THE ROLE OF CHOLINERGIC NEURONS OF THE DORSOLATERAL PONTOMESENCEPHALIC TEGMENTUM IN SLEEP-WAKEFULNESS STATES

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ABSTRACT

Pontomesencephalic tegmental cholinergic neurons were destroyed in cats by local injections of kainic acid in order to assess the role of these neurons in sleep-wakefulness states and in the defining variables of these states: EEG (electroencephalographic) and EMG (electromyographic) amplitude, PGO (ponto-geniculo-occipital) spike rate, REMs (rapid eye movements) and (OBS) olfactory bulb spindles. Loss of cholinergic innervation to forebrain and brainstem structures was also assessed by histochemistry. Histological and histochemical analysis of the brains after the lesion showed a major destruction of the pontomesencephalic cholinergic neurons and a major loss of innervation to thalamic nuclei and brainstem regions, including the reticular formation. Whereas the states of waking and slow wave sleep were relatively unaffected, paradoxical sleep (PS) was reduced or eliminated immediately following the lesions. Two to three weeks later, incipient PS-like episodes returned with a reduced PGO spike rate and REMs, and an elevated EMG amplitude, marking the loss of muscle atonia. Such results suggest pontomesencephalic cholinergic neurons and their projections to thalamic and brainstem regions are important for the expression of PS and its defining variables.

RESUME

Le rôle des neurones cholinergiques du tegmentum ponto-mésencéphalique, dans les états de veille et de sommeil chez le chat, a été déterminé en mesurant des paramètres caractéristiques de ces états (électroencéphalogramme, électromyogramme, et pointes ponto-géniculo-occipitales) après une lésion avec l'acide kainique. La perte ou la diminution de l'innervation cholinergique des structures localisées dans le prosencéphale et le tronc cérébral a également été estimée par immunohistochimie. Une destruction importante des neurones cholinergiques pontomésencéphaliques ainsi qu'une diminution de l'innervation des noyaux du thalamus et des régions du tronc cérébral, dont la formation réticulée, ont été observées. Bien que les états de veille et de sommeil a onde lente soient peu modifiés, le sommeil paradoxal est diminué voire éliminé immédiatement après la lésion. Cependant des périodes ressemblant au sommeil paradoxal réapparaissent deux à trois semaines après la lésion. Elles sont caractérisées par un nombre réduit de pointes ponto-géniculo-occipitales et de mouvements rapides des yeux, ainsi que par une augmentation de l'amplitude de l'électromyogramme caractérisant la disparition de l'atonie musculaire. Ces résultats suggèrent l'importance des neurones cholinergiques de la région pontomésencéphalique ainsi que de leurs projections au thalamus et aux régions du tronc cérébral dans l'expression du sommeil paradoxal.

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Claim to Original Work.

This is the first report of a direct approach to the study of brainstem cholinergic neurons and their importance in sleep-wakefulness states, involving the specific localization and identification of these neurons by immunohistochemical means in the dorsolateral pontomesencephalic tegmentum of the cat. This is also the first report in which kainic acid, a neurotoxin which destroys cell bodies but not fibers, has been employed to show that neurons located in the dorsolateral pontomesencephalic tegmentum are important for the expression of PS. The quantification of the number of cholinergic neurons and subsequent statistical correlation between this number and the amount of PS and PGO rate is also original work. This result contrasts with the lack of correlation between the number of noradrenergic neurons located in the same area, and the amount of PS. This is also the first study in which the combined histochemical and immunohistochemical assessment of the loss of cholinergic innervation to forebrain and brainstem target areas in the same animal reveals potential cholinergic pathways which may be involved in the system controlling PS.

In conclusion, this is the first study to provide direct evidence of the importance of pontomesencephalic cholinergic neurons and their projections in the generation of PS.

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Sleeping and waking are regularly recurring states during which physiological, behavioural and mental changes take place within an organism. The state of an organism can be operationally defined by a set of numerical values which may be assigned to relevant physiological and behavioural variables at any one instant in time (Ashby, 1954, 1964; Hobson & Steriade, 1986). On the basis of investigations that took place from the 1930's through to the 1950's, three distinctly recognizable sleep-wakefulness states have been defined objectively in mammals according to criteria determined by behavioural and physiological parameters (for reviews see Jouvet, 1969; Koella, 1985). These states are waking, slow wave sleep and PS, and following their phenomenological description, much research has been directed towards understanding their underlying mechanisms. One avenue of these investigations has focussed on the effect of pharmacological agents. When administered systemically or injected directly into the brain these agents yield information on the type of neurotransmitters which might be involved in the mediation or modulation of a particular state. Jouvet referred to the pharmacological method as the indirect approach to the study of sleep-waking states. A second focus of these physiological studies has been on the effects of lesions or electrical stimulation of specific brain structures on a particular state. The tran-

section of fibers connecting different regions of the brain is also included in these methods of investigation. By the late 1950's and early 1960's, several monoamines, which had been shown to have effects on vigilance states, were localized within or near structures in the brain that had previously been found to participate in the control of sleep-wakefulness states. The localization of these monoamines in the brain made it possible to use lesions to destroy specific structures in which a particular transmitter predominated. Jouvet referred to the lesioning of specific structures containing known neurotransmitters as the direct approach to the study of these transmitters in the control of sleep-wakefulness states.

Ever since its discovery as a transmitter in the brain, acetylcholine has been implicated in the control of vigilance states, which had also been the case for the monoamines (for reviews see DeFeudis, 1974; Krnjević, 1974; Jouvet, 1972). However, unlike the monoamines, the histochemical localization of cholinergic neurons in the brain could not be performed reliably. At the time, histochemical methods had only been developed for detecting the presence of acetylcholinesterase, the degradative enzyme for acetylcholine. Because acetylcholinesterase was known not to be specific to cholinergic neurons and fibers, more specific methods for identifying cholinergic neurons were sought, and by the early 1980's, through the development of monoclonal

antibodies against choline acetyltransferase (ChAT), it became possible to specifically identify cholinergic neurons in the brain with greatly improved accuracy and confidence over what had been possible only a decade before (for reviews see Butcher & Woolf, 1984). This development was of great significance because even though acetylcholine has figured prominently in theories of vigilance states, especially PS and waking, ever since the 1930s, the methods used to test these theories, such as injection of acetylcholine or its agents, agonists and inhibitors, or implantation of acetylcholine crystals in the brain, had seldom yielded repeatable and satisfactory results (Jouvet, 1972). Now, with the new immunohistochemical methods, it is possible to visualize these neurons in their specific locations, to study their interactions with other cells in the same or nearby locations, to assess their major projections to other areas, and to assess the effect of selectively destroying these neurons upon sleep-waking states. These points will be the focus of the present research. In the Introduction, I will present a general review of the mechanism of waking, slow wave sleep, and PS and will lead into an examination of the early pharmacological and histochemical studies that had indicated an important role for acetylcholine and monoamine neurons in the sleep-waking states. Such studies form the background for the present research.

A. Discovery, Description, Definitions, and Measurement of Sleep and Waking States.

The study of sleep in the nineteenth and early twentieth century depended upon behavioural measures to distinguish a waking state from a sleeping state. Different depths of sleep were distinguished by the different levels of intensities of a stimulus necessary to awaken the sleeping subject (see for review Kleitman, 1963). Although Caton (1875) in England and Danilewsky (1891) in Russia were studying brain waves of animals, it would take until 1929 before Hans Berger would demonstrate the importance of these waves for the study of brain mechanisms. He recorded from the scalp of human subjects and reported that their EEG during sleep was different from that during waking. Subsequently, Loomis, Harvey and Hobart (1937) reported that the EEG of a waking subject consisted of high-frequency, desynchronized, low-amplitude waves; whereas during sleep the EEG waves were synchronized, of high amplitude and low frequency. The pattern of the waves varied predictably in terms of amplitude and frequency depending on the arousal level of the subject (Blake & Gerard, 1937). While the linking of arousal levels to EEG patterns was useful and relatively reliable, it was not perfect, yet some researchers (Gibbs & Gibbs, 1950) went on to create elaborate divisions of the sleep-waking continuum, ranging from the stage of coma on the sleep end of the continuum, to that of mania on the

waking end (for review see Dement & Mitler, 1974). Loomis and his associates (1937) divided the sleep state into four different stages: 1, 2, 3, and 4, ranging from the lightest to the deepest stage respectively, and this classification is still used in the humans. Waking was divided into two stages: a) quiet waking, when mainly alpha activity is present, and b) active waking, when beta activity predominates (for reviews see Brazier, 1961 and Glaser, 1961). In addition to the desynchronization of the cortical EEG, the olfactory bulb spindle (OBS) is another electrographic reliable variable which is used to distinguish the state of waking from sleep states (Lavin, Alcocer-Cuaron & Hernández-Peón, 1959). Lavin and his associates found that during waking, olfactory bulb recordings from cats showed high-frequency, high-amplitude, spindle-shaped waves which were replaced during sleep by low-amplitude waves similar to those seen in the neocortex during deep sleep. The fast spindle-shaped waves occurred only during waking and independently of respiration or olfactory stimulation.

As research on the sleep-waking cycle progressed, definitions of sleeping and waking came to encompass both electrographic and behavioural criteria. One important criterion of sleep which has endured is that it is a reversible state during which the threshold for response is elevated relative to waking, and the electrographic variables conform either to the definition of slow wave sleep or

PS. The inclusion of the precondition of "reversibility of state" describes a property of sleep which helps to distinguish sleep from other states such as coma, in which some electrographic variables may be similar, but consciousness is not regained, and other characteristics of sleep, such as transition from one state or stage to another, do not appear (for review see Moruzzi, 1972).

