure whose walls were lined with steel and copper screening. A common ground connected the shelving and screening. Inside each running box were three 24v/10w light bulbs, two clear (light cycle) and one red (dark cycle). On one wall of each box was a one way glass where a television camera was sometimes mounted (Sachel-Carlson).

A 2cm hole was drilled into the top of each box. Above this hole a 16 channel commutator (Air Flyte) connected to a shielded cable was

mounted on a stand. The cable was then led out of the room to the polygraph. The two machines used in the studies were: 1) a five channel Grass model #78 polygraph, and 2) a nine channel Grass model #6 electroencephalograph.

Leads were attached to the connectors on the subject's head during light ether anesthesia. The animal was placed in a sawdust bedded glass aquarium tank (16"x8"x10") with food and water. The tank was then placed inside the running box. The lead was put through a hole in the wire mesh tank cover, the hole in the running box, and connected to the commutator. A loop was made in the lead and held by a spring. The box was closed leaving the subjects to adapt to the recording situation for at least two days. Food and water were checked daily.

General Recording and Scoring Procedures

Electrophysiological data was collected on an ink writing polygraph with a paper speed of 10mm/sec. Using high impedence input AC amplifiers (Grass 7P511) with 60 cycle notch filters on, the following filter setting were employed: (EEG) low frequency (LF) filter- 1Hz, high frequency (HF) filter- $\geq$ 30 KHz; (EMG) LF-3Hz, HF- $\geq$ 60 KHz; (EOG) LF- 0.1Hz, HF-100Hz; (depth electrodes) LF-3Hz or 5Hz, HF-$\geq$ 60 KHz. The recordings were deemed acceptable only if there was an absence of a sizable amount of 60 cycle (or multiple) noise, assessed with a paper speed of 100mm/sec, and by the presence of changes in frequency and amplitude over time. The latter indicating responsiveness to electrophysiological changes.

The procedure used for sleep staging has been discussed. It

is based on an adaptation of the Rechtschaffen and Kales method. All records were scored for staging by one person utilizing the EEG, EMG and EOG only. Attempts were made to keep the knowledge of the experimental condition from the scorer.

The procedure for scoring central phasic waves from the depth electrode has been discussed. The sleep stage scorer developed the criteria for the waves of a particular subject based on the recordings of three REM periods. This criteria was then used by a different individual, who had no knowledge of sleep staging, to score the waves on all additional recordings of that subject. Attempts were made to conceal the experimental condition by removing all uncoded identification.

The Experiments

A. The mapping study

After recovery from surgery and an adaptation to the recording situation an initial recording test was performed. This was conducted in the early afternoon with a minimal duration of two hours containing at least three REM periods of at least 30 seconds duration. If the record was acceptable in quality it was scored to determine if a central wave criteria could be developed. The criteria for a positive assessment was that at least 80% of the waves be in REM sleep and in SW sleep two minutes before REM onset or a spontaneous awakening. This procedure was carried out on 40 subjects. Only those animals with suitable quality recordings were included in the study. For each subcortical electrode of each animal, data were recorded to note whether or not a central phasic wave criteria could be developed. If it could then the MAC, average signal to

noise ratio, and an interval scale characterization of the distribution was also recorded.

After all data had been collected the subject was sacrificed under Pentobarbitol anesthesia, and perfused intracardially with normal saline followed by 10% formalin. Frozen coronal sections (American Optical Freezing Microtome), mounted on glass slides, were stained with cresyl violet. These stained brain sections were examined under the light microscope to determine the position of the electrode tips by a comparison of the electrode tract terminus with brain atlases of the rat (Craigy; Swanson; Pelegrino and Cushman). In this procedure, no reference was made to the type of data recorded from the subject. Confirmation of the electrode placements were made by an independent judge. Any discrepancies in the calls were settled by discussion until full agreement was reached between judges. Only those subjects in which the electrode placement could be confidently determined were included in the study. There were 45 cases. Data from the histology and the initial tests were combined on a series of coronal sections of the rat brain to produce a "map" describing the topographical distribution of electrical activity.

B. The Normative Study

Assessment of the normative characteristics of the central phasic waves was based on 24 hour continuous polygraphic recordings from six subjects in which a criteria for a central phasic wave in the initial test could be developed. This condition also served as a baseline control for other experimental manipulations. Four of the subjects received a saline injection 24 hours before the start

of this condition.

The distribution of the waves was quantified as previously described. The frequency of occurrence of the waves in the five mutually exclusive categories as well as the time spent in each category was determined. Measures of frequency, rate, weighted rate and percentage of total were computed for each category.

The characteristics of the wave forms were examined by randomly selecting and recording gross potential changes from the deep electrodes and EOG onto FM magnetic tape (Ampex). The tape was later played back into a storage oscilloscope (Tektronics). In a random fashion, a single sweep was stored on the scope until a central phasic wave appeared. The screen was then photographed (Canon), for a permanent record, on 35mm film. In order to determine the amplitude of the signals, a 50uv calibration signal was also photographed.

In a similar manner the relationship between the occurrence of central phasic waves and eye movements during REM sleep was examined. Two channels of the storage oscilloscope were used and latencies between waves and eye movements determined.

C. Manipulations to effect Central Phasic Waves

1. The PCPA study

Four rats received an IP injection of a volume of sterile normal saline. 24 hours after the injection, a 24 hour baseline condition was recorded. Another IP injection of equal volume containing a solution of 300mg/kg dl PCPA methyl ester HCL (K&K) and sterile water immediately followed. 24 hours after the PCPA injection a

24 hour drug condition was recorded.

A comparison of the distribution of central phasic waves between the 24 hour baseline and 24 hour drug conditions were achieved by quantifying the distribution of waves into the five mutually exclusive categories (previously described).

The effects of PCPA on sleep stage amounts were examined in five subjects including the four subjects just described. Three categories of stage were used: awake, SW sleep, and REM sleep.

2. The REM deprivation study

Two rats, in which a criteria for central phasic waves could be developed were recorded continuously for 72 hours. Only one subject was run at a time. The first 24 hours constituted the baseline condition. During the second 24 hours a REM deprivation procedure was carried out using hand awakenings. The polygraph was closely and continuously monitored by an experimenter. Every time the animal was observed to enter into REM sleep, as determined from the EEG, EMG and EOG, the experimenter as quickly as possible went into the enclosure housing the running boxes and tapped on the box until the subject showed an EMG burst. As awakenings became more difficult, the awakening stimuli became more vigorous. During the final 24 hours the subjects were left undisturbed. This constituted the recovery condition.

Sleep stage amounts and the distribution of the central phasic waves for each 24 hour condition were quantified as previously described.

# IV. Results

## Subjects

A total of 40 subjects that under went successful surgical implantation of electrodes in 45 subcortical sites, were selected for the series of investigations described above. All displayed high quality polygraphic recordings in conjunction with histological material that allowed a high level of confidence in the localization of the electrode placements.

## Normative Study of Central Phasic Activity in the Rat

The object of this study was the determination of the distribution of the phasic waves in the brainstem of the rat and measurement of their waveform characteristics.

### A. Subjects

Six rats in which criteria for central phasic waves could be established in the multiple sites in which they were found, will be considered as a group in order to determine some generalizable properties of central phasic waves in the rat. The brain sites comprised the midline cerebellum, lateral locus ceruleus (lc), and nucleus tractus mesencephali (ntm), lateral fasciculus longitudinalis medialis (FLM), ventral lc, lc, and the nucleus tegmenti ventralis von Gudden (ntv).

### B. General Pattern of Appearance of the Central Phasic Waves

Figure 3 is a representative polygraph tracing showing the transition from slow wave (SW) sleep to REM sleep. On the channel labelled "R pons" (in this case the lc) appears readily discriminable wave forms several times larger than the background signal.

An MAC is easily developed to produce an objective criteria to measure the occurrance of this phenomenon to the exclusion of background signal and artifact.

Figure 4 shows the transition from SW to awake (AW). Notice that the central phasic waves are distributed in a very similar manner in pre-awake epochs as in pre-REM epochs. The waves cease at the onset of AW.

Figure 5 shows a representative segment of REM sleep. The waves can appear singly or in groups, the latter restricted only to REM sleep where they have a high association with the rapid eye movements characteristic of this stage.

C. Wave Form Characteristics

Figure 6, the channel labelled pons of figure 7, and the channel labelled L pons of figure 8, are representative oscilloscope patterns of REM sleep central phasic waves. They are monophasic, always of the same polarity, of approximately 100msec in duration. A second component of opposite polarity may be present, but its characteristics are very variable (as in figure 6), and is not considered in defining the wave. The amplitude of the wave is more variable between subjects than within. Table 4 reports the range of MACs used: 15.8$\mu$v to 66.7$\mu$v. Typically, waves appearing in REM sleep have higher amplitudes than waves in non-REM sleep.

The gross characteristics of the monophasic wave form are quite constant within and between subjects though some variability exists.

There appears to be bilateral synchrony in the occurrance of central phasic waves in the pons. Figure 8 illustrates the simultaneous appearance of a group of two waves in the right and left pons.

A gross correlation between the waves and eye movements has been determined from randomly selected REM periods: the probability that a single eye movement and a single wave appear together is 0.35, whereas for grouped waves, the probability is 0.86. Figure 7 shows the relationship of a central phasic wave to an eye movement. In this case, the wave precedes the eye movement by approximately 15msec (as measured by departure from baseline). This relationship is quite variable in the sites recorded and no statement about a fixed latency can be made in this study.

D. Wave Distribution with Respect to Sleep Stage

The 24 hour distribution of central phasic activity can be quantified in many different ways. The scheme used here was to divide the time into five mutually exclusive categories, with respect to sleep. It is hypothesized that the waves are not distributed in a random manner, but are rather concentrated mainly within REM sleep and the two minute periods preceeding REM sleep onset and spontaneous awakenings.

Four measures are used. The results of each are presented. Correlated "t" tests were used, with a 0.05 level of significance.

1. Absolute frequency

Table 1a describes the absolute frequency of occurrance of the waves as they are distributed in the five mutually exclusive categories throughout the 24 hour day. As hypothesized (one tail)

the frequency in REM sleep is singificantly higher than every other category (correlated "t" test minimum value 2.7, df=4). Other statistical differences are revealed in table 1a.

This reflects a non-random distribution of waves with respect to the five categories. The frequency in AW-2' is significantly greater than SW (cor t=2.7) and AW (cor t=3.1). The average total number of waves per 24 hours is 2,728.7 of which R-2' =166.7, AW-2'=290.5, REM=1,986.7, SW=138.3, AW-150.5. As predicted, the vast majority of the waves appear in REM sleep.

2. Rate per minute

The absolute frequency does not take into consideration the proportion of time occupied by each category. Accordingly, rate was also computed (table 1b). The rate in REM sleep is significantly greater (min cor t=3.3, df=4) and several times greater than any other category. The rate of each category as compared to each other is significantly different (two tail) except between R-2' and AW-2'. The average rates per minute are: R-2'=1.48, AW-2'=1.05, REM=15.72, SW=0.60, AW=0.22. This clearly indicates that the waves are highly associated with REM sleep. It should be noted that between subjects an exceedingly high variability is associated with the two preceding measures (see tables 1a and 1b). The standard deviation within each category is often in excess of 50% of the mean. In order to reduce variability two other measures were computed, weighted rates and percent frequency.

3. Weighted rate

Rate per minute was adjusted by multiplying, within each

subject, each category by a constant (2000/total frequency). This constitutes a relative measure so that the distribution across animals can be examined "as if" the rate was such to produce a total frequency of 2000 waves in 24 hours. As a result, the variability in REM was drastically reduced and only slightly so in awake. All categories are significantly different from each other (see table 1c). The average weighted rates per minute are: R-2'=1.26, AW-2'=0.90, REM=11.21, SW=0.53, AW=0.18. It is clear that the weighted rate transformation maintains the distributional qualities of the waves, again indicating the predicted distribution.

4. Percent frequency

Absolute frequency was transformed to a relative measure to compare distributions and reduce variability by computing the percent frequency, per subject, that each category was of the total frequency. Variabilities as a percent of the mean were reduced, except in AW-2' (see table 1d). The percent of waves in REM sleep is significantly greater than each of the other categories (min. cor t=6.8). Other differences are similar to the measure of absolute frequency. For the sake of comparison it is useful to note that: 7.67% of the time is spent in R-2' and 6.58% of the waves occur here; 19.24% of the time is spent in AW-2' and 12.6% of the waves occur here; 14.52% of the time is spent in SW and 5.28% of the waves occur here; and 50.01% of the time is spent in AW and only 6.52% of the waves occur here.

Table 2 shows the Pearson correlation matrix between the different measures. All measures correlate highly. The absolute

measures together, and the relative measures together, have coefficients in excess of 0.98.

Another consideration is that of time spent in each category. Table 1e shows that the distribution of time spent in each category is not random as evidenced by the significant differences in most of the categories. Hypothetically, if the waves were randomly distributed throughout the day, then one would expect the greater number of waves to occur in the category with the largest time. Table 2 demonstrates this not to be so since the sign of the correlation coefficient of every measure versus time is negative.

All of the four measures demonstrate a similar distribution of the phasic waves in the rat, but each measure examines the distribution in a different way. Frequency examines the absolute number of waves in each category. This measure introduces certain difficulties in comparisons between animals since the total number of waves occurring in 24 hours between animals differs greatly. This also introduces a high degree of variability within each category. Absolute frequency does not take into account the amount of time spent in each category so a rate was computed. Rate suffers from the same deficiencies as absolute frequency. Two animals can have the same exact distribution of waves yet because the waves occur generally more frequently in one of them, a comparison within the same category will reveal different rates. The relative distribution of central phasic waves is of primary importance here so it is more appropriate to use relative measures. Rate was transformed to a weighted rate and absolute frequency to a percent

frequency in an attempt to characterize the distribution of waves independent of the variability in the number of waves that occur across animals over a 24 hour period. In subsequent manipulations only the relative measures will be used to test for significant differences. Differences that are revealed do not necessarily reflect absolute differences in the number of waves but rather relative differences reflecting changes in distribution. The variability in the absolute number of waves may be due to differences in amplitude criteria, electrode placement, or in yet some unidentified factor and will require more research for its elucidation.

In summary, the central phasic waves recorded in the rat predominantly occur within REM sleep, within two minutes of REM sleep onset, and within two minutes of a spontaneous awakening.

E. The Distribution within REM Periods and by REM Period Length

Since most waves occur within REM periods, an analysis of the distribution of the waves within REM periods by REM period length was carried out. Table 3 is a summary of the distribution of central phasic waves within REM sleep based on six subjects recorded for 24 hours each. The summary includes REM periods equal to or greater than two 30 second epochs in length up to REM periods of six 30 second epochs in length. There were REM periods of greater length. However, they were rare and therefore excluded from this analysis.

There is a marked tendency for the frequency of waves within a REM period to start off slow, increase toward the midpoint of the period, and then trail off towards the end irrespective of REM period length. The majority of REM periods are two to three 30 second epochs in length, but they do not account for the majority

of REM phasic waves becuase of the marked tendency for the number of waves to increase with the length of the REM period. For example, REM periods of two epochs in length account for 24.8% of all REM periods, but account only for 9.8% of REM waves. REM periods of epoch length four account for 19.6% of all REM periods, and account for 22.8% of all REM waves.

F. Pre-REM and Pre-Spontaneous Awakening Waves

Central phasic waves almost always appear in SW sleep preceding REM sleep onset. The frequency of occurrence is lower than that of the first 30 second epoch of REM sleep. The waves in pre-REM always appear singly, never in groups. Since eye movements do not occur at this time, the association of the waves to eye movements is zero.

When the waves appear before spontaneous awakenings, central phasic waves have a very similar distribution in SW sleep pre-spontaneous awakenings. In contrast to the ever present pre-REM waves only 51.5%$\pm$ 11.6% of spontaneous awakenings are preceded by waves.

G. Awake and Slow Wave Sleep Waves

Isolated single waves do appear in AW and SW sleep. Some of the waves appearing in SW sleep occur within two minutes of a gross body movement. These arousals are of such short duration they are not considered an awakening, or can these transient arousals account for all the SW sleep waves. Waves that meet the criteria for central phasic waves do occur, although at low frequency, in AW and SW sleep.

In conclusion, the wave form and distribution of central phasic waves recorded in rats were determined to be associated with REM sleep and possess many similar characteristics to the PGO waves

recorded in cats. These similarities will be discussed in the final section.

The Mapping Study

Forty-five electrode sites were characterized as to their location and electrophysiological properties (see table 4). Figure 9 is a photomicrograph typical of the quality of the histological material from which the electrode sites were determined. Electrode sites assessed positive for the presence of REM sleep related central phasic waves are distributed in a widespread yet relatively discrete manner.

A. The Dorsal Pontine Tegmentum

Eleven sites located in the area of the nucleus lc and the ntm in frontal planes between 1.5mm and 2.0mm posterior to the interauricular line, were all assessed positive for REM central phasic waves. Seven sites immediately surrounding this area were assessed negative for central phasic waves (see figure 10a). These negative areas include: medially, the nucleus tegmenti dorsalis von Gudden (ntd); laterally, the pedunculus cerebellaris superior (PCS) and nucleus parabrachialis dorsalis (npd); dorsally, the cerebellar vellum above ventricle IV; and ventrally, the nucleus parabrachialis ventralis (npv) and just lateral to the FLM. The high density of the electrode placements in this area revealed a discrete distribution differentiating positive and negative sites. Other areas, with lower densities of electrode placements could not be as well defined.

Another area differentiated at this level is the lateral and

dorsal aspects of the FLM. Four electrodes were positive for central phasic waves. Just lateral to this fiber tract negative sites were found as posterior as 2.8mm from interaural zero. A negative site was revealed just ventral to the FLM at p1.0mm. Another negative site, at the level of the decussation of the PCS (P0.5mm), was judged to be just impinging on the ventral aspects of the FLM. At level P0.1, a site clearly within the limits of the FLM, was assessed positive.

Two other electrode placements yielded positive results in this region. Negative controls are lacking, however, so no statement can be made as to how localized this activity is. An electrode tip judged to be confined within the limits of the small ntv at the level P2.0 was assessed positive. Another site located at the medial margin of the nucleus reticularis pontis oralis (rpoo) at p1.5 was also positive.

B. Area of the Mesencephalic-Metencephalic Junction (P1.0-P0.1)

The dorsomedial aspects of the rpoo at level P0.5 presented the only clear conflict in assessed activity. At level P1.0 the dorsomedial rpoo is negative. At P0.5 one site is negative and one positive. A possible explanation lies in the fact that the positive site is slightly more medial and may impinge upon the tractus tectospinalis (TT) as it courses from the colliculi to the spinal cord dorsal to the medial lemniscus (see figure 10b).

At level P0.5 another positive site is located at the dorsomedial margin of the nucleus parabrachialis dorsalis just ventral to the decussation of the PCS. This region is also located

within the border of the dorsal noradrenergic bundle as described by Palkowitz and Jacobavitz (1974), but since we can not visualize this structure in our histological material this is a speculative reference.

Two additional positive sites not previously discussed can be found in this region at level P1.0. One is located medially to the dorsal aspects of the ntm in the substantia greisea centralis or central grey. I could not visualize the nucleus lc at this anterior level, however, Palkowitz and Jacobvitz (1974) found catecholamine cell bodies associated with the ceruleur system at this level. Another positive site is located along the midline dorsal to the ventricle in the colliculus inferior (ci).

C. Area of the Mesencephalic-Diencephalic Junction (A2.2)

Two sites located in the frontal plane, 2.2mm anterior to the interauricular line (A2.2) yielded negative results with respect to REM sleep central phasic waves. One is located in the dorsal central grey and the other in the nucleus centralis corporis geniculati medialis (see figure 10c).

D. The Anterior Lobe of the Cerebellum (P2.0-P2.6)

Five electrode sites were located in the anterior lobe of the cerebellum: four were assessed positive for central phasic waves and one negative (see figure 10d). There are neither enough sites nor a distribution such that a definitive statement can be made as to discrete localization of activity. The positive sites are located in (notation from Larsell, 1952): (1) the lingula (cI), (2) spanning the ventral lobe of lobus centralis (cII) and

cIIIa, (3) sublobe cIIIa, and (4) cII. The negative site is located in the ventral lobe of the lobus centralis (cII). The differentiating characteristic between the positive and negative sites may be that the negative site was the only one to be primarily situated in white matter whereas the positive sites all impinged on granular and purkinje cell layers.