It had also been reported early that during sleep the slow, synchronized, high-amplitude waves of the EEG were periodically replaced by low-amplitude, high-frequency waves, and that during behavioural observation of sleeping subjects, the low muscle tonus in sleep was often interrupted by myoclonic twitches (Klaue, 1937). Kleitman (1939) classified these episodes of low-amplitude, high-frequency waves as vestiges of infantile polycyclical sleep, and called them desynchronized or D-sleep. In addition to the cortical desynchronization occurring in D-sleep, Aserinsky and Kleitman (1953, 1955a, 1955b) also noted that during this stage, the eyes of the sleeping subjects darted back and forth rapidly under the closed lids. This led to the adoption of an alternate name to describe this state: Rapid Eye Movement or stage-1 REM sleep. The REM stage of sleep was subsequently correlated with dreaming, and is now also known as Dream Sleep (Aserinsky & Kleitman, 1953, 1955a, 1955b). Similar discoveries, in which cortical desynchronization occurred in association with rapid eye movements,

were being made in cats (Dement, 1958). Shortly after, during electrographic recordings in various CNS regions and the neck muscles of cats, Jouvet and his associates described the desynchronization of the EEG in these animals in association with rapid eye movements and complete loss of neck muscle tonus as a state of "paradoxical sleep" (Jouvet & Michel, 1959; Jouvet, Michel & Courjon, 1959). The total absence of muscle tonus was known to occur during REM sleep initially from behavioural observations (Dement, 1958). This process is now called muscle atonia, and is one of the main indicators of REM sleep in cats. These electrophysiological processes, which occur in predictable ways in correlation with sleep and waking states, still constitute the principal criteria used to describe waking, slow wave sleep, and PS. Jouvet et al. (1959) also recorded certain phasic, short-duration, high-amplitude waves in the pontine reticular formation of cats just before and during the REM stage of sleep. Subsequently, these waves were also recorded in the lateral geniculate nucleus, (Mikiten, Niebel & Hendley, 1961) and in the occipital cortex (Mouret, Jeannerod, & Jouvet, 1963) among other places (for review see Jouvet, 1972). These waves were referred to as ponto-geniculo-occipital (PGO) spikes or waves because of the order in which they were discovered in these three different locations (Jeannerod, Delorme & Jouvet, 1965; Jouvet, 1972) and are now commonly considered to constitute one of the most reli-

able variables used to distinguish waking from REM sleep in cats.

The term paradoxical sleep is often preferred over others for describing REM sleep because it expresses more completely and accurately the confluence of the several events, and does not focus exclusively on any one of them in particular. Terms such as REM sleep or D-sleep (for Deep, Desynchronized or Dream-sleep) are potentially misleading because they imply that either rapid eye movement, dreaming, a high arousal threshold in sleep, or cortical desynchronization fulfills the condition for defining this state of sleep (Moruzzi, 1972). All the same, it has also been argued, that because some EEG parameters are not identical during REM sleep and waking, the term paradoxical sleep is also inappropriate for describing this state of sleep (Tönnies, 1969).

B. Mechanisms of Sleep and Waking States.

An early and particularly interesting concept concerning the mechanisms underlying sleep-waking states was proposed by Brown-Sequard (1889). He suggested that certain parts of the brain were more active during sleep than during waking because the act of sleeping entailed the inhibition of active intellectual processes. The idea that active,

inhibitory processes were involved in bringing about the states of sleep and waking was an important step in furthering the understanding of the underlying mechanisms. This idea was pursued by Pavlov (1927, 1960), who refined and expanded it to include the notion that sleep came about as a result of spreading, cortical inhibition. The impetus for the development of this theory evolved primarily from Pavlov's observation that during his experiments on conditioned inhibition in dogs, the dogs usually fell asleep. He attributed the dogs' falling asleep to his belief that in order for the animals to learn the inhibitory response, certain cortical elements had to enter an inhibitory state. He believed that when the stimulus was repeated frequently the cortical elements entered the inhibitory state quickly, causing inhibition to spread from the inhibitory elements throughout the entire cortex. Experimental results did not support Pavlov's theory, for it is now known that when one set of neurons are inhibited, they cannot in turn directly inhibit (or excite) another set of neurons. However, it is now fully acknowledged in sleep-waking theories that the process of inhibition is involved in certain sleep-waking events, such as bringing a state to conclusion.

Study of the central mechanisms of sleep and waking is usually said to have begun with the clinico-anatomical observations of Gayet (1875) in France and Mauthner (1890) in Austria. They observed that patients suffering from a

lethargic syndrome showed degeneration of the rostral mesencephalon. Later, von Economo (1917, 1929) observed that patients suffering from encephalitica lethargica and exhibiting extreme somnolence, showed degenerative changes in the tegmentum and posterior hypothalamus, whereas in those suffering from extreme sleeplessness, the degeneration was seen in the anterior hypothalamus, the basal forebrain and adjacent structures. One important implication of von Economo's observations was that waking was an active state, promoted centrally, and that sleep was probably due to cerebral or thalamic inhibition of the waking center. So the concept was put forward that the structures underlying sleep and waking were intimately interrelated and balanced antagonistically.

There was no general, immediate acceptance of von Economo's idea on the anatomical locations of the sleeping and waking centers, or even of their existence. Many physiologists, including Kleitman, defended the notion that wakefulness was maintained by continuous sensory input to the brain (Kleitman & Camille, 1932). Nevertheless, Ranson and his associates conducted studies showing that lesions of the posterior hypothalamus in cats and of the lateral hypothalamus in monkeys led to intense somnolence (Ranson, 1939; Ingram, Barris & Ranson, 1936). The animals could be roused from this state with strong stimuli, but once awake they appeared lethargic and passive. Thus, these findings con-

firmed von Economo's (1929) idea of a waking center, though there was no evidence for a sleeping center in the anterior hypothalamus as he had proposed. In extrapolating from the results of their experiments, Ingram et al. (1936) suggested that normal sleep was due to the elimination of an emotional hypothalamic drive that strongly influenced waking by discharging rostrally to the cortex via the thalamus as well as caudally via the subthalamus and the brainstem. However, this hypothalamic activity was not considered to be absolutely essential for maintaining the state of wakefulness. Rather, wakefulness was attributed more to the influence of the subthalamus and the brainstem (Ranson & Magoun, 1939).

Even stronger support for the active theory of sleep-wakefulness states came from a study by Nauta (1946) in which he noted the effects of various brain lesions on sleep in rats, followed by meticulous descriptions of the neural degeneration studies after the lesions. Transections through the posterior hypothalamus and mammillary bodies of the rats led to lethargy and somnolence, whereas transections through the anterior hypothalamus at the level of the suprachiasmatic-preoptic region resulted in complete insomnia, followed in a few days by death. Nauta's results therefore agreed with von Economo's (1929) idea that the sleeping center was in the anterior hypothalamus, and with von Economo's (1929) and Ranson's (1939) idea that a waking center was located in the posterior hypothalamus. He believed

that increases in discharge from the sleep center influenced the waking center in the posterior hypothalamus and mesencephalic tegmentum to decrease its activity and bring about a state of sleep. Using the Marchi degeneration technique, Nauta traced fibers originating in the hypothalamic regions in and around the mammillary bodies, and found that these fibers ascended through the lateral hypothalamus and joined the medial forebrain bundle. They could also be traced to the anterior nucleus of the thalamus, to the septal nucleus, as well as to several other regions. Nauta also speculated that a second group of fibers ascended from the lateral hypothalamus to activate the cortex.

By the time Nauta (1946) had conducted these experiments it had become evident that hypothalamic structures were involved in important ways in sleeping and waking, but in addition, it was also obvious that structures in other areas of the brain were involved in promoting and controlling these states. In fact, soon after von Economo's (1917, 1929) observations, Hess (1931) showed that low-frequency stimulation of the massa intermediata of the thalamus, the anterior hypothalamus, and also of the preoptic and supraoptic hypothalamic areas, resulted in slow wave sleep. In contrast, high-frequency stimulation in the same areas resulted in transient waking. Hess' interpretation of his results were strongly criticized partly because of his methodology. The stimulating sessions lasted a long time,

often the cats had to be stimulated several times, and frequently they did not fall asleep until several hours after the stimulation, which made it impossible to judge whether the cats would not have fallen asleep regardless of the stimulation (for reviews see Bremer, 1971 and Moruzzi, 1972).

The interpretation of these experiments were also critized because of Hess' contention that the thalamus was the principal center controlling sleep (Akert, Koella & Hess, 1952), and in fact, it was later shown that lesion of the thalamus had no lasting effect on sleep or waking (Naquet, Denavit, Lanoir, & Albe-Fessard, 1965), and therefore that the area could not be considered as either a sleep or waking center. Nonetheless, as stated by Moruzzi (1972), the results were important for the notion that sleep and waking were controlled by specific brain mechanisms, and that like other states, such as thirst or hunger, they could be triggered by stimulation of specific areas of the brain. The same could be said about von Economo's work on the hypothalamus, for even though this structure is no longer considered as a center of sleep or waking, it is known to be involved in state control, and as such is a component of a larger anatomical system, different parts of which are involved in the control of various aspects of the sleep-waking states (for reviews see Hobson & Steriade, 1986; Jones, 1988; Jouvet, 1972; and Moruzzi, 1972).

1. Waking and the Ascending Reticular Activating System.

Bremer (1935, 1936, 1937) believed that sensory stimulation was needed to engender and maintain the state of waking and that by eliminating most of the sensory information reaching the brain a state of sleep would predominate. He wished to know how brain stuctures, rostral to the colliculi would function after being separated from the rest of the neuraxis (Bremer, 1971), so he performed intercollicular transections in cats (the cerveau isolé preparation) which interrupted all somatosensory input and eliminated all signs of cortical waking. The eyes of these cats were immobile and the pupils were fissurated (miotic). The 6-10 Hz EEG from the cortex showed an unending, monotonous, synchronous wave pattern, which Bremer (1935, 1936, 1937) interpreted as slow wave sleep. On the other hand, a transection at the spinomedullary junction (the encéphale isolé preparation), which left most of the sensory input from the head intact, had little effect on cerebral signs of the normal sleep-waking states. Bremer interpreted the results as showing that a minimal flow of ascending sensory influence was necessary in order to maintain the normal cortical activation of waking. Though some research (Claes, 1939; Roger, Rossi & Zirondoli, 1956) would show that prevention of visual and somatosensory information from reaching the brain resulted in sleep, which supported Bremer's position, the vast majority of the experiments that followed

lent support to the idea that the brain played an active rather than a passive role in the generation and maintenance of sleep and waking.

The findings which most definitely established the idea that sensory stimulation was not needed to maintain waking took place around the middle part of this century. Jasper, Hunter and Knighton (1948) and Moruzzi and Magoun, (1949) showed that electrical stimulation of the reticular formation was adequate to evoke high-frequency, low-amplitude waves characteristic of waking during slow wave sleep in cats. These results therefore indicated that continuous sensory stimulation was not essential for the induction and maintenance of waking. It was believed that behavioural and cortical arousal could be induced and maintained through ongoing sensory stimulation from the environment or from the internal milieu, but would also persist from the reverberating activity preserved within the ascending reticular activating system (ARAS) from the result of previous sensory stimulation.

In a follow-up series of experiments, Lindsley, Bowden and Magoun (1949) destroyed the midbrain reticular formation in one group of cats but spared the classical ascending sensory pathways (medial lemniscus, spinothalamic tract and lateral lemniscus). These lesions led to permanent synchronization of the cortical EEG. In a different group of animals, large lesions were performed which disrupted the

sensory pathways but spared the midbrain reticular formation. Sleep and waking were only minimally disrupted for a short while in these cats. This series of experiments thus showed that the integrity of the ARAS was essential for the onset and maintenance of wakefulness, whereas sensory input was not.

Lindsley and his colleagues (Lindsley, Shreiner, Knowles & Magoun, 1950) have also shown that in addition to the ARAS in the pons, the caudal diencephalon and midbrain are part of the arousal system of the brain, because electrolytic lesions of the tegmentum at the level of the pontomesencephalic junction, of the midbrain, or of the junction between the thalamus and hypothalamus, resulted in permanent slow wave activity in the cortex. This slow activity could be interrupted and replaced by fast cortical activity after somatic or auditory stimulation was applied, but the fast cortical activation persisted only for the duration of the stimulus.

Further evidence for the existence of a midbrain-diencephalic component of the ARAS was obtained from studies in which electrical stimulation of the sensory nerves showed that transmission from the peripheral nervous system to the cortex occurred along routes other than the classical sensory pathways. These alternate routes were concerned with the ascending transmission of information through the reticular formation, which was believed to send projections

along a dorsal route to the thalamus, and from there projections ascended to the cortex. In addition, it was believed that a ventral route to the cortex existed, because lesions of the thalamus did not prevent the recording of evoked potentials in the cortex following sensory stimulation (Morison, Dempsey and Morison, 1941). It was proposed that the ventral projection ran through the ventral thalamus, subthalamus and hypothalamus, rostrally to the forebrain, because lesions in these ventral structures abolished cortical activation (Morison et al., 1941). Both the ventral and dorsal pathways derive from neurons in the reticular formation, which in turn receives input along its entire length from collaterals of somatic, visceral and special sensory systems (Jones, 1988; Starzl, Taylor & Magoun, 1951). In accordance with its designation as a component of the ARAS, the possibility was also raised that the midbrain and diencephalon might themselves directly influence cortical activity (Dempsey, Morison & Morison, 1941; Zernicki, 1968).

C. Sleep, Forebrain and Brainstem Systems

In spite of earlier demonstrations (von Economo, 1917, 1929; Nauta, 1946; Ranson, 1939) indicating that

sleep, like waking, was an active state, many researchers believed that sleep came about because of a passive deactivation of the ARAS. However, Moruzzi and his colleagues, (Batini, Moruzzi, Palestini, Rossi & Zanchetti, 1959) showed that transections located in the midpons, rostral to the roots of the fifth cranial nerve (the pretrigeminal preparation) reduced sleep by more than 70%. These results therefore suggested that a system with strong influence on the induction of sleep was present in the lower brainstem, located in the region between the midpons and the spinobulbar junction and near the nucleus of the tractus solitarius. The sleep-promoting properties of this medullary region was to be confirmed subsequently through lesions (Ronvallet & Allen, 1963), electrical stimulation (Magnes, Moruzzi & Pompeiano, 1961) and later by single unit recording (Eguchi & Satoh, 1980a).

Besides the structures in the medulla (Magnes et al., 1961) and midbrain (Hess, 1931), which were believed to promote sleep, it was later found that the sleep system might also incorporate forebrain regions. For example, it was shown that lesion of the basal forebrain led to a decrease in sleep (Lucas & Sterman, 1975; McGinty & Sterman, 1968), whereas stimulation of the same area induced sleep (Sterman & Clemente, 1962a, 1962b). It was also shown that excision of the frontal cortex led to a decrease in sleep (Villablanca, Marcus & Olmstead, 1976), and extirpation of

the sensorimotor cortex deeply depressed or even eliminated the spontaneous background electrical activity in the mesencephalic reticular formation (Zernicki, Doty & Santibanez, 1970), and decortication led to a loss of slow wave sleep, but not PS signs in the brainstem (Jouvet, 1962; Villablanca, 1966a, 1966b).

The evidence reviewed above indicates that the sleep-wake system is extensively represented throughout the entire brain, and thus the implication is that different parts of the system might be able to control a state or some aspect of that state independently of other parts. For example, Villablanca (1966a, 1966b) showed that during the first week after mesencephalic transections cats displayed only the high-amplitude, synchronized EEG normally seen in cerveau isolé cats and during slow wave sleep. Late in the second week the low-amplitude, fast waves typical of waking began to reappear, and subsequently, the electroencephalographic recordings were similar to those of the normal cat, except for the EEG during PS. During this state of sleep, the cats displayed atonia, muscle twitches, pontine PGO spikes, and REMs - all typical signs of PS within the brainstem. Cortical activity, however, remained of the high-voltage and low-frequency type normally seen during slow wave sleep. Thus, the forebrain recovered signs of waking and slow wave sleep, while the brainstem recovered signs of waking, quiet sleep (the brainstem equivalent of

slow wave sleep) and PS. This partial recovery of function at two different levels of the brain shows that while the entire system controlling a state may function as one unit, when a part of that system is inactivated, the remaining components may or may not be able to compensate.

The series of experiments described above represent the culmination of a first stage in the endeavour to identify the anatomical and physiological mechanisms of sleep and waking states. Both of these states were shown to be largely under the control of brainstem mechanisms: waking was dependent to a large extent on the integrity of the ARAS, and sleep was controlled in part by structures in the lower hindbrain that interacted with sleep mechanisms in the midbrain and forebrain.

1. The Dual Process of Sleep

After it had been firmly established that sleeping and waking were both active processes of the central nervous system, Jouvet (1965) suggested that in order to form a conceptual framework within which to delimit more specifically the mechanisms underlying the sleep-waking cycle, it was necessary to establish whether slow wave sleep was qualitatively different from PS, or whether PS was a more intense form of classical sleep, as had been posited by some authors (Bremer, 1971; Dement, 1960; Hernández-Peón, 1965a, 1965b; Hernández-Peón & Chávez-Ibarra, 1963). Under

normal conditions slow wave sleep always precedes PS, and PS never follows waking directly. In view of the regularity of this fixed, temporal relationship between the two types of sleep, and also because lesions of the raphe system that caused a decrease of slow wave sleep also caused a concomitant reduction of PS, Jouvet proposed that mechanisms involved in slow wave sleep act as priming mechanisms in triggering PS (Jouvet, 1969).

The process whereby slow wave sleep becomes increasingly more intense as it progresses into PS (Aserinsky & Kleitman, 1953; Dement, 1960; Jouvet, 1962; Koella, 1985) can be deduced from the corresponding changes that take place in various physiological parameters. For example, the arousal threshold is low at the onset of sleep, but increases gradually as a subject passes from the first stage of sleep to the later stages. In addition, tonus of the neck muscles is high during waking but decreases continuously as slow wave sleep progresses and virtually disappears with the onset of PS (Jouvet & Jouvet, 1963). Other evidence shows that blood pressure drops with the onset of slow wave sleep, continues to decrease as slow wave sleep proceeds, and reaches its lowest levels during PS (Rossi, 1963; Shiromani, Siegel, Tomaszewski & McGinty, 1986).

It was also thought that PS was a more intense form of slow wave sleep because both were assumed to depend upon

the same mechanism. One of the best examples of how this mechanism might be organized was expressed in a theory by Hernández-Peón (Hernández-Peón, 1965a, 1965b; Hernández-Peón & Chávez-Ibarra, 1963). These investigators proposed that the sleep system was composed of an ascending and a descending unit and that the sleep process was initiated when peripheral sensory stimulation related to sleep, such as warmth, comfort, fatigue or quietness activated the ascending system, and conditioned stimuli, such as the sight of familiar surroundings associated with sleeping at bedtime, in the descending system combined in the pons and established an inhibitory process which spread progressively. As the inhibition ascended it inhibited the mesencephalic neurons, whose inhibitory influences on the thalamic recruiting neurons would be lifted. These latter neurons would then be free to organize thalamocortical activity to produce the spindles and slow waves characteristic of slow wave sleep. The inhibitory process would proceed, and eventually the thalamic recruiting neurons themselves would be inhibited once more, which would result in a release of their control of thalamocortical activity. The cortex would then revert back to the low-amplitude, fast waves, seen in waking, except at this time as indicative of PS, because the whole brainstem system, including the ARAS would be under a blanket of inhibition. This model which maintained that slow wave sleep

and PS were a unitary process seemed appealing at the time it was proposed, probably because it incorporated the idea of behavioural conditioning and the Pavlovian concept of spreading inhibition, which were popular at the time. However, there was no direct, experimental evidence of spreading inhibition in the brainstem, nor that the mechanism as a whole would function as predicted by the model. Apparently, however, under certain specific conditions, slow wave sleep, PS and wakefulness can be shown to follow a continuum, representing increasing levels of arousal as suggested by Moruzzi (1972), which are dependent on the infusion of either small, medium or high doses of physostigmine, a cholinesterase inhibitor, in humans (Sitaram, Wyatt, Dawson & Gillin, 1976).