E. The Distribution of Electrophysiological Parameters.

Table 4 lists each subject including a description of an ordinal scale characterization of the distribution of central phasic waves between the mutually exclusive categories, the MAC in microvolts, and the ratio of the MAC to the average background signal, (averaged over SW, AW, REM).

Figures 10a-c depict the anatomical distribution of the quality of the distribution of waves within categories. 20 sites were considered to have a good distribution, four fair and three poor. At the level P1.5, two sites impinging on the lc: (1) at the extreme dorsal aspect and (2) at the extreme ventral aspect, were considered fair. At the same level, the dorsal FLM and medial rpoo were also considered fair. Again at level P1.5, the two lateral FLM sites were considered poor. Finally, at P0.5, the dorsomedial rpoo, possibly also impinging on the TT, has a poor distribution.

In conclusion the anatomical distribution of positive and negative brain loci with respect to central phasic waves seem to indicate a discrete yet widespread distribution.

Manipulations to Effect the Distribution of Central Phasic Waves

The object of these studies was to determine the effect on

central phasic waves in the rat. A pharmacological manipulation (PCPA administration) and a behavioral manipulation (REM deprivation) were used.

A. PCPA on Central Phasic Waves

1. Distribution between categories

A group of four subjects received 300 mg/kg PCPA methyl ester HCL. Each subject was used as its own control. Tables 5a-b compare the effects of PCPA to a vehicle control period on the absolute measures of frequency and rate. The means of the sample indicate an increase in the number of waking waves under PCPA (166 vs 513), eventhough there was a 55% reduction in the total number of waves occurring in the 24 hour PCPA condition as compared to the vehicle control. The mean rate at which waves appeared in waking was increased 2.5 fold under the drug.

Statistical tests were carried out using the relative measures of weighted rate and percent frequency. Correlated "t" tests with significance levels less than 0.05, df=2 were used. Tests between conditions in categories AW and SW were one tail, all others two tail tests.

Quantifying the distribution of central phasic waves by weighted rate revealed a significant increase in rate in categories pre-REM and pre-spontaneous awakening due to PCPA (1.18 vs 2.53, 0.92 vs 1.89, cor t=3.3, 3.5 respectively). The rate in SW was also significantly increased (0.54 vs 1.75, cor t=3.1). PCPA tended to increase the rate of awake waves (t=2.3, $t_{crit}$=2.96). The average awake weighted rate was 3.7 times greater under the drug then the

vehicle control and all subjects showed an increase (0.19 vs 0.71). Statistical significance using the correlated "t" test, was not reached in this category (see table 5c).

Percent frequency revealed a significant increase in the proportion of waves occurring pre-spontaneous awakening (13.18 vs 23.68, corr t=5.1), a decrease in REM sleep (67.94 vs 31.85 corr t=3.0) and an increase in awake (6.44 vs 31.06 corr t=3.2) (see table 5d). It is clear that PCPA has an effect on the distribution of central phasic waves. The drug causes a proportional increase in rate in SW, a proportional increase in the number of waves that appear in waking, and a proportional decrease in the number of waves appearing in REM sleep.

For a comparison of the change in distribution of the number of waves and time in each category see figure 11. PCPA caused a 36.4% increase in time spent awake and a 209.0% increase in the number of awake waves, even though the absolute number of waves in 24 hours was reduced. Figure 12 shows central phasic waves appearing in wakefulness 36 hours after a single injection of 300 mg/kg PCPA.

It appears that PCPA has two effects with respect to waking. One is to increase the amount of time spent awake and the other to increase the proportion of waves appearing in waking. This latter effect cannot be accounted for solely by the increased time spent awake due to the vast differences in their proportional changes. If the percentage of waves occurring in awake were increased solely due to an increase in waking under the drug, then the proportional

change in this percentage should equal the proportional change in time spent awake. Using this assumption, expected values for the percentage of waves occurring in awake were computed for each subject in the PCPA experiment based on each subject's proportion of increased waking seen after drug administration. The mean control percentage of awake waves was 6.44 percent. Taking the proportion of increased waking into account, the expected mean percentage increased to 8.82%. The observed mean percentage of waking waves after the drug was 31.06%. There is still a significant increase in the percentage of waking waves after PCPA when increased awake time is taken into account (cor "t"=2.78, $P < .05$, one tail).

2. Waves within REM periods

Table 6 shows an analysis of the distribution of waves within REM periods. As can be seen, PCPA did not effect the proportional distribution of waves within REM periods. The tendency to have the highest rates in the middle of a REM period was still maintained. There was a tendency to shift to shorter REM periods under PCPA, but no significant differences could be demonstrated: 24.2% of all REM periods on baseline were of epoch length two, on PCPA, 49.6% were of this length. The total number of REM periods was significantly reduced under the drug: the baseline mean is 53.75 and PCPA mean, 21.5 (cor t=9.3, df=2). The decrease in rate of waves within REM could be interpreted as being due to the decrease in REM period length. The decrease in number of waves could be seen as a combination of decreased length and decreased number of REM periods. Grouped waves were still seen only in REM sleep even under the PCPA condition.

3. Pre-REM and pre-spontaneous awakening waves

The characteristics of the pre-REM and pre-spontaneous awakening waves remained relatively constant across baseline and drug conditions. The absolute rate at which they appeared was not altered. The relationship of waves to spontaneous awakenings also seemed not to be affected with 54.5% of all spontaneous awakenings being preceded by waves on baseline and 48.8% preceded by waves during the PCPA condition.

4. PCPA on sleep stage amounts

A group of five subjects received 300 mg/kg PCPA. Correlated "t" tests were used to detect differences in means in categories AW, SW and REM between the vehicle baseline condition and the PCPA drug condition. One tail tests were used with significance levels having a probability less than 0.05, degrees of freedom equals three, and $t_{crit}$=2.35.

Using the measures, number of epochs and percent total recording time spent in each category, all categories underwent significant changes as a result of drug administration. AW was increased (1410 vs 1920 epochs, cor t=8.0), SW sleep decreased (1210 vs 832 epochs, cor t=6.2), and REM sleep decreased (243 vs 87 epochs, cor t=22.3) (see tables 7a-b). The REM percent of total sleep time was also decreased (17.0 vs 9.4, cor t=6.6) (see table 7c). The reduction in REM sleep was accomplished primarily by a reduction in the number of REM periods (53.75 - 21.5, cor t=9.3). There was also a marked tendency towards REM periods of shorter length (8.4% of epoch length two vs 26% of epoch length two). The increase in waking was almost totally due to an increase in the length of each awake period since the average number

of spontaneous awakenings remained fairly constant between baseline and drug conditions (246.3 awakenings on baseline, 250.8 on PCPA).

5. Relationships between central phasic waves and changes in sleep

An examination of certain possibly meaningful relationships between central phasic waves and sleep parameters as they are influenced by PCPA was accomplished by the computation of a Pearson product moment correlation coefficient and "t" test to determine if the coefficient is singificantly different from zero (df=2, $t_{crit}$-r $= \pm$ 0.95, two tail). These relationships were investigated in the four subjects, assessed positive for central phasic waves, receiving 300mg/kg PCPA.

To test the relationship between the degree that waves are naturally associated with spontaneous awakenings and the ability for PCPA to reduce sleep, the correlation coefficient between the percent frequency of waves in category AW-2' on baseline and the SW sleep percent of baseline under PCPA was computed. This tests the hypothesis, that if central phasic waves are highly associated with spontaneous awakenings, and if PCPA redistributes these waves, then their occurrance at times when they would not normally occur should serve either to wake the animal up or to keep it awake. The association between these variables is significant, r=0.97. The more highly waves are associated with spontaneous awakenings in a particular subject, the greater will the decrease in SW sleep be due to PCPA administration.

If after PCPA, increased waves occurring in SW sleep are causing more awakenings, then the increase in waves in SW sleep associated with spontaneous awakenings should be associated with decreased SW sleep under PCPA. The correlation coefficient between waves in category AW-2' as a percent of baseline and the amount of SW sleep after PCPA as a percent of baseline is 0.47. This is not significantly different from zero and is in the wrong direction to support the hypothesis. SW sleep is also not reduced due to more spontaneous awakenings, but rather due to longer periods of awake. Could increased awake waves be causing the longer awake periods? The answer is negative since there is no correlation between PCPA's ability to cause increased waves in waking and an increase in time spent in awake across subjects.

Another hypothesis concerned with the reduction in REM sleep, states that if PCPA disrupts the distribution of central phasic waves causing them to occur predominantly outside of REM sleep, then this should lead to a reduction in the need for REM sleep and subsequently lower amounts of REM sleep under PCPA. The correlation coefficient between an increase in the number of SW and AW waves as a percent of baseline under PCPA and the concurrent reduction in REM sleep as a percent of baseline is 0.62. This is not significantly different from zero and it is in the wrong direction to support the above hypothesis.

These data fail to support these hypotheses of the redistribution of waves causing the sleep effects of PCPA. Since correlations are reciprocal these data also fail to support the idea that the

effects on sleep by PCPA cause the redistribution of waves. The correlation coefficients obtained from this sample indicate that larger effects on sleep amounts produce smaller effects on waves and smaller effects on sleep amounts produce larger effects on waves. This finding is in contrast to the effects of PCPA within sleep categories across subjects. There is a direct relationship ($r=0.952$) between the reduction in REM sleep and the reduction in SW sleep due to PCPA administration. These data support the concept that there are at least two mechanisms mediating the effects of PCPA, one on sleep and the other on the distribution of central phasic waves.

In conclusion PCPA causes a redistribution of central phasic waves in the rat. There is a drastic decrease in the proportion of REM waves and a drastic increase in the proportion of waking waves. There is also an increase in the proportion of waves preceeding spontaneous awakenings. PCPA causes changes in sleep amounts with increased waking and decreased SW sleep and REM sleep. The drug's effect on the distribution of waves and on sleep amounts appear to be somewhat independent of each other.

B. REM Deprivation on Central Phasic Waves

Depriving subjects of REM sleep by hand awakenings proved to require a large degree of effort. During the 24 hour deprivation condition one subject required 206 awakenings the other 199. The magnitude of stimulation required to arouse the subjects increased as REM deprivation ensued. At the beginning of the condition a tap on the cage was sufficient. Towards the end of the condition

vigorous and extended banging and shaking of the cage was necessary. Even with this, the animal might immediately return to REM sleep. REM sleep was reduced by 47% (cor t=100.0). In each subject there were seven REM periods of two epochs in length and two of three epochs in length. Time awake increased by 4% (cor t=14.9), and time in SW sleep remained fairly constant. One odd result was that one subject did not exhibit a REM sleep rebound during recovery sleep (see tables 8a-c).

Trends observed in the absolute measures comparing the wave distribution during the baseline control condition and the REM deprivation condition are a 78% increase in absolute frequency in category R-2' and a 65% decrease in waves occurring in REM sleep. The deprivation condition reduced the total number of waves occurring by almost half (see table 9a). The rates between deprivation and baseline remained about the same with a 34% decrease in REM sleep. Rates in categories R-2' and AW-2' were increased. SW and AW rates were decreased (see table 9b).

Statistical tests were carried out using the relative measures of weighted rate and percent frequency. Correlated "t" tests with significance levels less than 0.05, df=1, were used. Tests between categories of R-2 and REM were one tail, all others two tail.

Weighted rate in category R-2' was significantly increased during the REM deprivation (cor "t"=12.1) and restored to baseline levels in recovery (cor "t"=22.8). The weighted rate in AW-2' was increased during the deprivation condition, however, not significantly. A significant decrease was observed between the deprivation

condition and recovery (cor "t"=54.3) (see table 9c). Using percent frequency as a measure, a significant increase can be seen in the proportion of waves falling in category R-2' (cor t=9.0) during REM deprivation as well as a restoration in recovery (cor t=22.9). The proportion of waves falling into REM sleep during REM deprivation was reduced significantly (cor t=6.8) (see table 9c).

It is clear that depriving a rat of REM sleep has an effect on the distribution of REM related central phasic waves. Proportionately, fewer waves occur in REM sleep and more occur in SW sleep two minutes within REM onset. For a comparison of how the REM deprivation procedures effect the wave distribution and time spent in each category see figure 13. There is a 47% decrease in the amount of time spent in REM sleep and a 65% decrease in the number of waves occurring in REM sleep. There is a 46.5% increase in the time spent in category R-2' and 78% increase in the number of waves occurring in this category as a result of REM deprivation.

In conclusion both manipulations of PCPA administration and REM deprivation have an effect on the distribution of central phasic waves in the rat. The effects, however, differ in many respects. Both manipulations reduce the amount of REM sleep, the proportion of REM sleep waves, and the total number of waves. REM deprivation mainly increases the proportion of waves appearing in SW sleep two minutes before REM onset whereas PCPA has very little effect on this category. PCPA drastically increases the proportion of waves appearing in awake whereas REM deprivation has very little effect on this category. To contrast the effects of these manipulations,

PCPA causes a shift in the distribution of waves. This shift causes the waves to occur at times when they normally would not. REM deprivation causes a shift in the distribution of the waves so that they just occur earlier than they normally would.

Scoring Considerations

A. 15 Versus 30 Second Epochs.

There was some concern that scoring the polygraph records in 30 second epochs would lead to inaccuracy and possible bias. To see if any great differences are dependent upon the choice of epoch length, RS-44, participating in the normative study and PCPA experiments, was scored using 15 second epochs.

Even though this subject had the highest values for time spent in awake and the lowest values for SW and REM sleep, these values are certainly close enough to the other values to be accounted for by individual differences and not scoring procedure. More convincing evidence lies in the effect of PCPA on sleep, which in this subject, is within the distribution of the size of the effect (see table 7b).

The quantification of the central phasic waves on this subject reveals every category to be within the other group member's range of values except that of SW sleep (see table 5d). The percentage of waves falling in SW seems to be much lower than the other subjects in the group. If a conclusion must be drawn it is that fewer waves appear in the category SW when epoch length is reduced from 30 to 15 seconds. Therefore, possible bias introduced by scoring in 30 second epoch lengths does not detract from the validity of any results supporting the hypotheses since it can only

bias against them.

B. Scoring reliability.

1. Sleep stage.

One experimenter scored every record for sleep stage. Internal consistency or reliability in scoring was determined by a rescoring of certain eight hour segments of polygraph records. The segments of records rescored were not totally chosen at random but rather assigned to test the limits of scoring reliability. The first eight hours (960 epochs) of a condition were chosen since more variability in scoring should be present before the scorer has had enough experience with a subject's record to establish a definitive set. Rescoring was done at least six months after the original scoring so the scorer had no recollection of the segment. With these constraints, one segment was chosen at random from a baseline condition and one from a PCPA drug condition. As chance would have it the EMG was not usable on the chosen baseline segment, so the measure computed probably reflects the lowest limits of reliability.

Two measures chosen to assess the scoring reliability are: (1) Pearson-product moment correlation coefficient between the number of epochs scored in AW, SW and REM, on the original scoring and rescoring, and (2) percent agreement on an epoch by epoch basis. Scores of AR, SR, and mixed were considered independent of period number assigned, e.g. and AR-3 and an AR-2 were considered in agreement.

Overall sleep stage reliability independent of condition is

0.9809 for the stage totals. Epoch by epoch agreement is 92.2%. For the non-drug conditions, category reliability is 0.9996. Epoch by epoch agreement is 94.46%. The drug condition presents slightly less reliability in scoring: overall reliability is 0.9436 and epoch by epoch agreement is 87.34%. By far, disagreements between scoring and rescoring arise between AW and SW, and most of these from transition periods of AW to sleep. Disagreement in REM related measures accounted for less than 0.2% of all disagreements. This finding is consistent with that of Monroe (1969) who reports that REM sleep has the highest human scoring reliability.

2. Central phasic waves

Scoring central phasic waves is a rather straightforward task. Compared to sleep staging it does not involve any complex judgments. The wave scorer was given the MAC and was familiarized with the wave form characteristics, primarily the duration, and applied these criteria to a single channel of the polygraph record. The scorer was also alerted to eliminate movement artifact. Since the signal to noise ratio of the waves is usually between three and five, the individual waves are quite discriminable.

Wave scoring reliability was determined by randomly choosing an eight hour segment of record and rescoring it, using the original criteria, on an empty set of scoring sheets. The original sleep staging data was then placed onto these sheets and the waves divided into the five mutually exclusive categories. A Pearson-product moment correlation coefficient was computed on the five categories between the original scoring and rescoring totals

r=0.9975, reflecting the straightforward nature of identifying a wave and placing it in the appropriate category.

# V. Discussion

## Review

### A. Purpose and Objectives

The purpose of this series of studies was to determine if a REM sleep associated phasic event system is present in the rat. The specific objectives of the studies were as follows:

1. To identify a PGO type wave from subcortical EEG recordings in the rat
2. To demonstrate a widespread yet discrete distribution of brain loci in the dorsal pontine tegmentum from which a PGO type wave can be recorded
3. To demonstrate an alteration in the distribution of PGO type waves with the manipulations of PCPA administration and REM deprivation.
4. To determine the relationship between PCPA induced alterations in wave distribution and sleep stage amounts.

### B. Overview of What is Known

Investigations into the REM sleep phasic event system have been carried out using the cat as the primary experimental subject. Evidence has been reported supporting the view that during REM sleep many sensory, motor and autonomic mechanisms come under the influence of a phasic event system. This system provides a source of endogenous stimulation to widespread yet discrete areas all along the neuraxis. One measure of its operation is the PGO wave.

The mechanisms involved in the phasic event system are not well understood. However, it is known that the integrity of cells

in the pons appears to be necessary for the occurrence of PGO waves, that discrete pathways subserve the discharge propagating characteristics of the system and that a gating mechanism, at least in part serotonergic, partially or totally exercises inhibitory control over the generation or propagation of the pontine activity.

Though phasic discharge has been identified as a basic process within REM sleep,as defined by the tonic characteristics, the occurrence of phasic events is not restricted solely to REM sleep, for example, phasic events such as PGO waves typically occur also just before REM sleep onset and before many spontaneous awakenings. In addition, the association of PGO waves and REM sleep may be demonstrated to alter in the face of certain manipulations. Among the effective manipulations, PCPA has been shown to increase greatly the frequency of PGO waves in waking presumably by affecting the serotonergic inhibitory gating mechanism. REM deprivation also has been shown to affect the distribution of PGO waves; an increase in wave frequency before REM sleep onset is a characteristic effect. The mechanism for this effect is presumably not on the gating mechanism since PGO waves in waking are not appreciably affected, rather it appears to cause an increase in "pressure" for the waves to occur in REM sleep.

If a phasic event system constitutes a basic process of REM sleep, then evidence for its existence should be found in every species in which REM sleep has been identified to date. PGO waves, reflecting the activity of the phasic event system, have been identified in cat, man, and monkey. However, a controversy exists as

to their presence in the rat. In order for the notion that a phasic event system is fundamental, or even instrumental to the process of REM sleep, evidence for its existence must be found in the rat. Perhaps the simplest way to acquire this evidence is to find PGO waves in this species. Of course, peripheral phasic events such as muscle twitches and eye movements have been known to be associated with REM sleep in the rat, but evidence for phasic activity within the central nervous system has not been identified.

C. The Hypotheses

The null-hypotheses posed for testing in these studies are summarized below:

1. The subcortical EEG recordings of rats will not reveal a discriminable waveform similar in its characteristics and distribution to the PGO wave recorded in cats.
2. Recordings from many macroelectrodes aimed at the dorsal pontine tegmentum of rats will not reveal an anatomical distribution of loci that differentiate positive and negative areas with respect to the ability to identify PGO type waves; that is, a discrete yet widespread anatomical distribution will not be found.
3. The administration of PCPA to rats will not cause a redistribution of PGO type waves such that the proportion of waves in waking is significantly increased.
4. REM deprivation in the rat will not cause a redistribution of PGO type waves such that the proportion of waves in SW sleep two minutes preceeding REM sleep onset is significantly

increased.