Jouvet (1965, 1972) rejected the unitary concept of sleep because he was able to demonstrate that slow wave sleep and PS were two distinct states. Based on phylogenetic and ontogenetic evidence, and also on anatomical, functional and behavioural criteria, Jouvet (1965) reached the conclusion that PS was dependent on mechanisms which were relatively independent of slow wave sleep mechanisms. For example, functional studies had shown that human subjects (Dement, 1960) and other animals (Jouvet, 1962) could be deprived of PS independently of slow wave sleep, after which there was a selective rebound of PS lasting over several subsequent episodes of sleep. Such a rebound effect

suggested to Jouvet that the organism was attempting to recapture the lost PS time. If a cat is deprived of PS for more than 72 hours it resembles a newborn kitten in its recuperative sleep in that up to 80% of the sleep time is spent in PS, and this latter follows waking without being preceded by any slow wave sleep. Next, by means of lesion studies, Jouvet (1965) showed that slow wave sleep could be eliminated selectively in the brainstem by a transection through the mesencephalon and that after aspiration of the cerebral hemispheres and the thalamus, a hypothalamic island was created by making a section passing from the rostral pons through the midbrain to the hypothalamus. In this preparation, slow wave sleep signs were completely abolished from the pons independently of the signs of PS. The state of PS was evident through EEG desynchronization in recordings from the pons and medulla oblongata and muscle atonia in the EMG recorded from the neck muscles. Large lesions of the reticularis pontis oralis (RPO) and reticularis pontis caudalis (RPC) were found to eliminate the state of PS and all its component variables (Jouvet, 1962), but these other brainstem lesions which affected PS did not diminish slow wave sleep.

Thus, by the middle part of the 1960s it was apparent that the two states of sleep, though obviously related in some important ways, were nonetheless dependent on separate mechanisms for their initiation and maintenance.

Slow wave sleep was mainly dependent on the caudal brainstem and on forebrain structures, while PS was dependent on structures located principally in the pontine tegmentum (Jouvet, 1962, 1965; Moruzzi, 1972; Villablanca, 1966a, 1966b).

D. Independent variables of Paradoxical Sleep.

Prior to the realization that PS and slow wave sleep were different states (Jouvet, 1962), investigation of the mechanisms underlying the descriptive variables of PS were in progress, though not necessarily from a standpoint of sleep-waking research. These data which had been amassed, particularly concerning motor mechanisms, muscle tonus, and the EEG, would contribute eventually to the explanation of the PS variables, which were altered so distinctively compared to their appearance during slow wave sleep (for reviews see Morrison, 1979, and Steriade & Hobson, 1976). The study of the similarities between certain variables during waking and PS (EEG desynchrony, PGO-EMPs, REM-saccades) or the complete contrasts (atonia-tonus), has led to a better understanding of the mechanism controlling a particular variable involved, as well as the relationship between a particular variable and the sleep-waking cycle as a whole (for review see Morrison, 1979).

1. EEG Desynchrony in Paradoxical Sleep.

Following the discovery of PS in cats (Dement, 1958; Jouvet & Michel, 1959), on the basis of electrical stimulation and pharmacologic treatments Jouvet and Michel (1960) asserted that these waves were not dependent on the ARAS of the midbrain, then hypothesized to be the system responsible for the maintenance of cortical activation of wakefulness, as had been described earlier by Moruzzi and Magoun (1949). Jouvet and Michel (1960) proposed instead that cortical activation of PS was dependent on a cholinergic mechanism in the pons. Later, upon analysis of electrophysiological, lesion and physiological data collected over three years, Jouvet (1962) identified the RPC as the PS center. Cortical activation and all other signs of PS were abolished by means of lesions to this structure. The RPO was identified as appertaining to the ARAS, and therefore was associated with EEG activation of waking. After identifying the RPC as the PS pontine center, Jouvet (1962) suggested that influence from the RPC ascended to the forebrain via the limbic midbrain circuit of Nauta (1958) in order to bring about the cortical activation of PS. As noted above, Hernández-Peón and his colleagues (Hernández-Peón & Chávez-Ibarra, 1963; Hernández-Peón, Morgane & Timo-Iaria, 1963) were engaged in studies showing that application of acetylcholine to the basal forebrain evoked cortical desynchrony and PS, which supported Jouvet's predictions.

However, Carli and his colleagues (Carli, Armengol & Zanchetti, 1963; Carli, Armengol & Zanchetti, 1965 and Carli & Zanchetti, 1965), after assessing the effects of a series of lesions within the brainstem and forebrain, suggested that there was no effect of the lesions on EEG desynchrony of PS. The lesions involved several locations along the ascending limb of the limbic midbrain circuit, as well as the destruction of its descending components, such as the medial forebrain bundle. The conclusion was that a diffuse system of brainstem pathways must underlie cortical desynchrony during PS. Such results would later be confirmed by Hobson (1965). Similar negative results were obtained after lesions were made in the septum and hippocampus, which reduced, but did not abolish cortical EEG activation of PS (Parmegianni & Zanocco, 1963). The only area which when destroyed affected PS and EEG activation was in the pons in the mediolateral and posterior two-thirds of the RPO and possibly the RPC (Carli et al., 1963; 1965; Carli & Zanchetti, 1965), or the ARAS according to Parmegianni and Zanocco (1963).

Rossi and his colleagues (Candia, Rossi & Sekino, 1967; Rossi, Minobe & Candia, 1963) agreed with these conclusions, because they found that only when the brainstem was transected rostral to the pons was it possible to eliminate PS. Electrolytic lesions in specific (well-defined) structures, such as the lateral lemniscus and superior olive,

or in unspecific ones such as the RPC, RPO and locus coeruleus (LC), could not replicate the effects obtained by unilateral transections in the brainstem. These lesions only produced mild disturbances in light sleep, or nonspecific effects on cortical desynchronization during PS, which might be either increased or decreased or unaffected relative to baseline.

Hobson (1965) also believed that the systems related to cortical desynchrony of PS were widely distributed within the caudal brainstem, because only a total transection at the pontomesencephalic junction could block the influence of the desynchronzing mechanism. However, he saw no reason to assume that the system underlying EEG desynchrony in PS was different from that underlying desynchrony in waking. Nonetheless, it also appeared that after the transections, the EEG desynchrony of PS occured more readily or with greater facility than that of waking. Because Hobson (1965) believed that the same system was responsible for cortical desynchrony of waking and of PS in the forebrain, in order to account for the difference in facility that appeared to favour the reestablishment of desynchrony in PS after pontomesencephalic transections, he suggested that the triggering mechanism for the desynchrony of PS and of waking might be different. The subthalamus appeared to be more important for triggering the desynchrony of waking, while the pontine brainstem seemed to be neces-

sary for triggering the desynchrony of PS. Therefore, the general consensus was that the final common pathway at the forebrain level was used by the cortical activating systems of both PS and waking, but no clear agreement was reached concerning the structures at the pontine levels, where the triggering mechanisms for cortical activation of PS were conjectured to reside. Hobson would later conclude, in agreement with Jouvet, that the RPC or gigantocellular tegmental field (FTG) was specifically necessary for cortical desynchrony of PS (Hobson, McCarley, Freedman & Pivik, 1974a; Hobson, McCarley, Pivik & Freedman, 1974b). By this time, Jouvet (1969) had proposed that the LC was one of the main components in the system responsible for the expression of PS, including cortical desynchronization.

2. EMG Atonia During Paradoxical Sleep.

In seeking to delineate the mechanism responsible for the extreme loss of muscle tonus which occurred during PS, Jouvet (1962) and his colleagues and others (Hobson, 1965) performed transections or electrolytic lesions in various brain regions as well as several pharmacological manipulations. The results showed that neither cerebellectomy, complete decortication, nor transection of the brainstem at the mesencephalic level abolished the periodic occurrence of EMG atonia in association with other aspects of PS (Hobson, 1965; Jouvet, 1962; Jouvet & Michel, 1959).

However, atonia was eliminated by transections posterior to the RPC (Jouvet, 1962; Jouvet & Delorme, 1965) and at the midpons (Hobson, 1965) and pontomedullary junction as well (Jouvet, 1962). Therefore, the overall conclusion was that in the rostral brainstem, structures within and anterior to the mesencephalon are not required for the control of EMG atonia, and that in the caudal brainstem, cells of the reticular formation of the lower pons, medulla and the spinal cord are not sufficient for the control of atonia. However, it appeared that the rostral pontine region was necessary for such control. In order to identify the critical site for control of atonia within the rostral pons, Jouvet (1962) made electrolytic lesions to the RPC, and Hobson (1965) lesioned the RPO. In both cases EMG atonia was eliminated for at least ten days. Similar effects were reported by Carli and Zanchetti (1965), who eliminated all aspects of PS, including muscle atonia, with electrolytic lesions of the RPO.

Even though the pontomedullary lesions (Jouvet, 1962) and the midpontine lesions (Hobson, 1965) showed that the medullary structures were not sufficient to maintain atonia, it was believed that the medulla contained structures which must participate in the development of the phenomenon of atonia. Magoun and Rhines (1946) had discovered a region in the bulbar reticular formation of decerebrate cats, which when stimulated, led to the inhibi-

tion of spinal motoneurons. This area has ipsi- and contralateral connections with several other regions in the brainstem, including the pontine facilitatory area (Magoun & Rhines, 1946). Jouvet and Delorme (1965) speculated that these connections referred to by Magoun and Rhines (1946) might not all be important in atonia, but that an uncrossed tract, the lateral tegmentoreticular tract, that coursed laterally and ventrally through the pons to enter the ventral medullary reticular formation, which had been identified by Russel (1955) from degeneration studies in several mammalian species, might be important in atonia of PS. This tract connected the LC to the medullary inhibitory area of Magoun and Rhines (1946).

In later studies, after the discovery of the monoaminergic system and the localization of monoamines (Dahlstrom & Fuxe, 1964) in regions of the brainstem which lesions and stimulation work (Jouvet, 1962) had shown were involved in atonia of PS, Jouvet and his colleagues obtained evidence showing that these transmitters were involved in all aspects of PS and that the caudal part of the LC, rather than the RPO-RPC, might be the structure in the rostral pons that was critically necessary for the expression of atonia in PS.

3. PGO Spikes of Paradoxical Sleep.

As noted earlier, PGO waves were first reported to occur in the pons by Jouvet and Michel (1959), in the lateral geniculate bodies by Mikiten et al. (1961) and in the occipital cortex by Mouret et al. (1963), and the nomenclature **PGO** reflects the historical order in which PGO spikes were discovered in these structures. It also illustrates the order of occurrence of PGO spikes in these structures during PS, but does not imply that activity is propagated to the occipital cortex via the lateral geniculate nucleus. As an illustration, PGO spikes continue to occur in the cortex even after total ablation of the lateral geniculate nucleus because of the presence of alternate thalamocortical pathways (Hobson, Alexander, & Fredrickson, 1969). Besides the areas mentioned, PGO spikes are recorded from several other locations in the brainstem (Brooks, 1973; for reviews see Hobson & Steriade, 1986; Jouvet, 1965, 1972; Morrison 1979; Sakai, 1985a, 1985b and Vertes, 1984). PGO spikes are particularly prominent in the lateral geniculate bodies where they occur as monophasic (Brooks, 1967) or biphasic and even triphasic potentials (Steriade & Hobson, 1976) which are 100 to 400 uV in amplitude, and 100 msec in duration, occurring in bursts of two to eight spikes, or singly during PS at an average rate of about 50 spikes per minute. PGO spikes are at their highest amplitude during the transition from slow wave sleep to PS, after which they diminish in amplitude to about 70% of this value (Brooks,

1973). The first indication of the end of PS is cessation of firing in these neurons (Brooks, 1973). Under normal conditions they persist throughout the PS period. Therefore, PGO spikes may be said to constitute a more reliable phasic variable of PS than REMs because REMs do not necessarily occur continuously throughout PS (Hobson & Steriade, 1986).

PGO spikes are independent of primary visual input, and although they are correlated with eye movements (Kiyono & Jeannerod, 1967), the relationship is not a causal one. Brooks (1973) noted that one of the most interesting aspects of PGO spikes was that they represented a non-retinal input to the visual system from the brainstem. Nonetheless, there may be a functional relationship between PGO spikes and sensory input, because the sites in the lateral geniculate bodies of the cat from which PGO spikes are most reliably recorded are those which yield the maximum evoked response to light flash stimuli (Munson & Graham, 1973). PGO spikes may be related in some way to motor output, in view of the fact that they accompany active, but not passive eye movements, however, this relationship is not totally consistent (Jouvet & Michel, 1959).

Though it had been seen from lesion (Jouvet, 1962), transection (Hobson, 1965), and stimulation (Bizzi & Brooks, 1963) studies that the pons was the site containing the generator of PGO spikes, identification of the specific structure within the pons controlling these spikes has been

elusive. Over the years, several structures within the pons have been proposed as such a generator. Within the pons proper, the vestibular nuclei have received a lot of attention, and early work involving lesions to this complex revealed that it might be involved in the control of PGO bursts during PS, since when the medial and vestibular nuclei are lesioned, PGO spikes occur as isolated units (Morrison & Pompeiano, 1966). However, Perenin, Maeda and Jeannerod (1972) found that lesions to these nuclei disrupted PGO spiking for less than a week, after which time their rate was only slightly reduced.

The raphe serotonergic system has also been shown to be involved in the control of PGO spikes. There is general agreement that it exerts a tonic inhibitory influence over these spikes (Ferguson et al., 1969; Jacobs & Jones, 1978; Jouvet, 1972; Steriade & Hobson, 1976). Cells in the lateral geniculate bodies receive substantial innervation from the dorsal raphe serotonergic neurons (Mackay-Simm, Sefton, & Martin, 1983; for review see Törk, 1985) which may inhibit cells' firing in these nuclei (Rogawski & Aghajanian, 1980). Lesions of the raphe nucleus readily cause PGO spikes to be released into slow wave sleep and waking (Renault, 1967; for reviews see Jacobs & Jones, 1978; Jouvet, 1969, 1972) a finding which supports the idea that the raphe normally exerts an inhibitory influence over the pontine PGO spike generator (Simon, Gershon & Brooks,

1974; Steriade & Hobson, 1976). In turn, the raphe may function in a state-dependent manner, seeing that Trulson, Jacobs and Morrison (1981) have shown that these neurons normally decrease their activity during PS to levels below those seen in waking and slow wave sleep.

Pharmacological studies show that PGO spikes are released after depletion or inhibition of 5-HT with the administration of reserpine (Delorme, Jeannerod, & Jouvet, 1965; Jeannerod et al., 1965) and after para-chlorophenyl-alanine (PCPA) and para-chloromethamphetamine (Delorme, Froment & Jouvet, 1966). This release of PGO spikes could be blocked by drugs that enhance the levels of serotonin, such as its precursor 5-HTP (Jouvet, 1972).

Many studies have suggested that PGO spikes originate within the pontine tegmentum in a central generator or pacemaker which is capable not only of enhancing the discharge of postsynaptic lateral geniculate neurons, but also of simultaneously suppressing transmission through primary afferent pathways during PS (Brooks & Bizzi, 1963; Jouvet, 1962, 1972; Michel, Jeannerod, Mouret, Rechtschaffen, & Jouvet, 1964 and Steriade & Hobson, 1976). Accordingly, during PS, pontine stimulation can elicit spikes in the lateral geniculate bodies almost identical to those occurring spontaneously (Bizzi & Brooks, 1963; Brooks, 1967). After transections through the mesencephalon or the

rostral pons (Jouvet, 1962, 1965) PGO spikes can still be recorded caudal to the transection within the pons, but not rostral to a hemisection in the same region (Hobson, 1965). PGO spikes can also be recorded from the lateral geniculate bodies when transections are made caudal to the abducens nucleus (Siegel, Nienhuis, Tomaszewski & Wheeler, 1981), or at the pontomedullary junction (Webster, Friedman & Jones, 1986). As noted earlier, on the basis of lesion experiments, Jouvet (1962) came to the conclusion that the RPC was the structure controlling the occurrence of the state of PS as well as PGO spikes. Jouvet observed the fact that the lesions eliminating PS destroyed at least four fifths of the RPC. However, as happened with the other PS variables, when the monoamines were discovered in the central nervous system, attention was focussed on the noradrenaline neurons of the LC as being responsible for PGO spikes. Lesions in this area and pharmacological studies had indicated that these neurons were involved in the control of several aspects of PS, including the generation of PGO spikes.

a. Eye Movement Potentials.[1]

Eye movement potentials (EMPs), and PGO waves may be similar to each other in some fundamental ways since both can be recorded from the same structures, however, they are distinguished from each other in that EMPs occur during waking in association with eye movements, are of much

smaller amplitude than PGO spikes, and are influenced by external conditions much more readily than PGO spikes (Brocks, 1967). For example, EMP amplitude is decreased in darkness but cortical PGO waves are not (Cohen, Feldman, & Diamond, 1969; Jeannerod & Putkonnen, 1970; Jeannerod & Sakai, 1970). In addition, PGO activity is strongly correlated with REMs during PS: every PGO spike is followed by a discharge in the external rectus muscle, even if an eye movement is not detected with EOG electrodes (Michel et al., 1964). Furthermore, retinal coagulation does not influence the recording of PGOs from the lateral geniculate body, but suppresses EMPs completely (Brooks, 1969).

EMPs are considered to be the peripheral manifestation of discharges (Teuber, 1971) that originate in pontine oculomotor structures during attention and can be observed only during cortical arousal (Jouvet, 1972). EMPs can also be distinguished from PGOs by their temporal relationship to eye movements, because EMPs typically follow eye movements, but PGO spikes generally precede them (Jouvet, 1972). Nonetheless, under conditions in which a cat is alert and attending to novel stimuli, PGOs and EMPs are identical in latency and amplitude (Bowker & Morrison, 1976). Bowker and Morrison reported that when a cat is placed in a dark ervironment, the amplitude of the EMPs are similar to the PGCs for the first 15-20 minutes. However, the amplitude of the EMPs decrease as the cat becomes more accustomed to its surroundings.

In spite of the many similarities between EMPs and PGOs, it is far from certain that the entire underlying mechanisms are the same. One possible fundamental difference is that the neurotransmitter mechanisms used by the two systems may be different, for example, PGO rate may be suppressed by 5-HTP, nialimide, pargyline, LSD, methysergide, alpha-methyl-DOPA and chlorimipramine, whereas the rate of EMPs, is increased by these agents (Brooks & Gershon, 1971; Delorme, 1966; Jouvet, 1972).

As to the functional significance of these waves, Morrison (1979) suggests that EMPs are central indicators of 'the startle response' to novel stimuli in waking, and that PGO spikes are the equivalent process in response to internally generated stimuli during sleep. This relation to attention or learning is plausible in view of the fact that such spikes may correspond to active but not passive movement. In this context, active movements are normally generated by the organism and some central processing of information can be assumed to have been involved in their execution. It is not clear at this point what the involvement of serotonergic systems may be in the control or induction of these potentials.