5. The administration of PCPA will not significantly increase time in AW while decreasing SW sleep and REM sleep.
6. A relationship of the alteration in PGO type wave distribution and sleep stage amounts after PCPA will not be consistant with altered wave distribution being causative to the changes in sleep stage amounts.

D. The Method

Forty Sprague-Dawley rats were surgically prepared for chronic sleep recording with the addition of one or two stainless steel bipolar macroelectrodes aimed at the dorsal pontine tegmentum. A candidate for a PGO type wave was defined by its gross duration and amplitude. The distribution of the waves was determined by computing the absolute frequency, rate per minute, weighted rate, and percent frequency for five mutually exclusive categories: 1) SW sleep within 2 minutes of REM sleep onset, 2) SW sleep within two minutes of a spontaneous awakening, 3) REM sleep, 4) SW sleep, 5) AW.

Six subjects in which a candidate for a PGO type wave was identified were recorded continuously for 24 hours each. The wave form characteristics were determined from randomly sampled oscilloscope tracings. The distribution of the characteristic waveform was determined by the quantification of the entire 24 hour record.

Forty subjects containing a total of 45 electrode sites were each recorded for a minimum of three hours and until at least three REM periods were obtained. These records were examined for the presence of a PGO type wave and assessed positive or negative for their

presence. Each subject was sacrificed and the histologically treated brain material was examined. The locus of each electrode tip was determined independently of the electrical activity. A characterization of the electrical activity was combined with the brain derivations to produce representations (maps) on frontal brain sections. The anatomical distribution of positive and negative sites were determined from these maps.

Four subjects in which a candidate for a PGO type wave was identified, received 300 mg/kg of PCPA methyl ester HCL. A continuous record was obtained during a 24 to 48 hour period following drug administration. The quantified record was compared to a vehicle control condition also 24 to 48 hours after IP injection.

Two subjects in which a candidate for a PGO type wave was also identified underwent a 24 hour REM deprivation procedure. The quantified record was compared to the 24 hour baseline condition preceding, and the 24 hour recovery condition following, the deprivation.

Summary of Experimental Findings, Interpretation and Literature Support

A. The Methodology

A significant contribution of this series of studies is the delineation of a methodology for the quantification of central phasic activity, and the delineation of criteria for determining whether REM associated central phasic waves exist in a species. This is in contrast to the abundance of essentially anecdotal reports in the literature. If quantification by count is taken, only categories of REM sleep, NREM sleep, and AW are used. In view of the data gathered in this study, I believe that the use of the five mutually exclusive

categories that are presented, with appropriate variation, is minimally necessary to capture the actual distribution of central phasic activity. A good measure of distribution is additionally important if the distribution variable is going to be used for comparison across species. In lieu of a further elucidation of the mechanisms involved with the phasic event system, it is important to have at least standard criteria for scoring its output. Aside from the capacity to assess certain basic properties of the system, established criteria also would facilitate the comparison of phasic emission across species.

B. The Normative Characteristics of REM Associated Central Phasic Waves in the Rat

1. Waveform characteristics

Readily discriminable monophasic waves of approximately 100msec in duration, recorded in the rat, conform to the description of pontine PGO waves recorded in the cat (Brooks and Bizzi, 1963). The amplitude of the waves recorded in the rat seem to be lower than the amplitude of cat PGO waves. Brooks and Bizzi report 200-300 uv amplitude waves around the abducens nuclei, however, more lateral and dorsal at the same A-P level, the amplitudes range between 25 to 75 uv. These latter voltages conform very well to the amplitudes found by me in the sites implanted in the rat (MACs 15.8 uv-66.7uv). The fact that the higher amplitude waves were not observed in the rat may be explained by the placement of electrodes anterior to the abducens which in the cat yields lower amplitude waves. Perhaps even more revealing than absolute amplitude, in a comparison of rat and cat waves, is the interaction of amplitude and distribution.

Bowker and Morrison (1976) report a 50 percent difference between the largest and smallest PGO waves, with the largest appearing pre-REM sleep. The waves recorded in the rat show the same amplitude variation across stages.

PGO waves within REM sleep show a gross correlation to eye movements, as measured by the EOG (Jeannerod and Sakai, 1970). A relationship also is found between central phasic waves and eye movements in the rat. Pompiano, (1970) using integrated potentials, has made a distinction between type I and type II PGO waves. Type I is associated with PGO waves that appear singly and type II with those appearing in busts. Type II only appears in REM sleep and are more highly associated with eye movements than type I. I have found a very similar relationship in the central phasic waves of the rat; grouped waves only appear during REM sleep and are more highly associated with eye movements than waves appearing singly.

In almost every respect, the wave form characteristics of central phasic waves recorded in the rat are the same as those of PGO waves recorded in the cat.

2. Wave distribution with respect to sleep stage

The distribution of central phasic waves in the rat indicates that their emergence is a REM associated phenomenon. As in the cat, the vast majority of waves occur in REM sleep and in SW sleep just preceeding REM onset (Dement, 1966). As in the cat, waves frequently appear at a relatively high rate before spontaneous awakenings (Thomas and Benoit, 1967). The literature concerned with PGO waves in the cat is not very clear as to the spontaneous occurrance of

waves in SW sleep and AW. Although not explicitly stated, the impression is given that PGO waves rarely, if ever, occur during waking (Jouvet, 1972). Observations of cat EEG recordings reveal that waves do occur during waking but the frequency is low and there is a possibility that these waves are EMPs (Personal Communicational Farber). This lack of information makes it difficult to compare 6.5 percent of waves in the rat during awake with waking PGO waves in the cat. The average AW rate of a 0.22 per minute in the rat is also a low frequency, whether this rate is significantly higher than in the cat is not clear.

Another similarity between the rat waves and cat PGO waves is their distribution within REM periods. There is a marked tendency for the waves to increase as REM sleep continues and then trail off by the end of the REM period (Dement, 1966). Also in both the cat and rat the frequency of waves is lower pre-REM than in REM sleep itself.

In summary, the evidence demonstrating the similarities in both waveform and distributional characteristics of PGO waves and central phasic waves in the rat makes it tenable that the two phenomena represent the same mechanisms. If central phasic waves in the rat are indeed PGO waves, then differences across species are of prime comparative interest.

3. Difference in PGO waves between the rat and cat

Besides the great number of similarities that exist between cat and rat PGO waves differences also have been demonstrated. Jouvet (1972) reports that under laboratory conditions the cat has a fairly constant number, 13,000 $\pm$ 1500, of PGO waves each day.

The computed mean number of waves in the rat is 2,729 $\pm$ 1,858. The rates at which the waves appear in REM sleep are also grossly different between these species; in the cat- 60 per/minute (Jouvet, 1972) compared to 15.7 per/minute in the rat. These differences are so large that they are probably significant. Research in Morrison's laboratory (Reiner et al, 1976) has shown that large cerebellar cortical lesions in the cat results in limb jerks that occur simultaneously with PGO waves. This group has performed similar lesions in the rat and find a reduced frequency in limb jerks as compared to the cat. The proportion of limb jerks between rat and cat after the lesions is comparable to the proportion of PGO wave frequency between these species. Thus, this is confirmatory evidence that PGO waves occur at a lower frequency in the rat than in the cat.

Any theory attempting to explain the functional significance of PGO waves will have to account for these differences. A lower PGO wave frequency might reflect a differential "need" for REM sleep processes. Future research will have to take into account the known differences between the rat and the cat in terms of PGO frequency. If the operation of the mechanisms reflected by PGO waves has functional significance, then species differences in PGO activity should be related to species differences in function. Just what this function, or functions are is not clear at this time, but the comparative approach may be giving us some leads.

Thomas and Benoit (1967) report that cat PGO waves are found in SW sleep prior to spontaneous awakenings. They interpret this phenomenon as the appearance of an aborted REM period. At these

times, the waves have a similar frequency and distribution to the waves occurring prior to actual REM periods.

The distribution of PGO waves in the rat suggest a different interpretation. About half of all spontaneous awakenings in the rat are preceeded by waves. I interpret this as a straightforward association between the occurrence of waves and spontaneous awakenings rather than put forward the idea that rats have 120 aborted REM periods per day, especially when there is no evidence for this. With the appearance of PGO waves before many spontaneous awakenings the possibility arises that the mechanisms producing the waves may be causative to the arousal process in general. One function served may be that of waking animals from sleep.

One question raised by the previous discussion is why PGO waves appearing at one time signal that a REM period is about to begin and at other times signal a spontaneous awakening. The outcome may be dependent on the internal state of the organism. Future research should investigate manipulations that can effect this internal state, such as stress or food deprivation, and their effect on the relationship of PGO waves to spontaneous awakenings.

Another question that can be raised is why do waves only precede about 50 percent of all spontaneous awakenings? It is known that sensory stimulation can arouse a sleeping animal. In the laboratory, eventhough visual and auditory stimuli are controlled, there is no control over tactile or proprioceptive stimulation. It is very possible that many spontaneous awakenings are produced by uncomfortable body position as muscle tone decreases. Therefore if the

phasic event system is involved with providing endogenous stimulation that leads to an arousal it need not account for every spontaneous awakening.

The mechanism by which the phasic event system may function to produce arousals is not known. However, Bowker and Morrison (1976) report that they can elicit PGO waves with auditory or visual stimuli. That is, the same stimuli that can awaken a sleeping animal can also produce PGO waves. By tapping on the cage of a rat I have elicited the waves as well. It is possible that the phasic event system provides endogenous stimulation to sensory systems that are capable of producing an awakening through a mechanism similar to that of exogenous stimulation.

In summary, I believe that as the phasic event system is studied in more species we will be in a better position to extract more generalizable properties of its operation. Some of the ideas I have discussed are quite speculative and more research is needed to further develop them. The main point, however, is that these ideas arise from the comparison of only two species. It is my conviction that the comparative approach can be a very powerful tool not only in the uncovering of relationships of the phasic event system to other phenomena but in uncovering its possible functional significance.

C. Anatomical Location of Sites Positive for PGO in the Rat

No systematic effort has been made to localize PGO waves to specific structures at the pontine level in the cat (Brooks, 1973). Most of the information we have is from a study using movable

macroelectrodes by Brooks and Bizzi (1963). It is difficult to compare their results in the cat to the results obtained here for they have only two electrode insertions corresponding to the anterior-posterior level in which I have my high density of electrode placements. The results, however, can be compared with the general nature of the distribution of brain loci from which PGO waves can be recorded in the cat. As listed in the introduction the brain loci from which PGO waves can be recorded are somewhat discrete but yet widespread. The maps produced of the pons of the rat indicate that such a distribution is present here.

The area of the lc and ntm consistantly yield sites positive for PGO waves, while adjacent medial, lateral, ventral, and dorsal sites are negative. Accordingly, at least one area from which PGO waves can be recorded in the rat has discrete neuroanatomical localization. The lateral aspects of the FLM and the ntv may be two other circumscribed areas in the dorsal pontine tegmentum that are positive for PGO waves. This conclusion must be qualified because of the small number of electrode placements. The widespread characterization of a central phasic tributary system is not only illustrated by the three areas just discussed but also by some isolated electrode sites that were assessed positive located in the rpoo, ci, npd, and cerebellum.

It is difficult to account for all the positive and negative brain loci with a single explanation in terms of known anatomical pathways. This will have to await a further elucidation of the mechanisms of generation and propagation of PGO activity. The findings, however,

are consistant with the general nature of PGO loci in the cat. With respect to a functional grouping of the positive sites, it is intriguing to consider the description by Lindvall and Björklund (1974) of a lateral branch of the noradrenergic dorsal periventricular system (DPS);

> "...running from the region of the locus coeruleus ventrorostrally through the lateral part of the medial longitudinal fasciculus. These fibers apparently give rise to a dense terminal network in the ventral tegmental nucleus, and then continue their ventrorostral course behind the decussation of the superior cerebellar peduncles" (p15).

Apparently, connections exist between the three main areas in which PGO waves have been localized in the pons of the rat. The involvement of norepinephrine in the propagation of PGO activity is not a new one. Jouvet (1972) sites evidence from his cat studies that noradrenergic mechanisms are central to the PGO phenomenon, if the DPS is involved with the phasic event system it is consistant with this view.

Lindvall and Björklund further describe some of the DPS fibers sweeping dorsally into the ci. A positive site has been found in the ci of the rat. Descending from the colliculi is the TT where another positive site has been tentatively located. The fibers of the TT are concerned with turning the head and moving the upper extremity in response to various kinds of sensory stimuli, in particular visual and acoustic signals (Zeman and Innes, 1963). This neuroanatomical link presents the possibility that some of the phasic paroxysmal motor movements seen during REM sleep may be caused by

a reaction to afferent stimuli whose origin is endogenous, that is, output of the phasic event system into sensory systems. Admittedly, this is retrospective fitting of the data into other schemas. Future research on the functional and anatomical connections of PGO positive areas is needed.

Positive sites have also been identified in the anterior lobe of the cerebellum. PGO waves have been recorded there in the cat (Jeannerod, 1965) and the lc is known to invest it with noradrenergic projections (Olson and Fuxe, 1971). The cerebellum is a very large structure and there are too few electrode sites in the present study to comment further on the anatomical distribution of positive and negative loci. There is a hint, however, that at least one differentiating characteristic may be the type of cell layer impinged upon by the electrode. This is a very tentative conclusion since only one negative case in the cerebellum is involved in the differentiation, but it is theoretically reasonable to assume that the ability of a macroelectrode to record gross activity is dependent upon the cytoarchitechtonics of the area being recorded. The negative site recorded was primarily situated in white matter. The paucity of neuronal perykaria in these regions may account for this finding.

All electrode sites in the cerebellum were placed in the anterior lobe. This area receives much of its innervation from the dorsal and ventral spino-cerebellar tract carrying afferent proprioceptive and tactile impulses (Zeman and Innes, 1963). It is possible that the phasic waves recorded here are the result of the peripheral motor movements. This explanation is considerably diminished if we

consider that PGO waves recorded in the cerebellum precede the onset of REM sleep while the peripheral movements do not.

D. The Effect of PCPA on the Distribution of PGO Waves in the Rat

The effects of PCPA on the distribution of PGO waves in the rat are comparable to the effects of PCPA on the distribution of PGO waves in the cat. PCPA causes a reduction in the proportion of waves that occur in REM sleep and an increase in those appearing during waking (Jouvet, 1972, Dement et al, 1970; Delorme, 1966, Ferguson et al, 1969, Koella et al, 1968). In the cat PCPA is thought to effect a serotonergic inhibitory gating mechanism regulating the propagation of PGO activity (Brooks and Gershon, 1971, Jouvet, 1972). Accordingly, the central phasic waves recorded in the rat not only have a similar waveform and distribution to PGO waves recorded in cats, but also seem to show a dependence on some serotonergic mechanism. This is further evidence in support of an identity between underlying mechanisms that produce the rat and cat PGO waves.

E. The Effects of PCPA on Sleep Stage Amounts

In the group of five rats tested, 300 mg/kg of PCPA had very clear effects on sleep stage amounts by reducing SW sleep to 69 percent of baseline, REM sleep to 36 percent of baseline, and increasing AW to 136 percent of baseline values. These values are quite comparable to the data in the initial study done by Mouret et al., (1968). The size of the effect is less than that obtained by Mouret et al, but in the Mouret et al. study a 500 mg/kg dose of PCPA was employed.

I cannot shed any light on the inconsistant results of

Rechtschaffen et al (1973) in rats with PCPA. I have administered other doses of PCPA to rats. Because of the many procedural difficulties with the multi-dose studies, which made the conclusions somewhat questionable, they were not included in the results section. With these limitations in mind, however, there was no evidence for a linear relationship between dose and effect on sleep stage amount. Two-hundred mg/kg of PCPA actually led to an increase in SW sleep amounts in one subject and 400 mg/kg led to a minute decrease of less than three percent in SW sleep in another subject. REM sleep amounts were always greatly decreased. This is somewhat reminiscent of the Rechtshaffen et al. findings.

The findings in the PCPA study that was described can be taken to support Jouvet's (1972) hypothesis that SW sleep is dependent on a serotonergic mechanism. The study equally supports the notion that REM sleep is dependent on a serotonergic mechanism as well.

F. The Effect of PCPA upon Relationships Between PGO Waves and Changes in Sleep Stage Amounts

It is clear that the increase in the proportion of PGO waves that appear in waking is not solely a function of the increased time spent in AW under the drug. The correlation coefficients obtained across subjects indicate that the effect on sleep stage amounts is inversely related to the effect on wave distribution. It is possible that this relationship may be dependent on the dose of PCPA used. But the fact that PCPA can differentially effect sleep stage and wave distribution indicates some degree of independence of the

mechanisms subserving these phenomenon as well as some degree of independence of the effects of PCPA. This finding is again consistant with the concept of a serotonergic gating mechanism of PGO waves, and supports the idea that this gating mechanism is different from the mechanism subserving sleep stage.

Little support was gained for any of Dement's hypothesis concerning a causitive relationship between PCPA's effect on wave distribution and sleep stage amounts. The data is not consistant with the concept that waves appearing outside of this normal distribution wake the rat up from sleep or keep it awake to produce the increased waking seen under the drug. Or, is the data consistant with the concept that increased volleys of waves appearing outside of REM sleep reduce the need for REM sleep and thus reduce the time spent in this stage under the drug. PCPA seems to have two partially independent effects: one on sleep stage and another on PGO wave distribution.

With the failure to support Dement's hypothesis I find it difficult to explain the very strong relationship between the normal proportion of waves that precede spontaneous awakenings and the degree of enhancing effect PCPA has on waking. In other words, the more PGO waves are associated with spontaneous awakenings the greater will be the insomnia produced by PCPA. It may be that animals who have a high association of waves preceding spontaneous awakenings respond to alterations in wave distribution by having longer awake periods. This explanation is very tentative and further research will be needed to explore this relationship.

G. The Effect of REM Deprivation on the Distribution of PGO Waves

The effects of REM deprivation on the distribution of PGO waves in the rat is comparable to the effect on PGO waves by REM sleep deprivation in the cat. The effect of REM deprivation is to shift the distribution of PGO waves so as to increase their occurrence in SW sleep just before REM sleep onset (Vimont-Vicary, 1966, Dement, 1966). The similarity in the effect of this manipulation on the central phasic waves recorded in the rat, and on PGO waves recorded in the cat, constitutes a firm support that the rat waves are indeed PGO waves.

The increase in pre-REM PGO waves due to REM deprivation has been explained in terms of an increased REM pressure(Dement et al, 1970). It is not necessary however, to invoke the construct of REM pressure. The increase in pre-REM waves can be accounted for by the increased pre-REM time. Normally a rat has about 50 REM periods per day and of course, 50 pre-REM periods. During the deprivation procedure the rat attempts to enter into REM sleep about 200 times. Therefore, there are 200 pre-REM periods. It does not necessarily follow that increasing the time spent in this category by a REM deprivation procedure will increase the number of waves that occur in it. The fact that this does happen for both cat and rat PGO waves is a product of the mechanisms subserving them. It does not, however, seem necessary to go beyond this explanation.

It is interesting to contrast the effects of REM deprivation with that of PCPA administration. In both cases REM sleep time was reduced approximately by half, time awake was increased, although significantly

but much less so due to REM deprivation, the effects on PGO wave distribution were quite different. With PCPA the proportion of waves appearing in waking and pre-spontaneous awakening were increased. This is consistant with the hypothesized effect of serotonin depletion on the gating mechanism. With REM deprivation, the proportion of pre-REM waves was increased with little or no effect on waking or pre-spontaneous awakening waves. This indicates that the gating mechanism was not affected by this latter procedure.

Conclusion

Central phasic waves have been recorded in the dorsal pontine tegmentum and anterior lobe of the cerebellum of the laboratory rat that has an almost identical wave form and distribution to that described for PGO waves recorded in the cat. The anatomical distribution of brain sites from which these waves can be recorded in the rat is discrete yet widespread. This also characterized the general anatomical distribution of brain sites from which PGO waves are recorded in the cat. The respective effects of the manipulations of PCPA administration and REM deprivation on the distribution of these waves in the rat also mirrors their respective effects on the distribution of PGO waves in the cat. I conclude that the central phasic waves I have recorded in the rat constitute a phenomenon identicle to PGO waves in the cat - The homologue of the PGO wave.