[1] Footnote: Even though EMPs are related to waking they are discussed under the PGO section because they are not critical for describing the state of waking and because it was felt that their inclusion here would facilitate the discussion of the mechanisms of both phenomena when contrasting and comparing them.

E. Chemical Neurotransmitters: Principal Agents of State Control.

With the discovery of neurotransmitters in the brain, attention shifted during the 1950's and 1960's to the involvement of these agents and their respective neuronal systems in states of sleep-wakefulness. Of particular interest were the catecholamines and serotonin because their location in the brain coincided with sites where lesions of fiber pathways and cell nuclei had led to state alteration. Acetylcholine was also thought to be important for the control of sleep-wakefulness states. Even though it could not be positively visualized within the brain by any histochemical method, multiple lines of evidence from pharmacological and biochemical studies suggested that acetylcholine played an important role in vigilance states.

1. Waking and Catecholamines.

Following the delineation of the ARAS and the recognition of its importance for behavioural waking and cortical arousal (Moruzzi & Magoun, 1949), many researchers attempted to identify which neurotransmitters were involved in acting upon the brain, and the ARAS in particular, to generate and maintain wakefulness. Primary among the transmitters studied were the catecholamines, which had been associated with waking even prior to the successful development of methods for their visualization in the brain (Jouvet, 1962, 1969, 1972). Pharmacological studies provided

early evidence of the important role of the catecholamines, including adrenaline, noradrenaline and dopamine, in control of the state of wakefulness and of their effect upon the ARAS.

During the early 1950's studies employing systemic injections of adrenaline in animals showed that it led to a long-lasting state of arousal (Jouvet, 1972). Because of the arousal effect, adrenaline was considered to be a "waking" hormone, which exerted its influence on the waking system via the reticular formation (Bonvallet, Dell & Hiebel, 1954; Jouvet, 1972). The effect on waking would have had to be indirect, because adrenalin does not readily cross the blood brain barrier. Nonetheless, evidence indicated that dopamine and noradrenaline played a role in waking because enhancement of their levels with injections of L-DOPA (L-hydroxyphenylalanine, the catecholamine precursor), led to increased waking (Jouvet, 1972; Monnier & Tissot, 1958) and also reversed the behavioural depression caused by injections of reserpine, a catecholamine depletor (Carlsson, Lindquist & Magnusson, 1957). In addition, inhibition of catecholamine synthesis led to increased somnolence and sleep (Weissman & Koe, 1965).

With the development of histofluorescent techniques for the identification of catecholaminergic neurons in the 1960's (Dahlstrom & Fuxe, 1964), it was observed that dopamine and noradrenaline neurons were concentrated in diffe-

rent, specific locations in the brainstem tegmentum. These findings facilitated the separate, experimental manipulations of these two transmitters, and made it possible to determine their respective roles in vigilance states.

a. The Dopamine System in Waking.

Dopamine neurons are located mainly in the diencephalon and mesencephalon. In the diencephalon, the cells are predominantly distributed in the periventricular region of the hypothalamus, in the posterior dorsal hypothalamus and in the zona incerta (for reviews see Bjorklund & Lindvall, 1984; Fallon & Loughlin, 1985; Moore & Bloom, 1978). In the mesencephalon the majority of the dopamine cells are located in the substantia nigra (A9), the ventral tegmental area (A10), and the adjacent tegmentum (A8). These cells project to the neostriatum, basal forebrain, nucleus accumbens, septum, amygdala and frontal cortex (Bjorklund & Lindvall, 1984).

Lesions of the mesencephalic dopamine neurons in cats lead to decreased behavioural arousal and responsiveness without significantly affecting EEG desynchronization of waking (Jones, Bobillier & Jouvet, 1969). Pharmacological studies involving depletion of dopamine (Jacobs & Jones, 1978; Jouvet, 1972), administration of the dopamine agonists (Wauquier, Clinke, Van den Broeck & De Prins, 1985) or injection of its precursor,

L-DOPA (Jouvet, 1972) suggest that dopamine is involved in behavioural activation, despite the fact that unit recording studies of substantia nigra cells show stable, low rates of firing across the states of quiet waking and sleep. Nonetheless, these cells of the substantia nigra do show an increase in firing during purposive movement, and a decrease in firing preceding the suppression of such responses (Jacobs, 1985). Reduction of firing in these dopaminergic neurons may be a necessary condition for waking behaviour to be reduced and cease, and thus permit sleep to ensue (Wauquier et al., 1985).

b. The Noradrenaline System in Waking.

Noradrenaline cell bodies are located throughout the pons and medulla (Brownstein & Palkovits, 1984; Moore & Card, 1984). The main group of noradrenaline neurons is located in the rostral pons within the LC nucleus and adjacent tegmentum (Dahlstrom & Fuxe, 1964). The LC neurons project to the entire central nervous system (Jones & Moore, 1977; Loughlin & Fallon, 1985; Moore & Card, 1984), passing rostrally into the thalamus, hypothalamus, forebrain and directly to the neocortex, paleocortex, hippocampus and olfactory bulb. Projections to the cerebellum, lower brainstem and spinal cord are also present.

Early evidence that noradrenaline was involved in controlling the state of waking was obtained following systemic

injections of L-DOPA that led to cortical activation in normal rabbits and cats (Delorme, 1966; Jones, 1971; Jouvet, 1972; Monnier & Tissot, 1958). Even though L-DOPA is a general precursor of catecholamines, the effect of these injections can be associated with noradrenaline inasmuch as other tests show there is a high correlation between cortical activation and noradrenaline levels in the brains after L-DOPA administration (Reis, Moorehead & Meslino, 1970).

Experimental lesion of the noradrenaline ascending pathways in the midbrain have also been used to test the importance of the noradrenaline innervation of the forebrain for wakefulness. These lesions produced a severe decrease in cortical activation of wakefulness (Jones, 1969; Jones, Bobillier, Pin & Jouvet, 1973). Small lesions of the rostral LC and its dorsal bundle also produced deficits in waking, which was associated with hypersomnia (Jones et al., 1973). From observation of clinical cases in which the somnolent state of coma was present, and which also involved a severe decrease of cortical activation, lesions of the ascending noradrenergic pathways in the midbrain were almost always observed (Schott et al., 1972). Furthermore, coma has been reversed in patients by administration of L-DOPA (Di Rocco, Maira, Meglio & Rossi, 1974).

In spite of the data from these varied sources supporting the argument for a role of noradrenaline in waking, there is evidence that indicates this transmitter may not play the essential role in cortical desynchrony originally attributed to it. Studies in which more specific lesions of the noradrenergic system have been performed, have not reproduced the same deficit in wakefulness as was originally obtained after electrolytic lesion of the ascending pathways. Electrolytic destruction of the LC, the source of most of the ascending fibers, had no effect upon cortical activation (Jacobs & Jones, 1978; Jones, 1988), nor did the intraventricular injection of 6-hydroxydopamine (6-OHDA) which destroys catecholamine neurons, with some degree of selectivity (Howard & Breese, 1974; Laguzzi, Petitjean, Pujol & Jouvet, 1972).

Results from unit recording of presumed noradrenergic cells in the LC support a role in waking for these neurons. These cells fire tonically during waking, at an intermediate rate during slow wave sleep, and are virtually silent during PS (Jacobs, 1985; Steriade & Hobson, 1976). The results of these electrophysiological studies have led to a reassessment of the role of noradrenaline in control of vigilance states. It has been shown that noradrenaline acts by modulating the activity of other neurotransmitters through enhancement, damping or prolongation of an effect over widespread areas of the

cortex, activities consistent with those exhibited by neuromodulators (Jacobs & Jones, 1978; Jones, 1988; Moore & Card, 1984). Therefore, as a neuromodulator, noradrenaline may enhance or prolong wakefulness but it is not considered essential for the maintenance of that state.

2. Waking and Acetylcholine.

Acetylcholine, the first neurotransmitter to be identified, was discovered in the peripheral nervous system, and shortly thereafter, in the brain (for review see Krnjević, 1974). Acetylcholine may act upon either muscarinic or nicotinic receptors in the brain and in the periphery (for reviews see Eglen & Whiting, 1986; MacIntosh, 1981; Rotter, 1984). Both muscarinic and nicotinic agents may promote cortical activation of wakefulness as well as of PS. The effect of any one of the cholinergic agents in the central nervous system on vigilance state is dependent on several factors, including the route and method of administration (Domino, Yamamoto & Dren, 1968; Rinaldi & Himwich, 1955a, 1955b), the potency of the drug in question, the vigilance state (waking, slow wave sleep, PS) during which the drug is administered, the mental and emotional state of the subject, as well as on a possible genetically predetermined sensitivity to acetylcholine (Gillin, Sitaram, Mendelson, & Wyatt, 1978a; Nadi, Nurnberger & Gershon, 1984;

Sitaram, Nurnberger, Gershon & Gillin, 1980; for review see Gillin, Sitaram, Janowsky, Risch, Huey & Storch, 1985).

a. Pharmacological Studies.

In order to study the role of acetylcholine in vigilance states, acetylcholine as well as muscarinic and nicotinic agonists have been administered systemically or into the cerebrospinal fluid or directly into the brain. In rabbits, intracarotid injection of acetylcholine led to cortical desynchronization of waking. The effect was immediate, and similar to that caused by sensory stimulation (Rinaldi & Himwich, 1955a). After the initial effect, if the administration of the drug was continued, it led to a decrease in cardiac activity and blood pressure as a result of its direct influence on the peripheral parasympathetic system.

Intracarotid administration of physostigmine (eserine), a reversible inhibitor of acetylcholinesterase, in cats, monkeys and rabbits produced a long-lasting, activated, low-voltage, EEG pattern (Funderburk & Case, 1951). When infused intravenously into the cat during slow wave sleep, physostigmine led to EEG and behavioural signs of waking (Domino et al., 1968). The EEG activation could be reversed or blocked by administration of the muscarinic antagonist, atropine, however, because it is a muscarinic agonist as well (Domino, 1968; for review see DeFeudis,

1974) additional administration of physostigmine counteracted the effect of atropine and reestablished cortical activation, and also caused the animal to pass directly from wakefulness into PS (Domino et al., 1968).

In normal human subjects, the effect of physostigmine may interact with the vigilance state of the individual, in view of the fact that intravenous infusion of this agent during PS caused subjects to awaken. After the first PS period had occurred, infusion of physostigmine caused waking (Sitaram & Gillin, 1980). However, when it was infused during slow wave sleep, it led to PS (Sitaram et al., 1976).

When DFP (di-isopropyl-fluorophosphate), an irreversible inhibitor of cholinesterase, was administered intravenously to cats and monkeys, it caused a drop in amplitude and an increase in frequency in the EEG activity (Wescoe, Green, McNamara & Krop, 1948). Also, systemic injections of DFP into sleeping or resting cats and monkeys quickly led to cortical activation, but not to behavioural arousal, and even transient, peripheral changes, such as fluctuation in blood pressure, seemed unrelated to the EEG activation (Bradley & Elkes, 1953, 1957). Intracarotid injections of DFP in curarized rabbits also produced a gradual decrease in the amplitude of the slow waves until the EEG was totally activated. At this stage, if more DFP was administered, muscular twitching, a nicotinic effect,

appeared. However, the muscular twitching was abolished by additional administration of curare (Rinaldi & Himwich, 1955a). Muscarinic effects, such as miosis and bradycardia were also seen.

Atropine, a muscarinic receptor blocker, when given in large systemic doses, prevented cortical activation during wakefulness. Wescoe et al. (1948) administered intravenous injections of atropine to curarized cats and a monkey, which caused an increase in EEG voltage and a decrease in frequency. In effect, the waves it produced were identical to those observed in natural sleep. A similar effect was achieved in behaving animals when Funderburk and Case (1951) administered atropine systemically to cats and monkeys and caused high-voltage and low-frequency waves in the EEG similar to that of slow wave sleep, even though the animals were behaviourally awake. Wikler (1952) obtained the same effect in studies in which this drug was used in dogs. When doses just slightly higher than needed for the induction of cortical synchronization were given, the powerful blocking effect of this agent became even more evident, in that the EEG could not be activated either by sensory stimulation or even by electrical stimulation of the reticular formation (for review see Longo, 1966).

Atropine also has a preventative effect upon cortical activation when it is given prior to the administration of muscarinic agonist drugs, such as

acetylcholine, arecholine, physostigmine or pilocarpine (Domino, 1966, 1968; Domino et al., 1968). It may also reestablish EEG synchronization and moderate muscle tonus in cats, rats and rabbits that have been previously injected with reserpine and eserine (Karczmar, Longo & Scotti de Carolis, 1970). In these cases, atropine has a blocking effect on the increased levels of acetylcholine brought about by the eserine treatment. When atropine is given to rabbits that have been pretreated with acetylcholine, their activated EEG gradually becomes synchronized. The rate and degree of synchronization depends on the amount of atropine administered, and the synchronized effect cannot be reversed by subsequent injection of acetylcholine (Rinaldi & Himwich, 1955a). A similar effect is obtained when atropine is administered to rabbits that have been pretreated with DFP and are showing an activated EEG. Notwithstanding such an effect, additional injections of DFP may reestablish the activated EEG that had been abolished by the atropine (Rinaldi & Himwich, 1955a).

The effect of atropine on human subjects is similar to that seen in experimental animals, in that there is a shift from rapid, low amplitude EEG waves to synchonization of the EEG that is dissociated from behaviour. As in animals, humans subjects also show drowsiness and slowed behaviour, as well as deficits in performing learning and memory-related tasks (for review see Longo, 1966).

The nicotinic agonists, nicotine and DMPP (1,1-dimethyl-4-phenylpiperidinium iodide), when injected systemically, may lead to cortical desynchronization of wakefulness. This EEG activation can be reduced but not necessarily abolished by atropine, suggesting that atropine may also act as a nicotinic antagonist (Domino, 1966, 1968; Domino et al., 1968). Nicotinic antagonists such as trimethidinium and mecamylamine, are not as effective as atropine in reversing the EEG activation of wakefulness induced by pilocarpine and physostigmine injected into the carotid artery (Domino et al., 1968). Hemicholinium bromide (HC-3), inhibits acetylcholine synthesis by inhibition of choline uptake and hence reduces the tissue content of acetylcholine. Intravenous injection of HC-3 led to synchronization of the EEG and a decrease in response to external stimuli, which suggests that the decrease in acetylcholine synthesis had caused a disruption of waking. It is to be noted, however, that HC-3 does not cross the blood brain barrier effectively (Domino et al., 1968), a fact that might explain why intraventricular administration of HC-3 lowers the level of acetylcholine, and disrupts PS more than waking.

b. Regional Specificity of Cholinergic Drugs.

The results from experiments employing systemic administration of acetylcholine or its agents, make it clear that cholinergic drugs have a behaviourally arousing or

cortically activating effect, however, this method of administering the drugs has serious drawbacks. For example, some of the cholinergic agents could not cross the blood brain barrier, and may have had their main effect on peripheral systems. Alternatively, they might have become hydrolysed by acetylcholinesterase (which is found abundantly in neural tissue and blood cells) before getting to the brain in sufficient quantity to cause a central effect. Perhaps the greatest drawback is that these drugs may have had potent effects on both central and peripheral systems, making confident interpretation of the central effects impossible.

An alternative approach was to inject the drug directly into the ventricles or the brain, where it would exert its action before being destroyed by cholinesterases. In addition, methods were developed whereby neuropharmacological, neuroanatomical and neurophysiological techniques were combined in order to determine the principal site of action of a cholinergic agent in eliciting the activation of the brain. Bonnet and Bremer (1937) had noted that intracarotid injections of acetylcholine-chloride awakened the encéphale isolé preparation. Likewise, Miller and colleagues (Miller, Stavraky & Woonton, 1940) found that direct application of acetylcholine to the cortex of cats caused cortical activation. In conjunction with the work being done on the brainstem activating system (Jasper, 1949;

Moruzzi & Magoun, 1949), it was becoming clear that cortical activation was dependent largely on the ARAS as well as on the diffuse ascending thalamic projection system. Using intracarotid injections of acetylcholine, Rinaldi and Himwich (1955a, 1955b) provided evidence that cholinergic mechanisms of the midbrain reticular formation were involved in cortical activation. Based on the premise that cortical activation through electrical stimulation of the ARAS was similar in appearance to that caused by cholinergic agents, Rinaldi and Himwich (1955b) suggested that the ARAS was the site of action of the cholinergic drugs.

An alternative explanation for the activation of the ARAS by acetylcholine included the suggestion that desynchronization of brain waves was secondary to its parasympathetic action, such as causing the dilatation of cerebral blood vessels; or indeed, by its enhancement of sensory input through stimulation of peripheral receptors (Darrow, Pathman, & Kronenberg, 1946). Such competing explanations began to lose their appeal when Rinaldi and Himwich (1955b) successfully showed by comparing the effect of acetylcholine in the isolated cortex to that in the isolated hemisphere preparation, that acetylcholine acted upon the ARAS. All sensory input or influence from the ARAS was excluded, but the circulatory system was spared in the isolated cortex. In this preparation neither sensory stimulation nor intracarotid injections of acetylcholine

produced activation of the EEG in the isolated cortex. These results contrasted with that from the cerveau isolé preparation (Rinaldi and Himwich, 1955b), which preserved the midbrain reticular formation, and in which EEG activation could be produced by the intracarotid administration of acetylcholine or by olfactory or visual stimulation. These results therefore provided strong support for the prediction that cortical activation was dependant on activation of the ARAS by cholinergic influences. Based on these results, Rinaldi and Himwich, (1955a, 1955b) concluded that in order for acetylcholine to activate the EEG it did not need to have its effect either at the cortical level or through parasympathetic action on the blood vessels.

Rinaldi and Himwich's conclusions, though convincing, must be tempered by the observation that in chronic cerveau isolé preparations, it is observed that structures in the rostral telencephalon are also capable of inducing and maintaining desynchronization which can alternate with synchronization (Moruzzi, 1972; Villablanca, 1965a, 1965b). Besides, Villablanca (1965b) found that intravenous injections of physostigmine could produce desynchronization in the isolated cortex preparation, and concluded that its action was at the cortical level because the transection, which was complete in most cases, was made either at the midcollicular or precollicular level (Villablanca, 1965a, 1965b, 1966a). Despite the latter

results, the mass of the accumulated data suggested that the normal route of cortical activation is via the brainstem reticular formation, and is neither initiated nor maintained by putative cortical cholinergic cells (Jouvet, 1972). This point was confirmed by Kawamura and Domino (1969), who by means of intravenous injections of nicotine, induced cortical activation, and then by subsequent lesion to the mesencephalic reticular formation, caused it to be abolished.

Seeking to establish more specifically the anatomical substrates of waking and sleep, Hernández-Peón and his collaborators (Hernández-Peón, 1965; Hernández-Peón & Chávez-Ibarra, 1963; Hernández-Peón et al., 1963) investigated the effects of implantation of acetylcholine pellets in particular sites in the midbrain-forebrain limbic system and in the hypnogenic system believed to exist in the spinal cord and to send ascending influences to the pontobulbar system (Moruzzi, 1972). Such applications usually led to aroused wakefulness prior to the onset of sleep, but at some sites, acetylcholine would induce only wakefulness for long periods of time. More recently, carbachol, a cholinergic agonist, injected into the midbrain and medulla was also found to produce cortical activation and behavioural wakefulness (Baghdoyan, McCarley & Hobson, 1985). However, these latter sites favoring waking may overlap with those favoring sleep in the same general

region, seeing that Hernández-Peón and his colleagues, and Shiromani and Fishbein (1986) report that slow wave sleep and PS were also produced after acetylcholine and carbachol stimulation of these structures.