The identification of PGO activity in the rat supports the concept that the phasic event process is universal to mammalian REM sleep, thus also supporting the basic nature of phasic events in

REM sleep. Ultimately, the understanding of the functional significance of REM sleep will have to be understood on the level of the functions of the component mechanisms. I believe that the phasic event system is one of these mechanisms that should be studied to achieve this end. Future research will have to attempt to determine the extent of the radiating phasic event system and eventually its hub, the generating and gating mechanisms. Such an approach may finally lead to the uncovering of the function of REM sleep itself.

FIGURE LEGENDS

Figure 1. Schematic representation of a hypothesized mechanism of central and peripheral phasic activities of REM sleep in the visuomotor and acoustomotor systems.

Figure 2. Electrophysiological recording from a cat during REM sleep, with PGO waves. Calibrations on right are 50 μv, at bottom 2 seconds. Channel labeled LGN was a miss. Vis. Cx., visual cortex; VIIth nuc., cranial nerve nucleus VII; Vth nuc., cranial nerve nucleus V; Vest., vestibular nucleus; T.T., tensor tympani muscle; Stap., stapedius muscle; Inte. Stap., integrated activity from the stapedius muscle.

Figure 3. Continuous electrophysiological recording from a rat showing the onset of REM sleep from SW sleep. PGO type waves appear on the channel labeled R Pons, histologically determined to impinge on the lc.

Figure 4. Continuous electrophysiological recording from a rat showing a spontaneous awakening from SW sleep. Notice the pre awake waves and the absence of waves during waking on the subcortical channel. Subcortical was determined to be in the anterior lobe of the cerebellum.

Figure 5. Continuous electrophysiological recording from a rat during REM sleep. Notice the PGO type waves appearing singly or in groups on the channel labeled Pons. Eye movements have a high association with grouped waves. The electrode in the pons was determined to be in the lc.

Figure 6. Sample oscilloscope tracing of a PGO type wave in the rat recorded from ntm.

Figure 7. Sample oscilloscope tracing showing a relationship between eye movements and the occurrence of PGO type waves in the rat.

Figure 8. Sample oscilloscope tracing showing bilateral synchrony in the occurrence of a pair of PGO type waves recorded from the left and right dorsal pontine tegmentum of a rat.

Figure 9. Representative sample of histological material from which electrode tip placement was determined. One electrode passed completely through the brain, the other was impinging on the ntm.

Figures 10a-c Schematic representations of the anatomical distribution of electrical activity. Frontal sections of rat brain adopted from Palkowitz and Jacobawitz (1974). Planes defined in relation to interaural zero.

Figure 10d. Schematic representation of the anatomical distribution of electrical activity in the cerebellum. Frontal sections of rat brain adopted from Pellegrino and Cushman (1967). Planes defined in relation to interaural zero. Pons shown stippled.

Figure 11. Bivariate bar graph depicting the mean frequency of waves and time spent (hatched) in the five mutually exclusive categories between the control and PCPA conditions.

Figure 12. Continuous electrophysiological recording from a rat which received 300mg/kg PCPA approximately 36 hours earlier. Shown is a spontaneous awakening from SW sleep. Notice the PGO type waves continuing to occur in waking. The Subcortical electrode was determined to be in the cerebellum.

Figure 13. Bivariate bar graph depicting the mean frequency of waves and time spent (hatched) in the five mutually exclusive categories between the control, REM deprivation, and recovery conditions.

FIGURE 1.

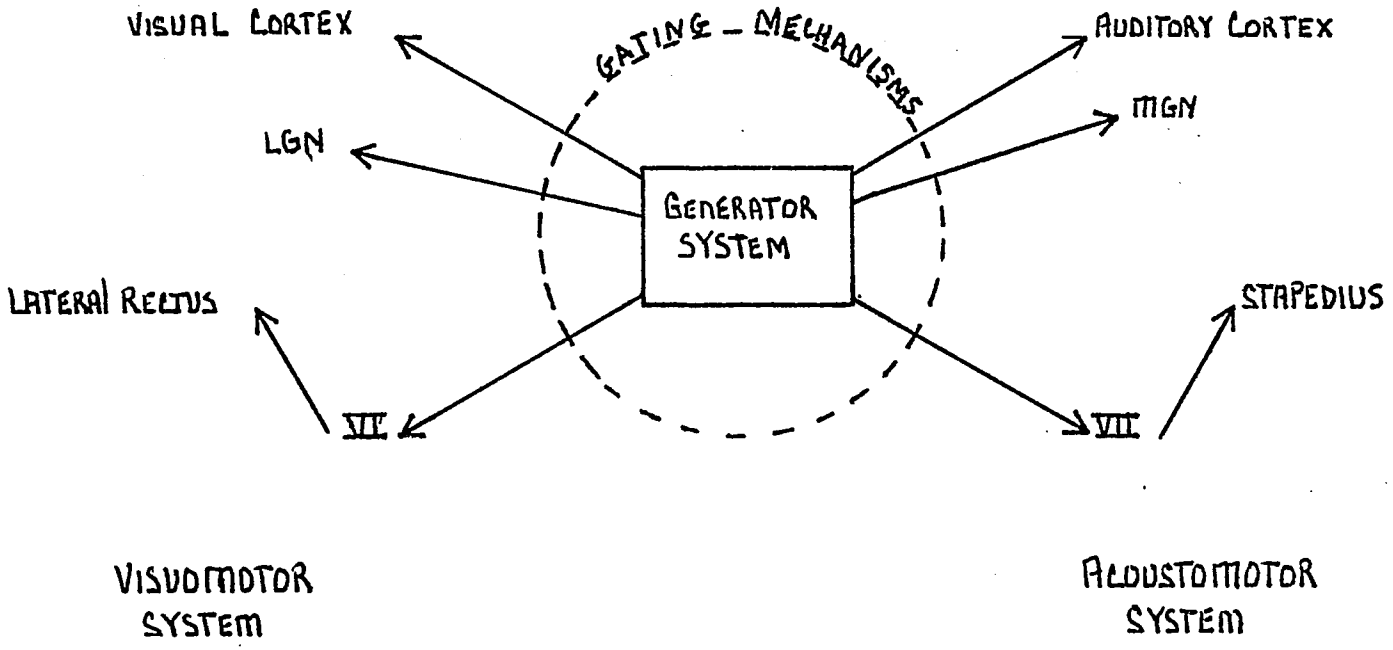

VISUAL CORTEX
AUDITORY CORTEX
GATING - MECHANISMS
MGN
LGN
GENERATOR SYSTEM
LATERAL RECTUS
STAPEDIUS
VI
VII
VISUOMOTOR SYSTEM
AUDIOMOTOR SYSTEM

FIGURE 2.

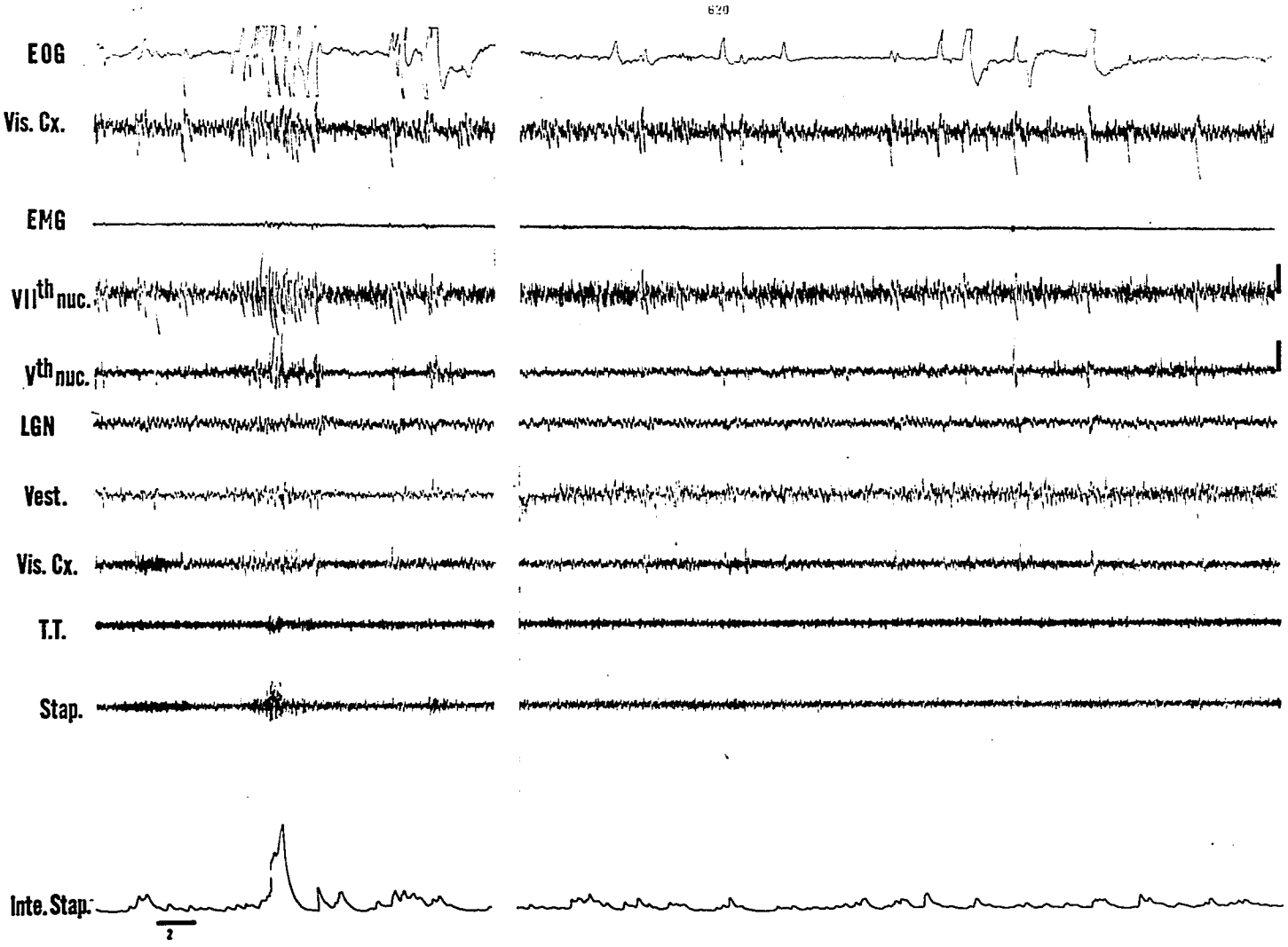
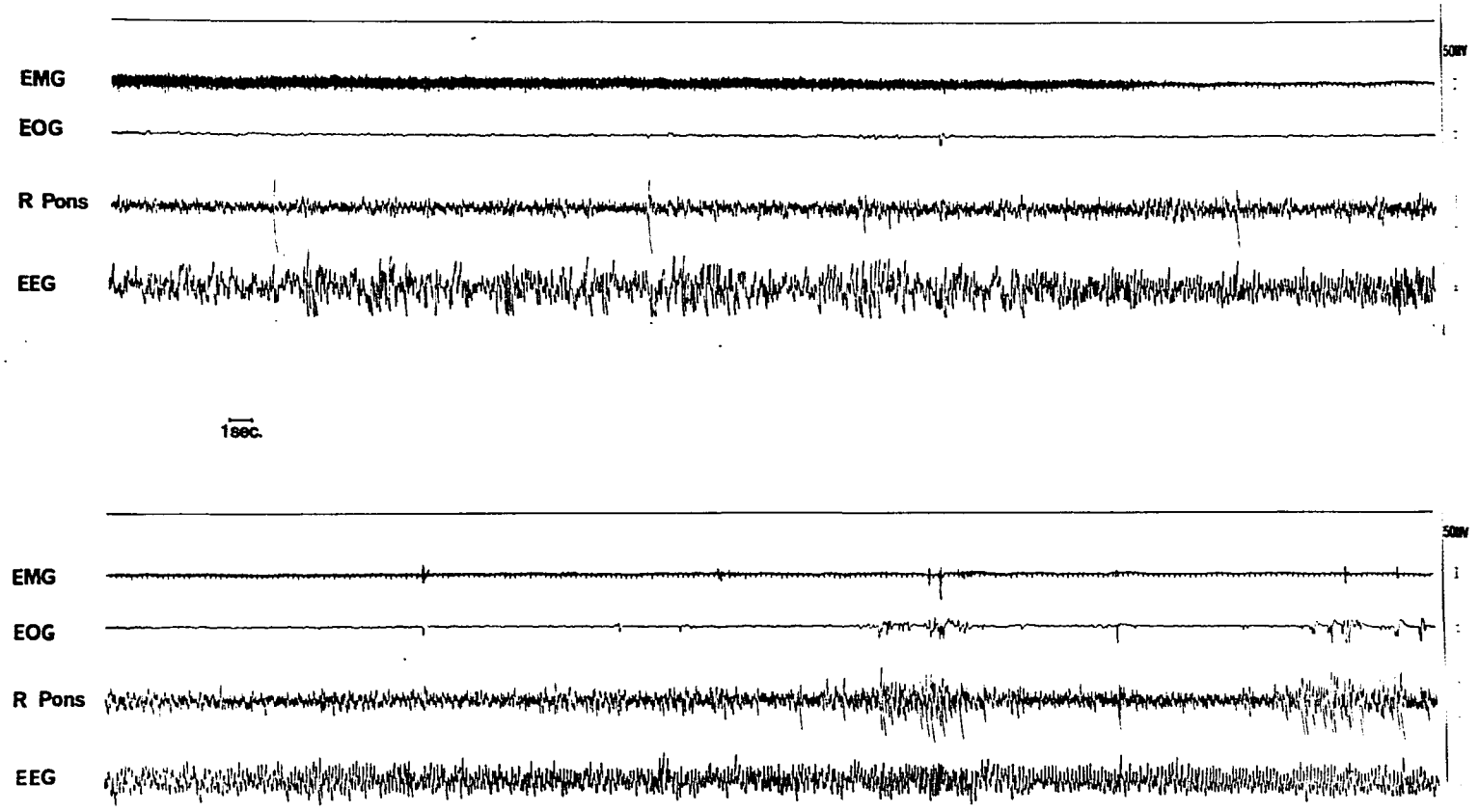

EOG
Vis. Cx.
EMG
VIIth nuc.
Vth nuc.
LGN
Vest.
Vis. Cx.
T.T.
Stap.
Inte. Stap.
2

FIGURE 3.




P-3

FIGURE 4.

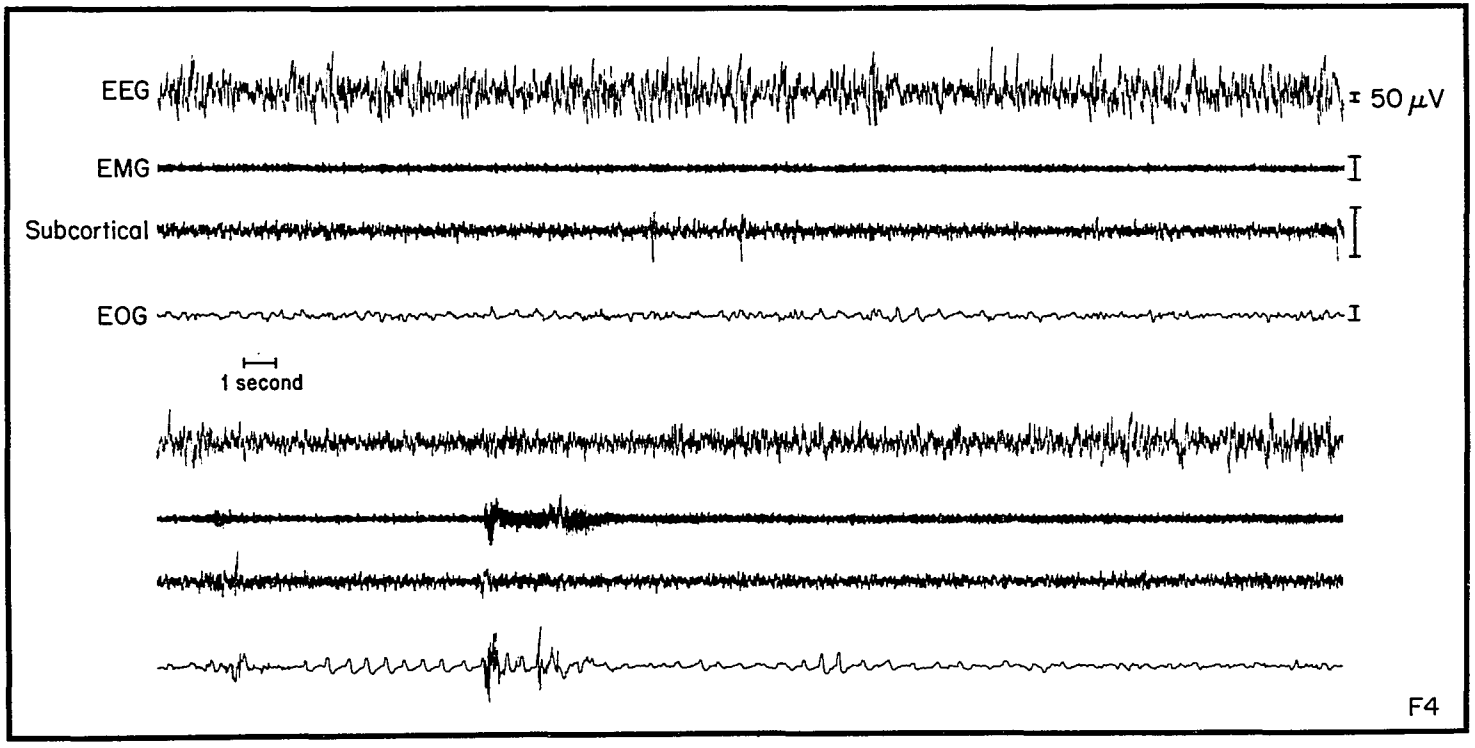


FIGURE 5.

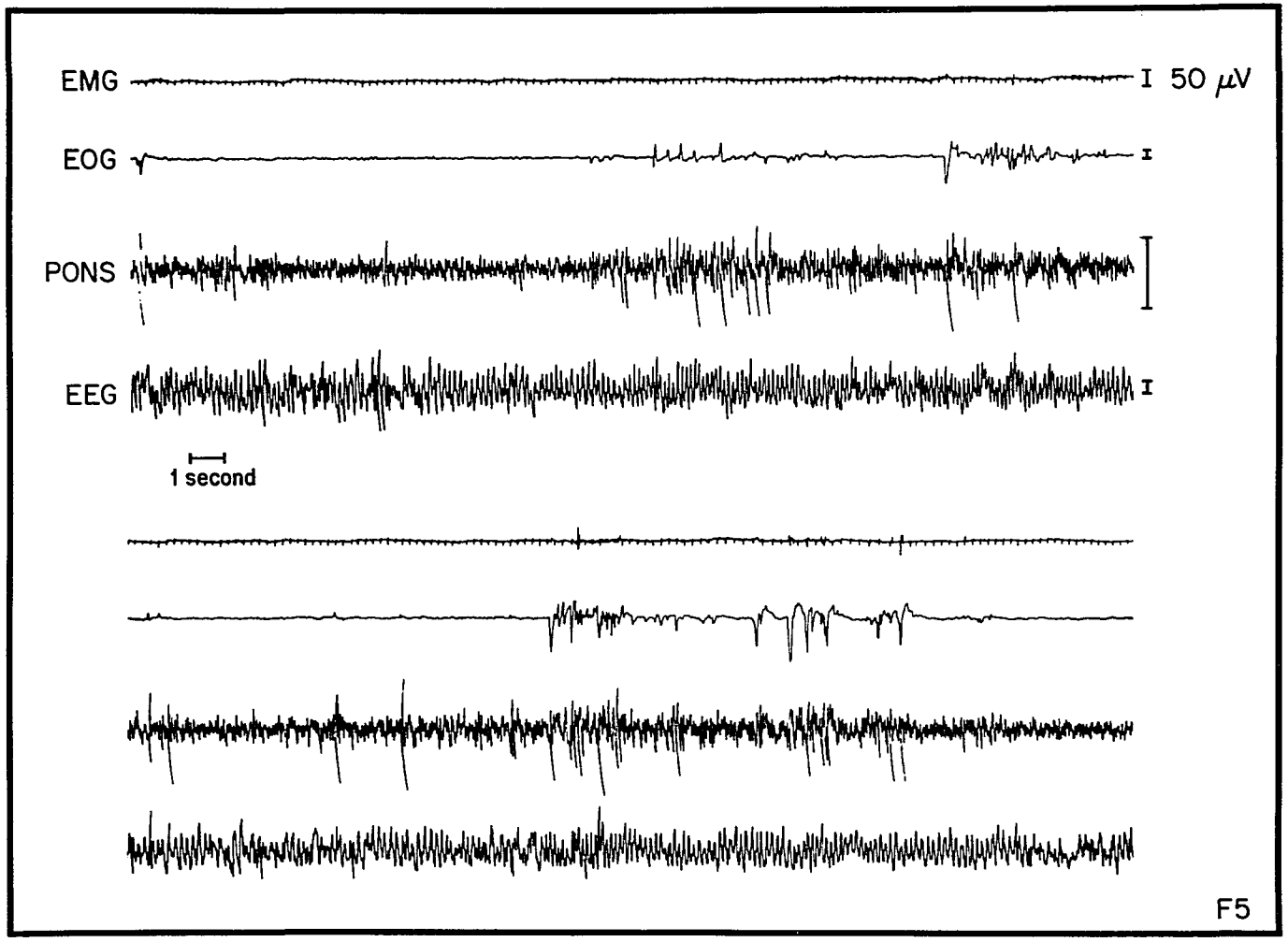


FIGURE 6.

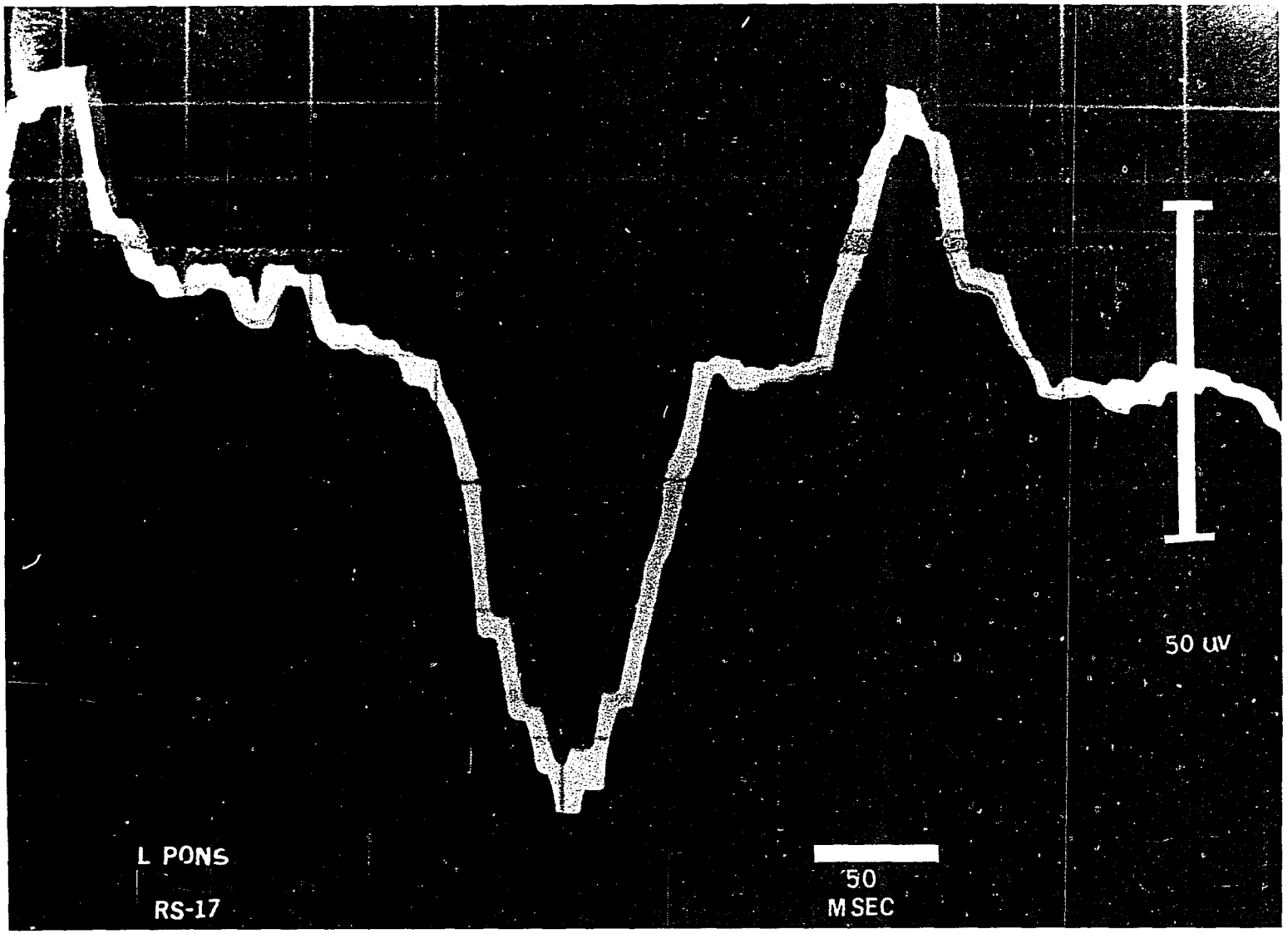


FIGURE 7.

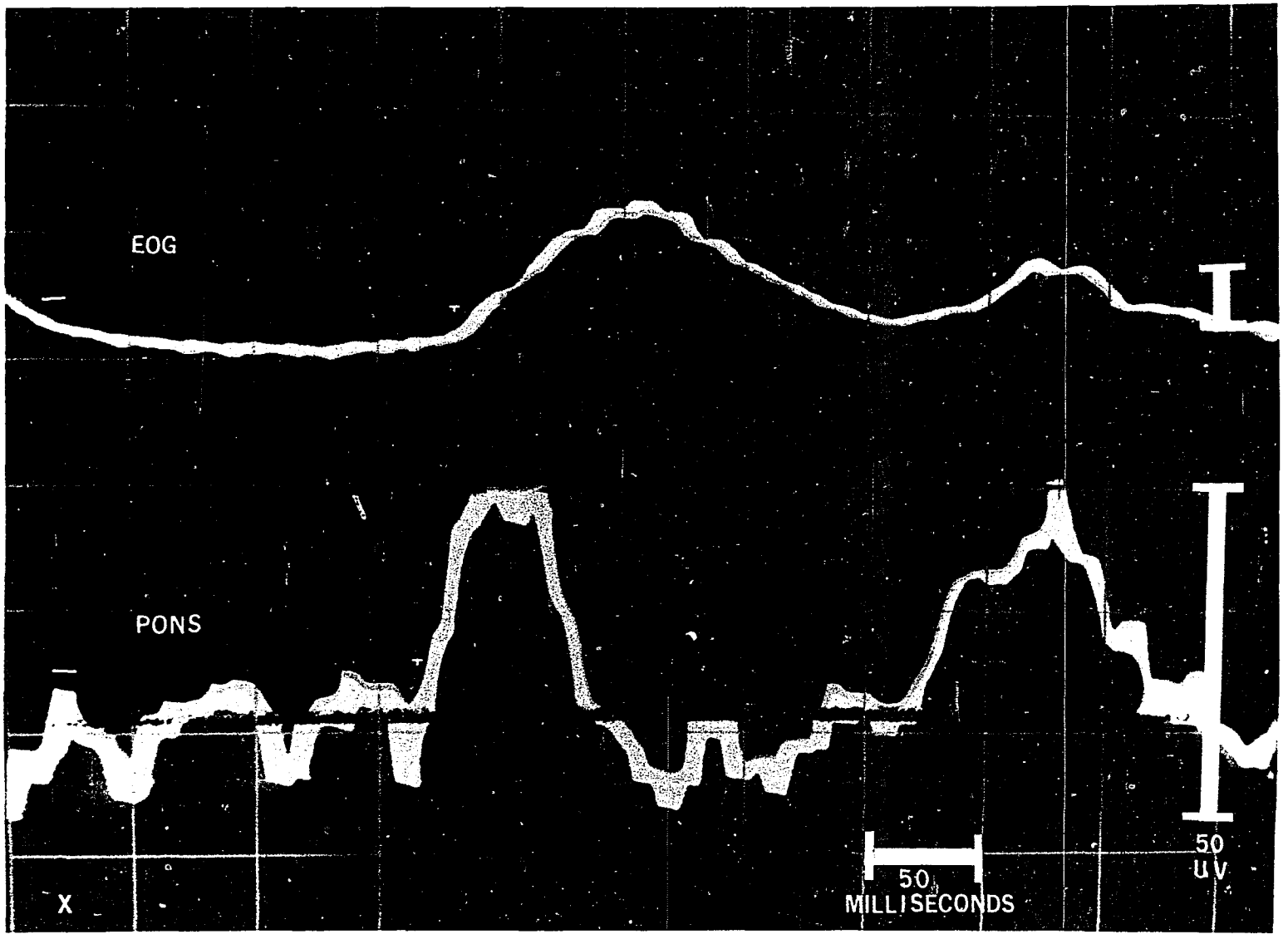


FIGURE 8.

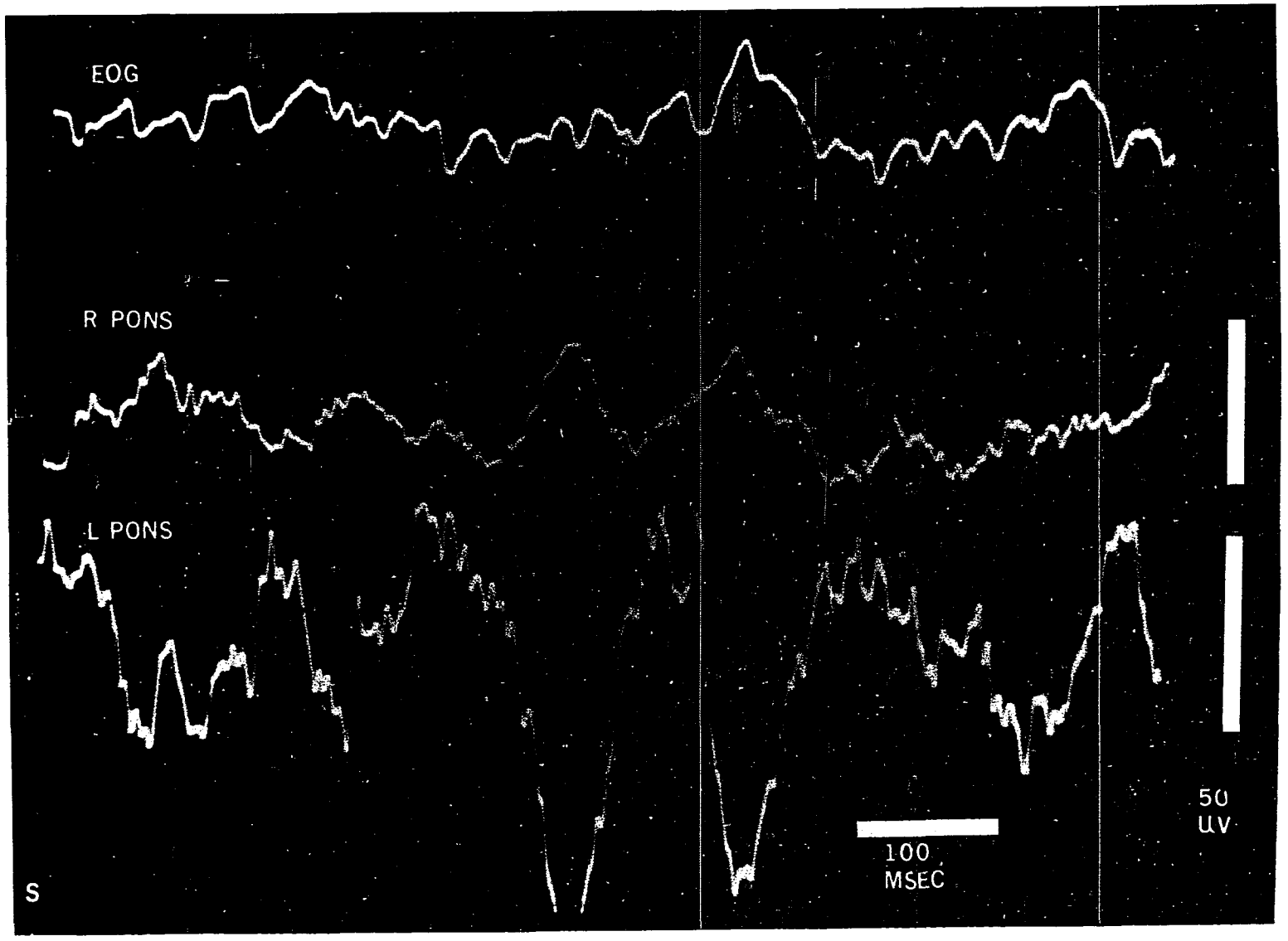


FIGURE 9.

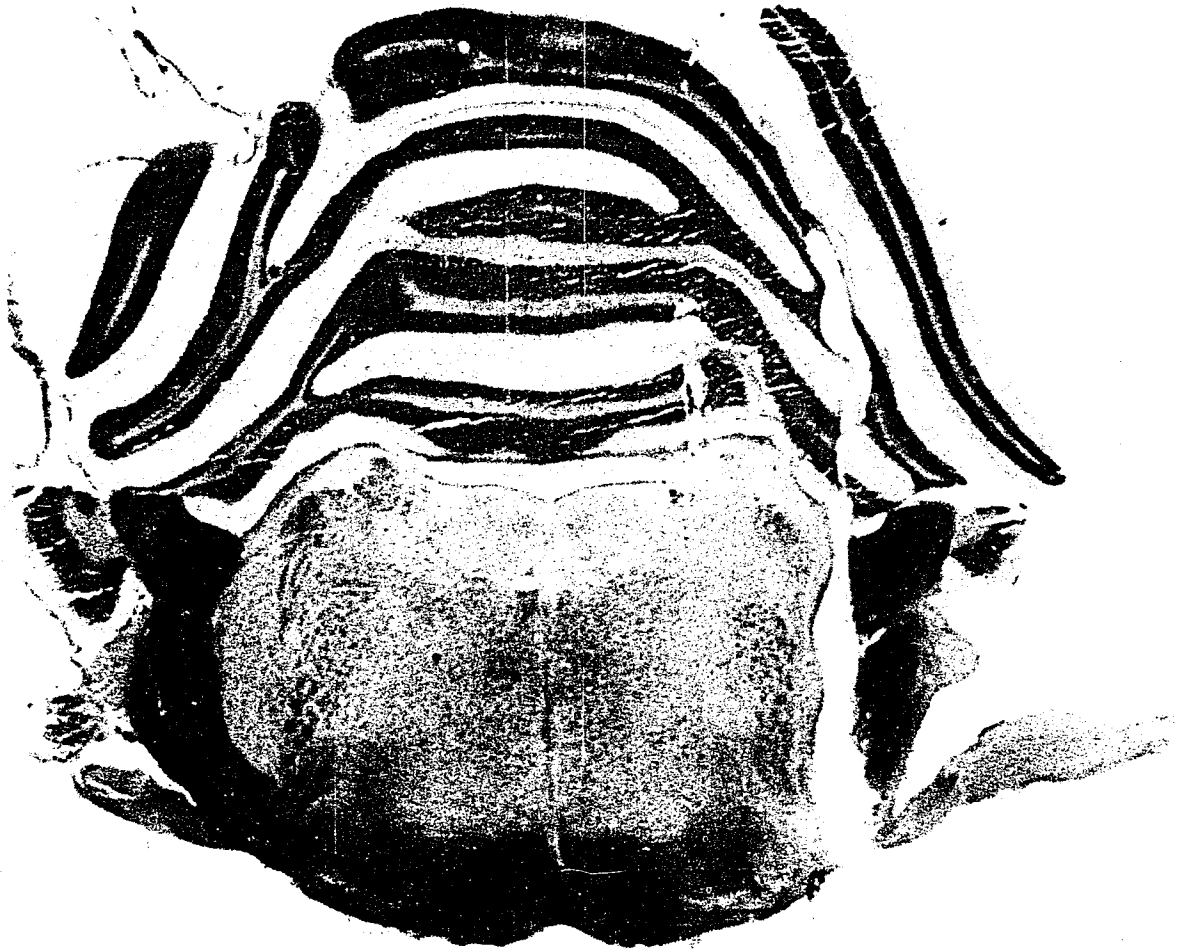


FIGURE 10a.

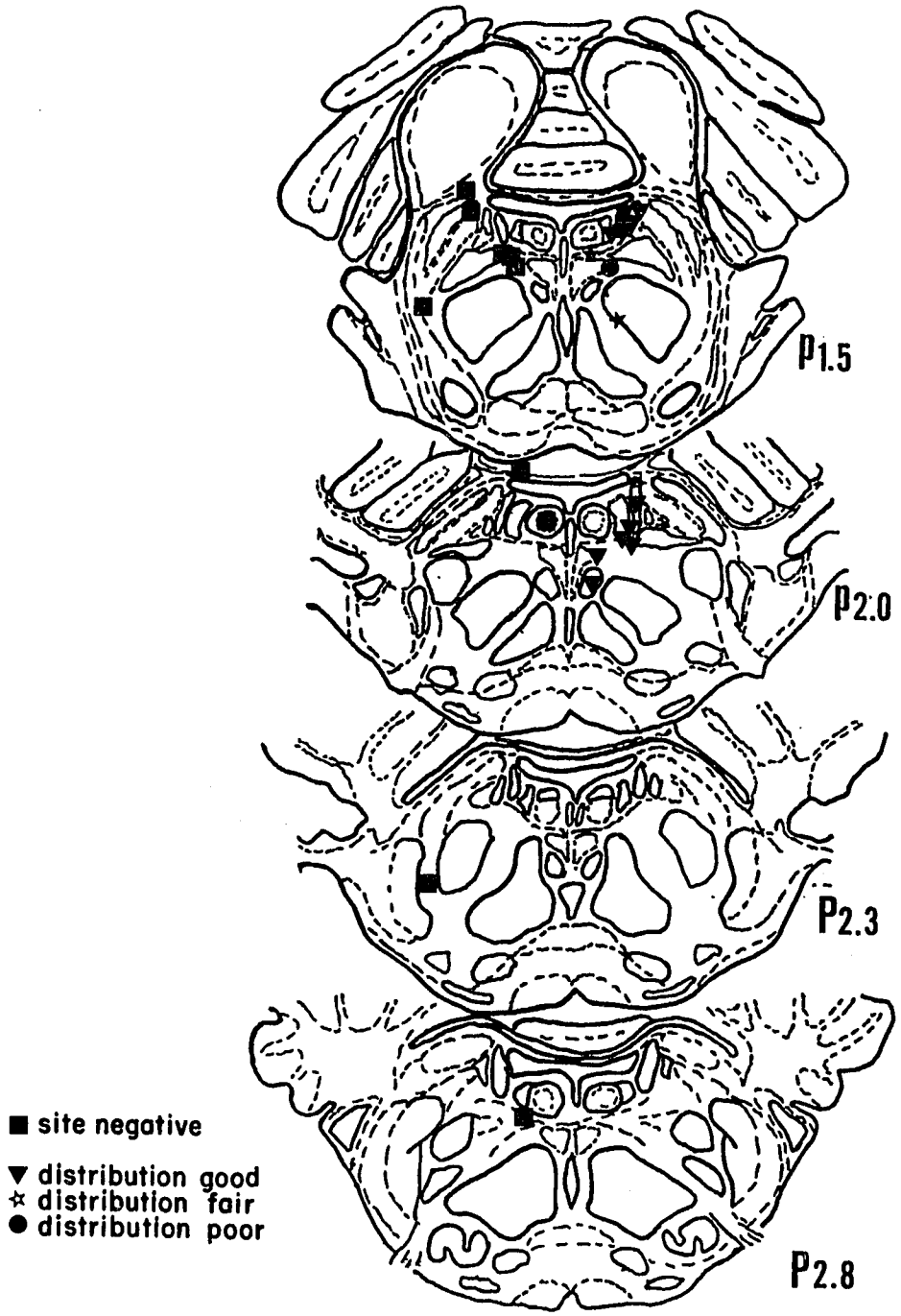

P1.5
P2.0
P2.3
P2.8
■ site negative
▼ distribution good
☆ distribution fair
● distribution poor

FIGURE 10b.

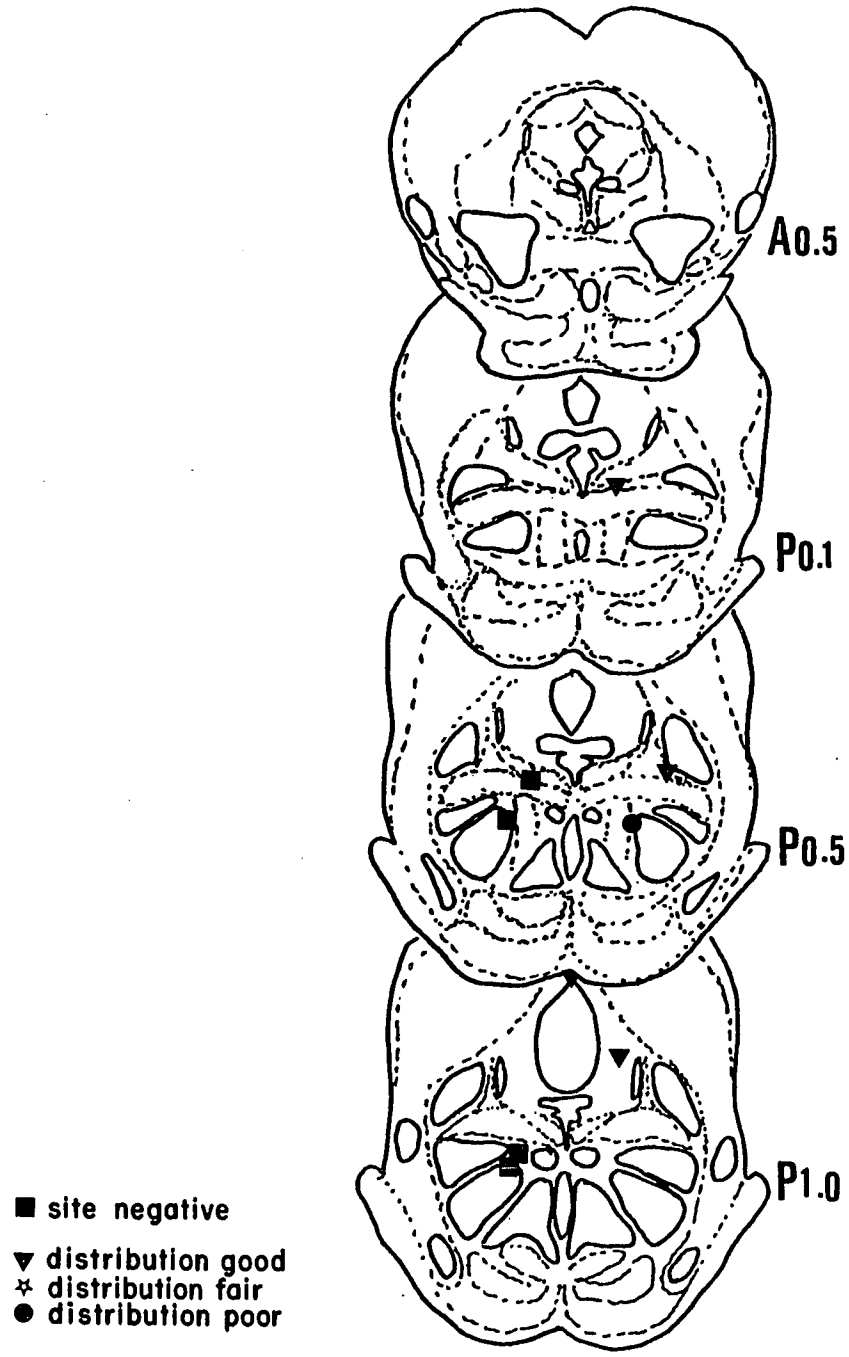
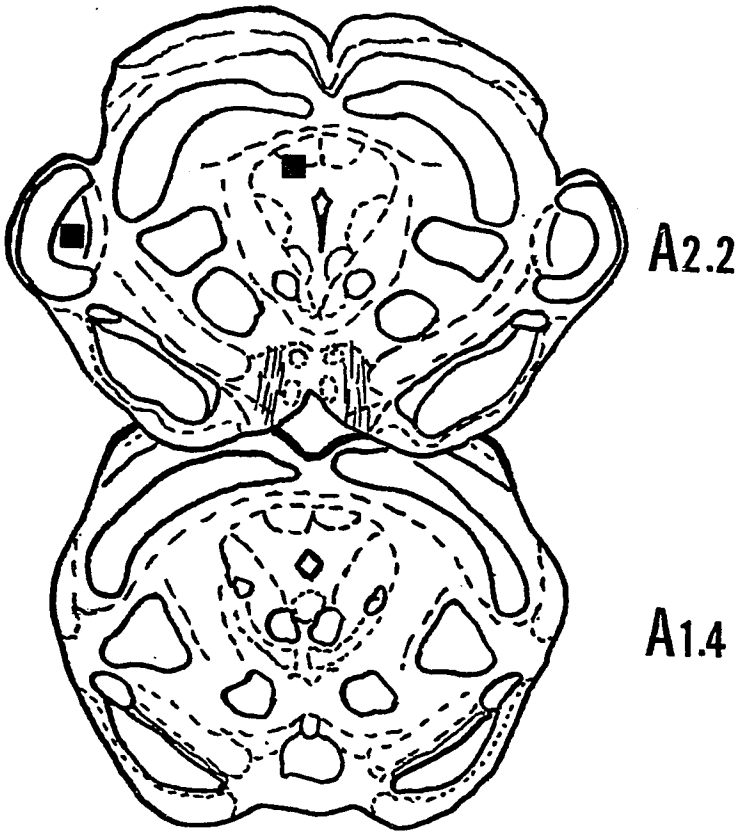
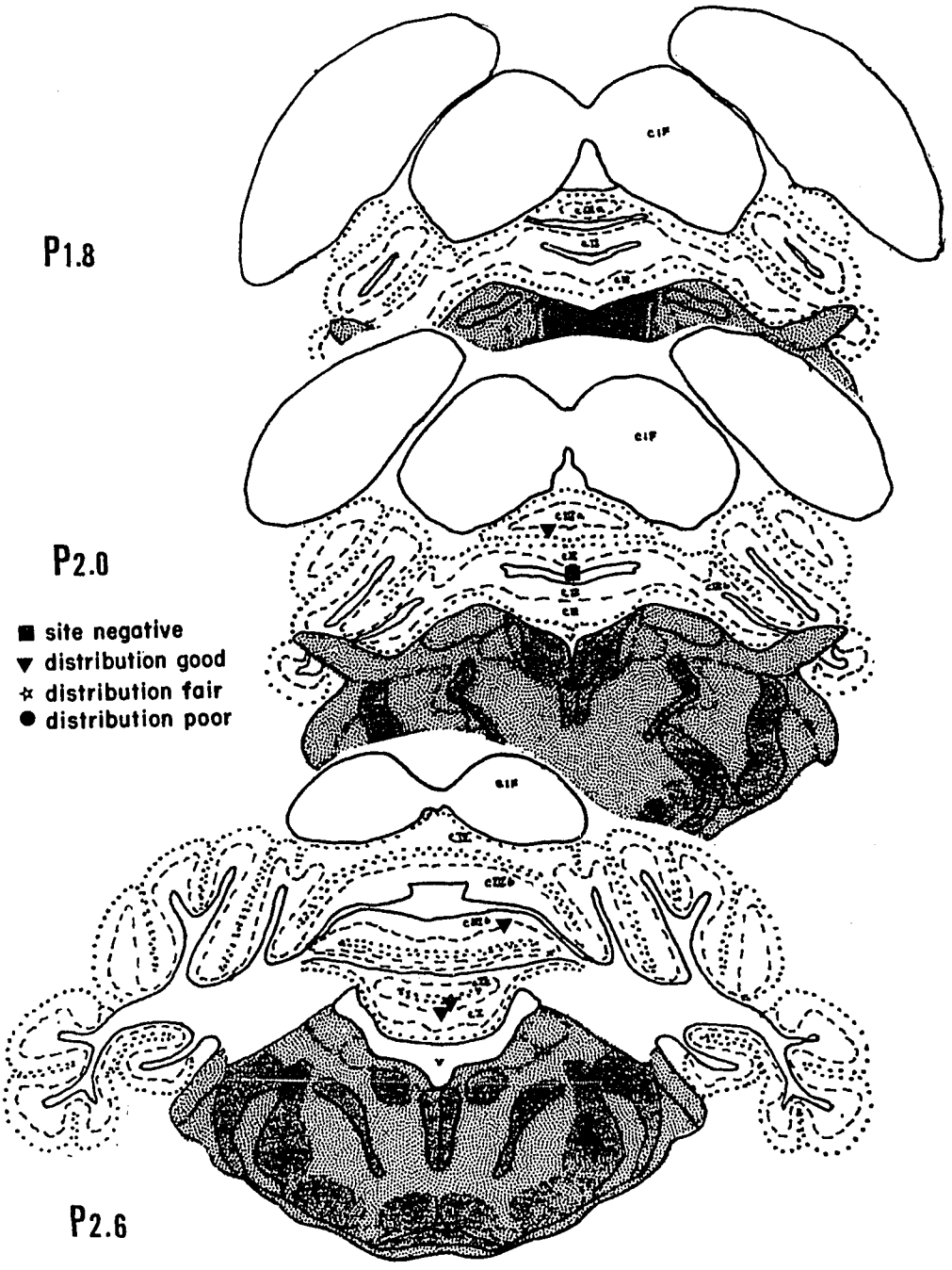

A0.5
P0.1
P0.5
P1.0
■ site negative
▼ distribution good
✲ distribution fair
● distribution poor

FIGURE 10c.




■ site negative

FIGURE 10d.


P1.8
P2.0
■ site negative
▼ distribution good
☆ distribution fair
● distribution poor
P2.6

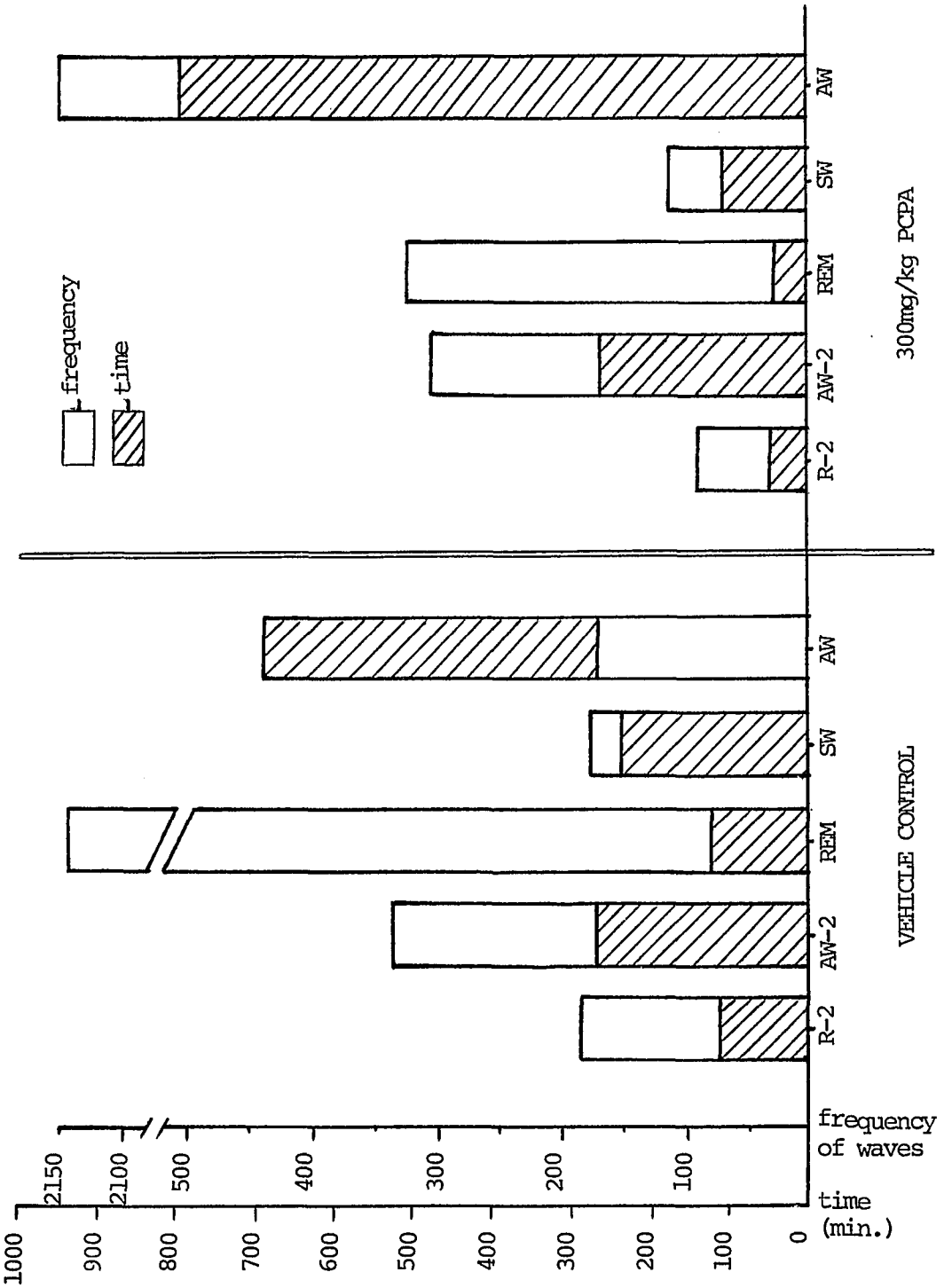
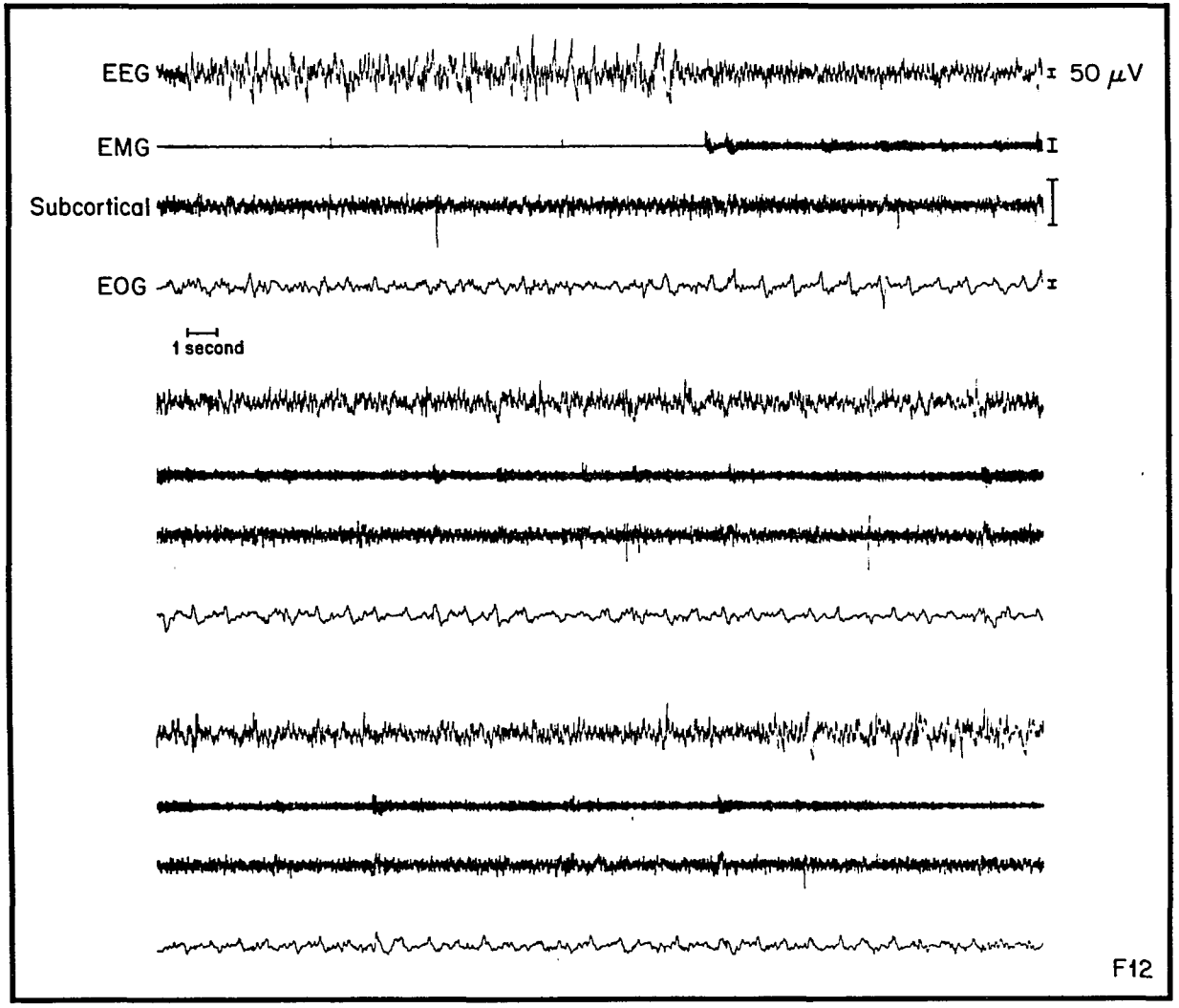

frequency
time
AW
SW
REM
AW-2
R-2
300mg/kg PCPA
VEHICLE CONTROL
frequency of waves
time (min.)
1000
900
800
700
600
500
400
300
200
100
0
2150
2100
500
400
300
200
100

FIGURE 11.

FIGURE 12.



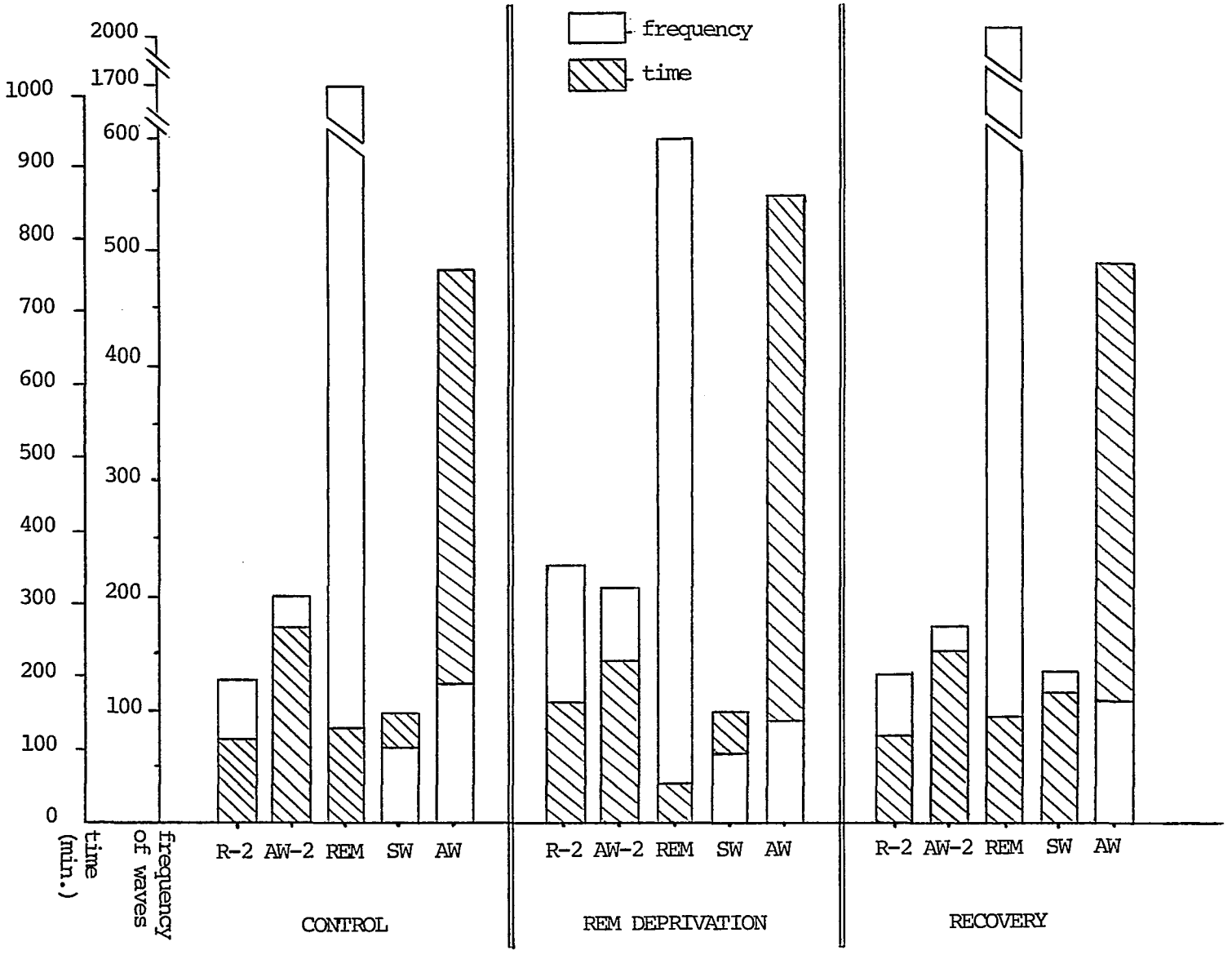

frequency
time
2000
1700
1000
600
900
800
500
700
400
600
300
500
400
200
300
200
100
100
0
time (min.)
frequency of waves
R-2 AW-2 REM SW AW
R-2 AW-2 REM SW AW
R-2 AW-2 REM SW AW
CONTROL
REM DEPRIVATION
RECOVERY

FIGURE 13.

TABLE 1a

Normative Wave Distribution

Absolute Frequency

| Subject | | R-2' | AW-2' | REM | SW | AW | TOTAL |
|---|---|---|---|---|---|---|---|
| RS-23 | | 331 | 423 | 4906 | 380 | 265 | 6305 |
| RS-37 | | 63 | 106 | 1008 | 70 | 110 | 1357 |
| RS-41 | | 141 | 371 | 981 | 221 | 123 | 1837 |
| RS-44 | | 185 | 444 | 1635 | 38 | 166 | 2468 |
| RS-42 | | 95 | 135 | 2604 | 42 | 90 | 2966 |
| RS-68 | | 161 | 264 | 786 | 79 | 149 | 1439 |
| $\bar{X}$ | | 166.7 | 290.5 | 1986.7 | 138.3 | 150.5 | 2728.7 |
| $\pm$ SD | | $\pm$93.6 | $\pm$146.0 | $\pm$1577.2 | $\pm$136.2 | $\pm$62.3 | $\pm$1857.6 |
| cor "t" values | R-2 | | 3.3 | 3.0 | N.S. | N.S. | |
| | AW-2 | | | 2.7 | 2.7 | 3.1 | |
| | REM | | | | 3.0 | 2.9 | |
| | SW | | | | | N.S. | |

TABLE 1b

Normative Wave Distribution

Rate (per minute)

| Subject | R-2" | AW-2" | REM | SW | AW |
|---|---|---|---|---|---|
| RS-23 | 2.75 | 1.62 | 35.74 | 1.02 | 0.50 |
| RS-37 | 0.69 | 0.43 | 7.49 | 0.30 | 0.15 |
| RS-41 | 1.35 | 1.18 | 8.86 | 0.88 | 0.19 |
| RS-44 | 1.68 | 1.55 | 14.71 | 0.54 | 0.19 |
| RS-42 | 0.69 | 0.47 | 20.89 | 0.22 | 0.13 |
| RS-68 | 1.69 | 1.02 | 6.64 | 0.62 | 0.18 |
| $\bar{X}$ | 1.48 | 1.05 | 15.72 | 0.60 | 0.22 |
| $\pm$ SD | $\pm$0.77 | $\pm$0.51 | $\pm$11.19 | $\pm$0.31 | $\pm$0.14 |
| cor "t" values | R-2 | N.S. | 3.3 | 4.1 | 4.7 |
| | AW-2 | | 3.3 | 3.5 | 4.7 |
| | REM | | | 3.3 | 3.4 |
| | SW | | | | 4.0 |

TABLE 1c

Normative Wave Distribution

Weighted Rate

| Subject | R-2' | AW-2' | REM | SW | AW |
|---|---|---|---|---|---|
| RS-23 | 0.87 | 0.51 | 11.34 | 0.32 | 0.16 |
| RS-37 | 1.02 | 0.63 | 11.04 | 0.44 | 0.22 |
| RS-41 | 1.47 | 1.28 | 9.65 | 0.96 | 0.21 |
| RS-44 | 1.36 | 1.26 | 11.92 | 0.43 | 0.15 |
| RS-42 | 0.47 | 0.32 | 14.09 | 0.15 | 0.09 |
| RS-68 | 2.35 | 1.42 | 9.23 | 0.86 | 0.25 |
| $\overline{X}$ | 1.26 | 0.90 | 11.21 | 0.53 | 0.18 |
| $\pm$ SD | $\pm$0.64 | $\pm$0.47 | $\pm$ 1.74 | $\pm$0.32 | $\pm$0.06 |
| cor "t" values | R-2 | 2.8 | 10.6 | 4.2 | 4.4 |
| | AW-2 | | 11.9 | 3.5 | 4.1 |
| | REM | | | 12.9 | 15.0 |
| | SW | | | | 3.1 |

TABLE 1d

Normative Wave Distribution

Percent Frequency

| Subject | | R-2' | AW-2' | REM | SW | AW |
|---|---|---|---|---|---|---|
| RS-23 | | 5.25 | 6.71 | 77.81 | 6.03 | 4.20 |
| RS-37 | | 4.64 | 7.81 | 74.28 | 5.16 | 8.12 |
| RS-41 | | 7.68 | 20.20 | 53.40 | 12.03 | 6.70 |
| RS-44 | | 7.50 | 17.99 | 66.25 | 1.54 | 6.73 |
| RS-42 | | 3.20 | 4.55 | 87.80 | 1.42 | 3.03 |
| RS-68 | | 11.19 | 18.35 | 54.62 | 5.49 | 10.35 |
| $\bar{X}$ | | 6.58 | 12.60 | 69.03 | 5.28 | 6.52 |
| $\pm$ SD | | $\pm$2.84 | $\pm$ 6.96 | $\pm$13.54 | $\pm$3.87 | $\pm$2.64 |
| cor "t" values | R-2 | | 3.1 | 9.5 | N.S. | N. S. |
| | AW-2' | | | 6.8 | 2.8 | 2.6 |
| | REM | | | | 9.6 | 9.8 |
| | SW | | | | | N.S. |

TABLE 1e

Normative Wave Distribution

Time in Minutes

| Subject | | R-2 | AW-2 | REM | SW | AW | TOTAL |
|---|---|---|---|---|---|---|---|
| RS-23 | | 120.36 | 261.11 | 137.27 | 372.55 | 530.00 | 1421.29 |
| RS-37 | | 91.30 | 246.51 | 134.58 | 233.33 | 733.33 | 1439.05 |
| RS-41 | | 104.44 | 314.41 | 110.72 | 251.14 | 647.37 | 1428.08 |
| RS-44 | | 110.12 | 286.45 | 111.15 | 70.37 | 873.68 | 1451.77 |
| RS-42 | | 137.68 | 287.23 | 124.65 | 190.91 | 629.31 | 1432.78 |
| RS-68 | | 95.27 | 258.82 | 118.37 | 127.42 | 827.78 | 1427.66 |
| $\overline{X}$ | | 109.9 | 275.8 | 122.8 | 207.6 | 717.4 | |
| $\pm$ SD | | $\pm$ 17.2 | $\pm$24.9 | $\pm$11.4 | $\pm$105.2 | $\pm$124.5 | |
| cor "t" values | R-2 | | 16.4 | N.S. | N.S. | 11.3 | |
| | AW-2 | | | 11.0 | N.S. | 8.4 | |
| | REM | | | | N.S. | 11.1 | |
| | SW | | | | | 5.5 | |

TABLE 2

Correlation Matrix Between Variables

| | | | | |
|---|---|---|---|---|
| Absolute Frequency | -.2741 | | | |
| Rate | -.2658 | .9957 | | |
| Weighted Rate | -.2241 | .8647 | .8571 | |
| Percent Frequency | -.2195 | .8187 | .7986 | .9848 |
| | Time | Absolute Frequency | Rate | Weighted Rate |

TABLE 3

Normative Wave Distribution

Within REM Periods

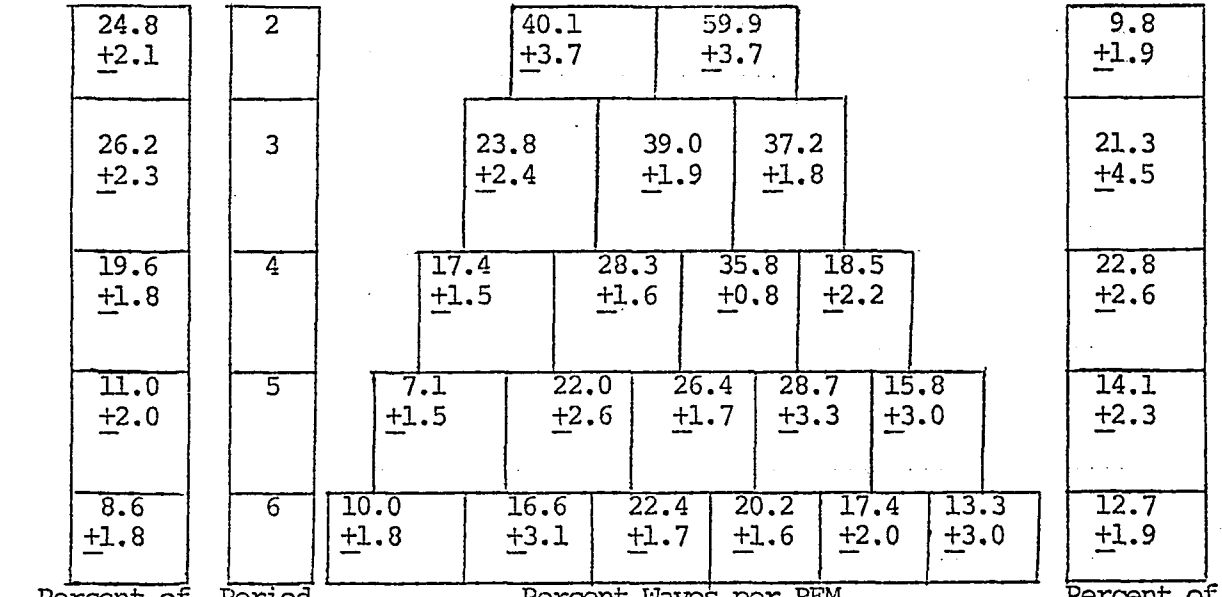


TABLE 4

Anatomical Distribution of

Electrophysiological Activity

| Subject | Site | | Electrophysiological Activity | | | |
|---|---|---|---|---|---|---|
| RS-# | Frontal plane Omm-interauricular line | Description in plane | Presence of waves | Distribution | S/N | MAC(uv) |
| 7 | P2.0 | PCS-ntm | + | good | 4.0 | 15.8 |
| | P1.5 | Lat to rpoo, med to PCM | - | | | |
| 9 | P1.5 | Vent to SGC Lat to FLM, med to npg | - | | | |
| | P2.3 | Med to npv, lat to nV | - | | | |
| 11 | P2.0 | dorsal lc | + | fair | 3.3 | 38.5 |
| 12 | A1.8 | ccgm | - | | | |
| | P2.0 | ventro med to lc | + | good | 4.0 | 30.8 |
| 15 | P1.5 | med to vent lc | + | good | 3.2 | 36.4 |
| | P2.0 | vent lc | + | fair | 3.5 | 58.3 |
| 17 | P1.0 | med to dors ntm | + | good | 3.0 | 46.2 |
| | P0.5 | vent to npd dors PCS | + | good | 5.0 | 19.23 |
| 23 | P2.6 | PCS-CI | + | good | 4.0 | 75.0 |
| 25 | A2.2 | SGCD-FLD | - | | | |
| 29 | P2.0 | lc | + | good | 4.0 | 36.4 |
| 33 | P0.5 | PCS-lattord | - | | | |
| 35 | P1.5 | vent to lc | - | | | |

TABLE 4 cont'd

| | | | | | | |
|---|---|---|---|---|---|---|
| 37 | P1.5 | lc- med ntm | + | good | 3.0 | 20.0 |
| 38 | P1.5 | vent to lc | - | | | |
| 41 | P1.5 | lac to FLM, dors to ntv, vent to ntd | + | poor | 3.3 | 66.7 |
| 42 | P1.5 | lc | + | good | 5.6 | 26.9 |
| 44 | P1.5+ | vent lc | + | good | 2.5 | 62.5 |
| 47 | P1.5 | vent to lc | - | | | |
| 48 | P1.5 | lat to ntm - PCS | + | good | 3.1 | 26.2 |
| 50 | P1.5 | lat to ntm - PCS | - | | | |
| 51 | P2.8 | cIIIa-cII off midline | + | good | 1.5 | 37.5 |
| 52 | P1.0 | midline ci | + | good | 4.0 | 21.1 |
| 54 | P2.0 | lat FLM | + | good | 3.2 | 34.8 |
| 55 | P1.5 | med rpoo | + | fair | 3.6 | 50.0 |
| 56 | P2.8 | radiation of Cn VII | - | | | |
| 57 | P1.5 | CN IV through velum | - | | | |
| 59 | P2.0 | vent to ntm | + | good | 4.0 | 60.0 |
| 60 | P2.0 | ntd | - | | | |
| 61 | P0.1 | ventromed FLM dorsal DPCS | + | good | 5.0 | 50.0 |
| 62 | P2.0 | vent lc | + | good | 3.5 | 31.8 |
| 63 | P1.0 | lat to ntv, med to npv dors to rpoo, vent to PCS | - | | | |

TABLE 4 cont'd

| | | | | | | |
|---|---|---|---|---|---|---|
| 64 | P1.5 | lat to FLM, vent to ntd dors to ntv | + | poor | 4.0 | 27.3 |
| 65 | P2.0 | velum-dors to lc | - | | | |
| 66 | P0.5 | dorsomed rpoo | - | | | |
| 67 | P1.0 | dorsomed rpoo | - | | | |
| 68 | P2.0 | ntv | + | good | 4.0 | 15.8 |
| 69 | P0.5 | med & slightly impinging on rpoo | + | poor | 4.0 | 26.7 |
| 70 | P1.5 | dors FLM | + | fair | 4.0 | 30.0 |
| 71 | P1.8 | cII midline | + | good | 4.0 | 19.5 |
| 72 | P2.0 | cII midline | - | | | |
| 73 | P2.0 | cIIIa off midline | + | good | 4.7 | 35.0 |

Table 4. abbreviations not in text: DPCS, olecusation of PCS; FLD, fasciculus longitudinalis dorsalis; $n\bar{v}$, nucleus originis nervi trigemini; PCM, pedunculus cerebellaris medius; rd, nucleus raphe dorsalis.

TABLE 5a

Control vs PCPA Wave Distribution

Absolute Frequency

| | Vehicle Baseline | | | | | PCPA 300mg/kg | | | | | BL | PCPA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | R-2' | AW-2' | REM | SW | AW | R-2 | AW-2 | REM | SW | AW | Total | Total |
| RS-23 | 331 | 423 | 4906 | 380 | 265 | 196 | 581 | 282 | 326 | 1307 | 6305 | 2692 |
| RS-37 | 63 | 106 | 1008 | 70 | 110 | 45 | 128 | 430 | 74 | 325 | 1357 | 1002 |
| RS-41 | 141 | 371 | 981 | 221 | 123 | 17 | 159 | 228 | 44 | 73 | 1837 | 521 |
| RS-44 | 185 | 444 | 1635 | 38 | 166 | 112 | 352 | 357 | 14 | 345 | 2468 | 1180 |
| $\bar{X}$ | 180 | 336 | 2132 | 177 | 166 | 93 | 305 | 324 | 115 | 513 | 2992 | 1349 |
| $\pm$ SD | $\pm$113 | $\pm$156 | $\pm$1873 | $\pm$157 | $\pm$70 | $\pm$80 | $\pm$209 | $\pm$88 | $\pm$143 | $\pm$544 | $\pm$2255 | $\pm$938 |

TABLE 5b

Control vs PCPA Wave Distribution

Rate (per minute)

| | Vehicle Baseline | | | | | PCPA 300mg/kg | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Subject | R-2' | AW-2 | REM | SW | AW | R-2' | AW-2 | REM | SW | AW |
| RS-23 | 2.75 | 1.62 | 35.74 | 1.02 | 0.50 | 2.47 | 1.91 | 4.52 | 1.71 | 1.77 |
| RS-37 | 0.69 | 0.43 | 7.49 | 0.30 | 0.15 | 0.83 | 0.45 | 7.28 | 0.60 | 0.36 |
| RS-41 | 1.35 | 1.18 | 8.86 | 0.88 | 0.19 | 0.72 | 0.62 | 6.17 | 0.47 | 0.07 |
| RS-44 | 1.68 | 1.55 | 14.71 | 0.54 | 0.19 | 2.27 | 1.69 | 9.94 | 0.71 | 0.31 |
| $\overline{X}$ | 1.62 | 1.20 | 16.7 | 0.69 | 0.26 | 1.57 | 1.17 | 6.98 | 0.87 | 0.63 |
| $\pm$ SD | $\pm$0.86 | $\pm$0.55 | $\pm$13.07 | $\pm$0.33 | $\pm$0.16 | $\pm$0.93 | $\pm$0.74 | $\pm$2.28 | $\pm$0.57 | $\pm$0.77 |

TABLE 5c

Control vs PCPA Wave Distribution

Weighted Rate

| | Vehicle Baseline | | | | | PCPA 300mg/kg | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Subject | R-2 | AW-2 | REM | SW | AW | R-2 | AW-2 | REM | SW | AW |
| RS-23 | 0.87 | 0.51 | 11.34 | 0.32 | 0.16 | 1.84 | 1.42 | 3.36 | 1.27 | 1.32 |
| RS-37 | 1.02 | 0.63 | 11.04 | 0.44 | 0.22 | 1.66 | 0.90 | 14.53 | 1.20 | 0.72 |
| RS-41 | 1.47 | 1.28 | 9.65 | 0.96 | 0.21 | 2.76 | 2.38 | 23.69 | 3.34 | 0.27 |
| RS-44 | 1.36 | 1.26 | 11.92 | 0.43 | 0.15 | 3.85 | 2.86 | 16.85 | 1.20 | 0.53 |
| $\bar{X}$ | 1.18 | 0.92 | 10.99 | 0.54 | 0.19 | 2.53 | 1.89 | 14.61 | 1.75 | 0.71 |
| $\pm$ SD | $\pm$0.28 | $\pm$0.41 | $\pm$0.96 | $\pm$0.29 | $\pm$0.04 | $\pm$1.00 | $\pm$0.89 | $\pm$8.45 | $\pm$1.06 | $\pm$0.45 |
| cor "t" values | 3.3 | 3.5 | N.S. | 3.1 | N.S. | | | | | |

TABLE 5d

CONTROL VS PCPA WAVE DISTRIBUTION

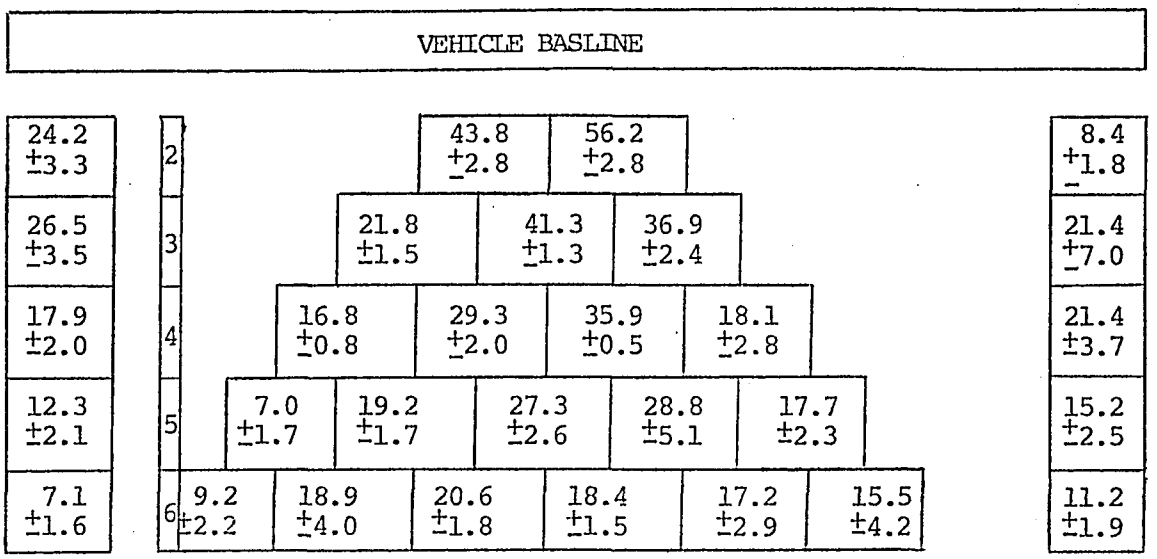
Percent Frequency

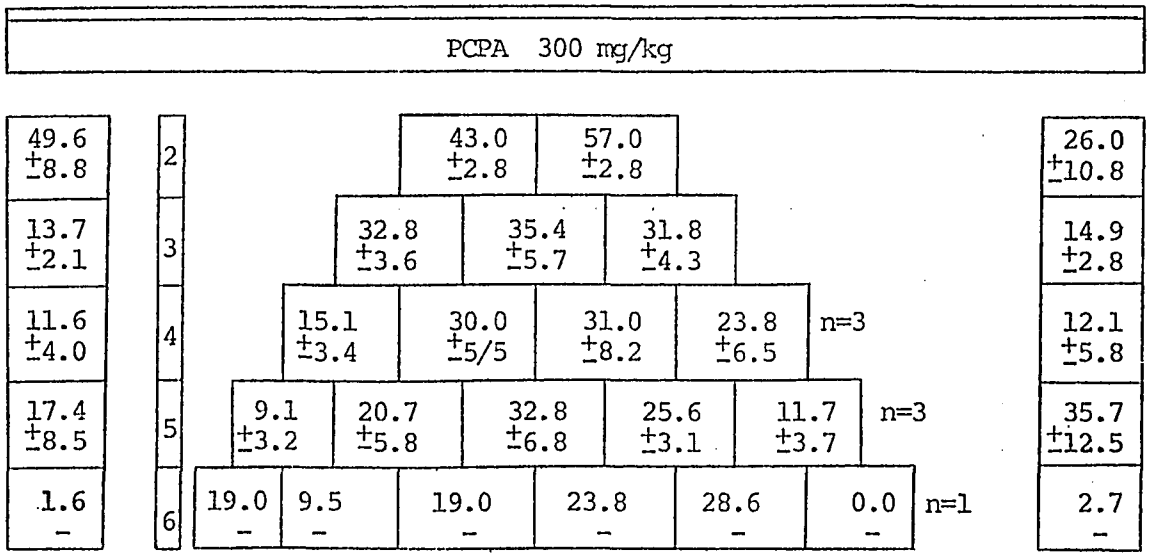
| | VEHICLE BASLINE | | | | | PCPA 300 mg/kg | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Subject | R-2 | AW-2 | REM | SW | AW | R-2 | AW-2 | REM | SW | AW |
| RS-23 | 5.25 | 6.71 | 77.81 | 6.03 | 4.20 | 7.28 | 21.58 | 10.48 | 12.11 | 48.55 |
| RS-37 | 4.64 | 7.81 | 74.28 | 5.16 | 8.12 | 4.49 | 12.77 | 42.91 | 7.39 | 32.44 |
| RS-41 | 7.68 | 20.20 | 53.40 | 12.03 | 6.70 | 3.26 | 30.52 | 43.76 | 8.45 | 14.01 |
| RS-44 | 7.50 | 17.99 | 66.25 | 1.54 | 6.73 | 9.49 | 29.83 | 30.25 | 1.19 | 29.24 |
| $\bar{X}$ | 6.27 | 13.18 | 67.94 | 6.19 | 6.44 | 6.13 | 23.68 | 31.85 | 7.29 | 31.06 |
| $\pm$ SD | $\pm$1.55 | $\pm$6.91 | $\pm$10.83 | $\pm$4.35 | $\pm$1.63 | $\pm$2.80 | $\pm$8.33 | $\pm$15.53 | $\pm$4.54 | $\pm$14.16 |
| Cor"t" VALUES | N.S. | 5.1 | 3.0 | N.S. | 3.2 | | | | | |

TABLE 6

CONTROL VS PCPA WAVE DISTRIBUTION

WITHIN REM PERIODS




Percent of total number of REM periods $\bar{x} \pm$ SEM

Period length 30 sec epochs

Percent waves per REM period length appearing in each epoch $\bar{x} \pm$ SEM

Percent of total waves $\bar{x} \pm$ SEM

Table 7a

Control vs PCPA Sleep Amounts

30 second Epochs

| | VEHICLE BASELINE | | | PCPA 300 mg/kg | | |
|---|---|---|---|---|---|---|
| Subject | AW | SW | REM | AW | SW | REM |
| RS-23 | 1049.75 | 1509.75 | 274.50 | 1473.34 | 1148.88 | 124.82 |
| RS-37 | 1453.29 | 1147.54 | 269.17 | 1809.62 | 930.29 | 118.09 |
| RS-42 | 1308.68 | 1342.91 | 221.41 | 2043.04 | 751.04 | 73.92 |
| RS-44 | 1708.50 | 936.25 | 222.25 | 2240.67 | 553.67 | 71.83 |
| RS-38 | 1530.05 | 1112.21 | 229.75 | 2034.79 | 776.21 | 45.00 |
| $\bar{x}$ | 1410.1 | 1209.7 | 243.4 | 1920.3 | 832.0 | 86.7 |
| ±SD | ±247.7 | ±221.3 | ±26.2 | ±292.8 | ±222.0 | ±33.8 |
| Cor"t" valves | 8.0 | 6.2 | 22.3 | | | |

Table 7b

Control vs PCPA Sleep Amounts

Percent Total Recording Time

| | VEHICLE BASELINE | | | PCPA 300 mg/kg | | |
|---|---|---|---|---|---|---|
| Subject | AW | SW | REM | AW | SW | REM |
| RS-23 | 37.04 | 53.27 | 9.69 | 53.63 | 41.82 | 4.55 |
| RS-37 | 50.64 | 39.98 | 9.38 | 63.32 | 32.55 | 4.13 |
| RS-41 | 45.55 | 46.74 | 7.71 | 71.24 | 26.19 | 2.58 |
| RS-44 | 59.59 | 32.66 | 7.55 | 78.18 | 19.32 | 2.51 |
| RS-38 | 53.27 | 38.73 | 8.00 | 71.25 | 27.18 | 1.58 |
| $\bar{x}$ | 49.22 | 42.28 | 8.47 | 67.52 | 29.41 | 3.07 |
| $\pm$SD | $\pm$8.48 | $\pm$7.92 | $\pm$1.00 | $\pm$9.38 | $\pm$8.38 | $\pm$1.23 |
| Cor"t" valves | 8.7 | 6.0 | 20.9 | | | |

Table 7c

Control vs PCPA

REM Percent of Total Sleep Time

| Subject | Vehicle | PCPA |
|---|---|---|
| RS-23 | 15.39 | 9.81 |
| RS-37 | 19.00 | 11.26 |
| RS-41 | 14.15 | 8.96 |
| RS-44 | 19.18 | 11.48 |
| RS-38 | 17.13 | 5.48 |
| $\bar{x}$ | 16.97 | 9.40 |
| $\pm$SD | $\pm$2.21 | $\pm$2.42 |
| Cor"t" value | 6.6 | |

Table 8a

Control vs REM Deprivation vs Recovery Sleep Amounts

30 second Epochs

| | BASELINE | | | REM DEPRIVATION | | | RECOVERY | | |
|---|---|---|---|---|---|---|---|---|---|
| Subject | AW | SW | REM | AW | SW | REM | AW | SW | REM |
| RS-42 | 1401.63 | 1229.05 | 249.32 | 1513.93 | 1223.93 | 135.16 | 1426.46 | 1142.04 | 326.49 |
| RS-68 | 1661.96 | 967.29 | 236.75 | 1791.87 | 949.28 | 120.83 | 1567.05 | 1071.09 | 230.49 |
| $\bar{x}$ | 1531.80 | 1098.2 | 243.0 | 1652.9 | 1086.6 | 128.0 | 1496.8 | 1106.6 | 278.5 |
| $\pm$SD | $\pm$184.1 | $\pm$185.1 | $\pm$ 8.9 | $\pm$196.5 | $\pm$194.2 | $\pm$10.1 | $\pm$99.4 | $\pm$50.2 | $\pm$67.9 |
| Cor"t" valves | 13.8 | N.S. | 130.7 | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. |

Table 8b

Control vs REM Deprivation vs Recovery Sleep Amounts

Percent Total Recording Time

| | BASELINE | | | REM DEPRIVATION | | | RECOVERY | | |
|---|---|---|---|---|---|---|---|---|---|
| Subject | AW | SW | REM | AW | SW | REM | AW | SW | REM |
| RS-42 | 48.66 | 42.68 | 8.66 | 52.70 | 42.60 | 4.70 | 49.27 | 39.45 | 11.28 |
| RS-68 | 57.99 | 33.75 | 8.26 | 62.61 | 33.17 | 4.22 | 54.63 | 37.34 | 8.03 |
| $\bar{x}$ | 53.33 | 38.22 | 8.46 | 57.66 | 37.89 | 4.46 | 51.95 | 38.40 | 9.66 |
| ±SD | ±6.6 | ±6.3 | ±0.3 | ±7.0 | ±6.7 | ±0.3 | ±3.8 | ±1.5 | ±2.3 |
| Cor"t" Valves | 14.9 | N.S. | 100.0 | | | | N.S. | N.S. | N.S. |

Table 8c

Control vs REM Deprivation vs Recovery

REM Percent of Total Sleep Time

| Subject | BL | Dep | Rec |
|---|---|---|---|
| RS-42 | 16.86 | 9.94 | 22.23 |
| RS-68 | 19.66 | 11.29 | 17.70 |
| $\bar{x}$ | 18.26 | 10.62 | 19.97 |
| $\pm$SD | $\pm$2.0 | $\pm$1.0 | $\pm$3.2 |
| Cor"t" values | 10.5 | N.S. | |
| | N.S. | | |

Table 9a

Control vs REM Deprivation vs Recovery Wave Distribution

Absolute Frequency

| | BASELINE | | | | | REM DEPRIVATION | | | | | RECOVERY | | | | | TOTALS | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | R-2 | AW -2 | REM | SW | AW | R-2 | AW -2 | REM | SW | AW | R-2 | AW-2 | REM | SW | AW | BL | DEP | REC |
| RS-42 | 95 | 135 | 2604 | 42 | 90 | 218 | 202 | 874 | 53 | 85 | 111 | 154 | 3471 | 89 | 95 | 2966 | 1432 | 3920 |
| RS-68 | 161 | 264 | 786 | 79 | 149 | 238 | 200 | 315 | 62 | 93 | 148 | 201 | 551 | 140 | 126 | 1439 | 908 | 1166 |
| $\bar{x}$ | 128 | 200 | 1695 | 61 | 120 | 228 | 201 | 595 | 68 | 89 | 130 | 178 | 2011 | 115 | 111 | 2203 | 1170 | 2543 |
| $\pm$SD | $\pm$47 | $\pm$91 | $\pm$1286 | $\pm$26 | $\pm$42 | $\pm$14 | $\pm$ 1 | $\pm$395 | $\pm$ 6 | $\pm$ 6 | $\pm$26 | $\pm$33 | $\pm$2014 | $\pm$36 | $\pm$22 | $\pm$1080 | $\pm$371 | $\pm$1947 |

Table 9b

Control vs REM Deprivation vs Recovery Wave Distribution

Rate (per minute)

| | BASELINE | | | | | REM DEPRIVATION | | | | | RECOVERY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | R-2 | AW-2 | REM | SW | AW | R-2 | AW-2 | REM | SW | AW | R-2 | AW-2 | REM | SW | AW |
| RS-42 | 0.69 | 0.47 | 20.89 | 0.22 | 0.13 | 1.18 | 0.79 | 12.93 | 0.31 | 0.11 | 0.91 | 0.59 | 21.26 | 0.48 | 0.13 |
| RS-68 | 1.69 | 1.02 | 6.64 | 0.62 | 0.18 | 1.52 | 1.10 | 5.21 | 0.46 | 0.10 | 1.33 | 0.94 | 4.78 | 0.67 | 0.16 |
| $\bar{x}$ | 1.19 | 0.75 | 13.77 | 0.42 | 0.16 | 1.35 | 0.95 | 9.07 | 0.39 | 0.11 | 1.12 | 0.77 | 13.02 | 0.58 | 0.15 |
| $\pm$SD | $\pm$0.71 | $\pm$0.39 | $\pm$10.08 | $\pm$0.28 | $\pm$0.04 | $\pm$0.24 | $\pm$0.22 | $\pm$5.46 | $\pm$0.11 | $\pm$0.01 | $\pm$0.30 | $\pm$0.25 | $\pm$11.65 | $\pm$0.13 | $\pm$0.02 |

Table 9c

Control vs REM Deprivation vs Recovery Wave Distribution

Weighted Rate

| | BASELINE | | | | | REM DEPRIVATION | | | | | RECOVERY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | R-2 | AW-2 | REM | SW | AW | R-2 | AW-2 | REM | SW | AW | R-2 | AW-2 | REM | SW | AW |
| RS-42 | 0.47 | 0.32 | 14.09 | 0.15 | 0.09 | 1.65 | 1.10 | 18.06 | 0.43 | 0.15 | 0.46 | 0.30 | 10.85 | 0.24 | 0.07 |
| RS-68 | 2.35 | 1.42 | 9.23 | 0.86 | 0.25 | 3.35 | 2.42 | 11.48 | 1.01 | 0.23 | 2.26 | 1.59 | 8.13 | 1.15 | 0.27 |
| $\bar{x}$ | 1.41 | 0.87 | 11.66 | 0.51 | 0.17 | 2.50 | 1.76 | 14.77 | 0.72 | 0.19 | 1.36 | 0.95 | 9.49 | 0.70 | 0.17 |
| $\pm$SD | $\pm$1.33 | $\pm$0.78 | $\pm$3.44 | $\pm$0.50 | $\pm$0.11 | $\pm$1.20 | $\pm$0.93 | $\pm$4.65 | $\pm$0.41 | $\pm$0.06 | $\pm$1.27 | $\pm$0.91 | $\pm$1.92 | $\pm$0.64 | $\pm$0.14 |
| Cor"t" valves | 12.1 | 11.5 | N.S. | N.S. | N.S. | | | | | | 22.8 | 54.3 | N.S. | N.S. | N.S. |

Table 9d

Control vs REM Deprivation vs Recovery Wave Distribution

Percent Frequency

| | BASELINE | | | | | REM DEPRIVATION | | | | | RECOVERY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | R-2 | AW-2 | REM | SW | AW | R-2 | AW-2 | REM | SW | AW | R-2 | AW-2 | REM | SW | AW |
| RS-42 | 3.20 | 4.55 | 87.8 | 1.42 | 3.03 | 15.22 | 14.11 | 61.03 | 3.70 | 5.94 | 2.83 | 3.93 | 88.55 | 2.27 | 2.42 |
| RS-68 | 11.19 | 18.35 | 54.62 | 5.49 | 10.35 | 26.21 | 22.03 | 34.69 | 6.83 | 10.24 | 12.69 | 17.24 | 47.26 | 12.01 | 10.81 |
| $\bar{x}$ | 7.20 | 11.45 | 71.21 | 3.46 | 6.69 | 20.72 | 18.07 | 47.86 | 5.27 | 8.09 | 7.76 | 10.59 | 67.91 | 7.14 | 6.62 |
| $\pm$SD | $\pm$5.65 | $\pm$9.76 | $\pm$23.46 | $\pm$2.88 | $\pm$5.18 | $\pm$7.77 | $\pm$5.60 | $\pm$18.63 | $\pm$2.13 | $\pm$3.04 | $\pm$6.97 | $\pm$9.41 | $\pm$29.20 | $\pm$6.89 | $\pm$5.93 |
| Cor"t" valves | 9.0 | N.S. | 6.8 | N.S. | N.S. | | | | | | 22.9 | N.S. | N.S. | N.S. | N.S. |

## Glossary of Abbreviations

| | |
|---|---|
| A | epoch scored awake |
| AR | epoch scored awake with REM sleep present |
| AW | awake |
| AW-2 | category of slow wave sleep within two minutes of a spontaneous awakening |
| A1.0mm | frontal plane of section one millimeter anterior to the interauricular line |
| β | sutura coronalis, Bregma |
| cm | centimeter |
| ci | colliculus inferior |
| cor "t" | value of the student "t" in a "t" test of correlated means |
| CNS | central nervous system |
| cI | lingula of the cerebellum |
| cII | ventral lobe of the lobus centralis of the cerebellum |
| df | degrees of freedom |
| DPS | dorsal periventricular system |
| EEG | electroencephalogram |
| EMG | electromyogram |
| EMP | eyemovement potential |
| EOG | electrooculogram |
| FLM | fasciculus longitudinalis medialis |
| FTG | giganto cellular tegmental field |
| 5HT | 5-hydroxytryptamine, serotonin |
| HCl | hydrocloride radical |
| HF | high frequency filter |

| | |
|---|---|
| HRP | horseradish peroxidase |
| $H_z$ | hertz, cycles per second |
| IP | intraperitoneal route of injection |
| $\lambda$ | sutura lambdoidea, lambda |
| $\lambda\lambda$ | lambda line, hypothetical line intersecting the lambda suture, two millimeters lateral to midline |
| lc | nucleus locus coeruleus |
| LF | low frequency filter |
| LGN | lateral geniculate nucleus |
| M | epoch scored mixed, contains awake, slow wave sleep, and REM sleep, none in majority |
| MAC | minimum amplitude criteria for determining central phasic waves |
| MEMA | middle ear muscle activity |
| MGN | medial geniculate nucleus |
| uv | microvolts |
| mg/kg | milligram per kilogram |
| ml | milliliter |
| mm | millimeter |
| msec | millisecond |
| npd | nucleus parabrachialis dorsalis |
| npv | nucleus parabrachialis ventralis |
| ntd | nucleus tegmenti dorsalis von Gudden |
| ntm | nucleus tractus mesencephali; mesencephalic V |
| ntv | nucleus tegmenti ventralis von Gudden |
| P1.0mm | frontal plane of section one millimeter posterior to the interauricular line |
| PCPA | para-chlorophenylalanine |

| | |
|---|---|
| PCS | pedunculus cerebellaris superior, brachium conjunctivum |
| PGO | ponto-geniculo-occipital (waves) |
| PRF | pontine reticular formation |
| R | epoch scored REM sleep |
| RAS | reticular activating system |
| REM | rapid eye movement |
| R-2 | category of slow wave sleep within two minutes of REM sleep onset |
| rpoo | nucleus reticularis pontis oralis |
| S | epoch scored slow wave sleep |
| $\pm$ SD | standard deviation |
| SEM | standard error of the mean |
| S/N | signal to noise ratio |
| SR | epoch scored slow wave sleep with REM sleep |
| SW | slow wave |
| SWS | slow wave sleep |
| TT | tractus tectospinalis |
| W | watts |
| $\bar{x}$ | arithmetic mean |

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