As mentioned above, bilateral lesions or transections of the caudal midbrain reticular formation in cats blocked the EEG activation that had been caused by intravenous nicotine infusions. Infusions of nicotine, however, did not cause cortical activation if the transection was at the rostral midbrain level, whereas arecoline could induce cortical activation in all cases. These results were interpreted as showing that the action of nicotine was primarily at the midbrain reticular formation level, while the action of arecoline was evident in the forebrain and cortex, as well as within the midbrain reticular formation (Kawamura & Domino, 1968, 1969).

Following the demonstration in the 1960's that the subcortical cholinergic system was important in the control of vigilance states, in order to obtain some estimate of the level of the relationship between acetylcholine metabolism and cortical activity, measurements were made of the amount of acetylcholine being released from the brain during various levels of cortical activation. It was found that acetylcholine is released in higher quantities from the cortex during waking and PS than during slow wave sleep (Celesia & Jasper, 1966; Haranath & Venkatakrishna-Bhatt,

1973). And that the amount released from the cortex during PS is generally lower (Haranath & Venkatakrishna-Bhatt, 1973) or equal to that released during waking (Celesia & Jasper, 1966). Acetylcholine is also released from the striatum during PS and waking in greater amounts than during slow wave sleep in non-medicated and unrestrained cats (Gadea-Ciria, Stadler, Lloyd, & Bartholini, 1973).

While cortical activation of waking and PS is associated with increased release of acetylcholine, cortical synchronization due to sleep or barbiturate anaesthesia is accompanied by a decrease in cortical acetylcholine release (Bartholini & Pepeu, 1967; Celesia & Jasper, 1966; Domino et al., 1968; Pepeu & Mantegazzini, 1964; Votava, 1967).

On the basis of acetylcholine collected from the cortex in awake, behaving cats, and during different states of sleep (Celesia & Jasper, 1966), it was proposed that the release of acetylcholine was associated more with cortical activity than with behaviour (Jasper & Tessier, 1971). However, DeFeudis (1974) proposed that in clinical cases the normal relationship between acetylcholine and the cortical and behavioural manifestation of its effects could break down under the influence of psychoactive drugs. In addition, atropinized animals showing cortical synchronization, may also exhibit increased cortical release of acetylcholine (Kanai & Szerb, 1965). There are also problems in determining the exact nature of the mechanisms which control how

acetylcholine is released from the cortex. For example, Krnjević (1965) has suggested that the level of cortical activation might be related to the amount of acetylcholine released from cholinergic neurons ascending from subcortical structures to innervate the deep pyramidal cells of the cerebral cortex.

Stimulation of the mesencephalic reticular formation causes enhanced release of acetylcholine from all areas of the cortex (DeFeudis, 1974). However, some of this acetylcholine released after stimulation of the subcortical structures is from cortical cholinergic neurons, since when the cortex is undercut the cortical release persists, though at a lower level (Szerb, 1967; Collier & Mitchell, 1967) and in addition, release may be increased by direct cortical stimulation (Phillis & Chong, 1965). It is also possible that the subcortical structures which must be stimulated in order to induce cortical activation and increased acetylcholine release may not be one and the same. For example, cortical acetylcholine output does not vary in a parallel fashion with cortical activation produced by electrical stimulation of the reticular formation. Besides, acetylcholine may also be released from the cortex after the stimulation of other subcortical regions, such as the septum, in the absence of such cortical activation (DeFeudis, 1974; Szerb, 1967).

In summary, several experimental approaches have been devised to investigate the mechanism whereby acetylcholine affects structures in the brain to bring about cortical activation of waking. In a classical study, Rinaldi and Himwich (1955b) showed that in acute cats acetylcholine acted upon the ARAS in order to activate the cortex, rather than at the periphery or directly upon the cortex. Even though this finding would be substantiated and generally accepted as the normal route of cortical activation, studies by Villablanca (1965a, 1965b, 1966a, 1966b) in cats with isolated forebrain, which were kept alive for almost two months, showed that in the chronic case, the isolated cortex could be activated by eserine. Work such as that by Hernández-Peón and his colleagues showed that cortical activation could be produced by local administration of cholinergic drugs to various sites within the brainstem, and that sites related to waking were often in close proximity to those promoting PS. In addition to the site specificity of the cholinergic drugs, several reports showed that there were regional specificities for these drugs also, in that some regions were more receptive to either nicotinic or muscarinic drugs. Nicotinic agonists were effective at the midbrain level, whereas muscarinic agonists were effective both within the midbrain as well as rostral to it. A further important test showing the relationship between acetylcholine and cortical activation was that this transmitter

was released in greater quantities during cortical desynchronization of waking and PS than during synchronization of slow wave sleep. This release is dependent upon influences from the brainstem reticular formation, as well as other subcortical structures and maybe even within the cortex itself.

c. Histochemical and Neuroanatomical Studies.

The histochemical identification of cholinergic neuronal systems was needed in order to understand more fully the role of this neurotransmitter in the brain. If the locations of these neurons could be identified, it would pave the way for the direct investigation of the effect of stimulating or destroying these neurons and their pathways. A histochemical technique for the detection of acetylcholinesterase, the catabolic enzyme of acetylcholine was developed during the 1950s. This technique provided an initial approach enabling the visualization of the cholinergic neurons (Koelle, 1954).

Although Koelle (1954) knew that acetylcholinesterase could be present in both cholinergic and non-cholinergic neurons, he believed it predominated at higher levels in the cholinergic ones. Therefore, it could be assumed that cells which stained deeply for acetylcholinesterase were cholinergic while those staining lightly were mainly cholinoceptive (for reviews see Bradley & Wolstencroft, 1965; Butcher

& Woolf, 1984; MacIntosh, 1981). Accordingly, Koelle (1954) described the localization of the cells which stained positively for acetylcholinesterase and constructed a map of the location of these cells.

Shute and Lewis (1963, 1967) used the acetylcholinesterase technique developed by Koelle (1954), to map the cholinergic systems in the central nervous system. Like Koelle (1954) before them, Shute and Lewis acknowledged that the stain could not be used to specifically identify cholinergic neurons, but they believed that it would provide a relatively accurate assessment of the cells and fibers of this system. Now it is widely accepted that the general distribution of cholinergic neurons may be estimated using acetylcholinesterase stain, but that it should not be thought of as an absolute indicator of acetylcholine neurons (Butcher & Woolf, 1984; MacIntosh, 1981).

The projections from the cholinesterase-positive neurons were mapped by Shute and Lewis (1963) by taking advantage of the phenomenon whereby acetylcholinesterase accumulated in the proximal portion of transected fibers projecting from the cholinesterase cells. Depletion of cholinesterase occurred in the distal portion where there was little or no activity of the enzyme. This research showed that the main projections were derived from two major groups of neurons in the brainstem which gave rise to two major tegmental pathways: the dorsal tegmental pathway, and

the ventral tegmental pathway (Shute & Lewis, 1963, 1967). Shute and Lewis (1963, 1967) and Lewis and Shute, (1967) saw these pathways as corresponding respectively to the thalamic and extrathalamic portions of the ascending reticular activating system described in the cat earlier by Starzl et al. (1951). Shute and Lewis referred to these two systems jointly as the "ascending cholinergic reticular system". These neuroanatomical observations lent support to the earlier pharmacological and electrophysiological findings by Rinaldi and Himwich (1955b), indicating that acetylcholine exerted its activating effect at the level of the mesencephalon on the ARAS (Rinaldi & Himwich, 1955b) which projected into the forebrain. The cholinergic cells contributing fibers to the dorsal tegmental pathway were identified as being in the region of the cuneiform nucleus, while cells in the general region of the ventral tegmental area of Tsai and in the substantia nigra were seen as supplying fibers for the ventral tegmental pathway (Shute & Lewis, 1963, 1967). Both pathways were seen to ascend rostrally to innervate structures in the forebrain, though no direct innervation of the cortex from the brainstem cholinergic neurons was observed. The cholinesterase-positive cells of the basal forebrain appeared to receive inputs from cholinesterase-positive cells in the brainstem. The cholinesterase-positive basal forebrain cells in turn

projected in a widespread manner to the cortex (Krnjević, 1965; Shute & Lewis, 1967).

Shortly after leaving the pons, the dorsal tegmental pathway splits into a dorsal and ventral sheaf, both of which continue on a rostral course into the forebrain. The dorsal sheaf, also known as the dorsal pathway, sends off fibers that innervate many nuclei of the thalamus, particularly the anterior, reticular and intralaminar nuclei, the pretectal nucleus as well as the corpora quadrigemina. The ventral sheaf, also known as the ventral pathway, bends ventrally in its rostral course and gives off fibers to nuclei of the hypothalamus, particularly the lateral hypothalamus, zona incerta, subthalamus, basal forebrain, and a small contingent of fibers goes on to the frontal cortex.

Although the early histochemical studies with acetylcholinesterase indicated possible, if not probable, cholinergic neurons and their projections, they did not provide absolute and certain identification of cholinergic neurons. Indeed, the cholinesterase-positive cells of the ventral tegmentum and substantia nigra were found to contain dopamine (Butcher & Woolf, 1984; Paxinos & Butcher, 1985; Woolf & Butcher, 1986) and thus the ventral tegmental pathway originating from those cells was also probably mainly if not entirely dopaminergic, rather than cholinergic. Similarly, the noradrenaline LC neurons stain darkly

positive for acetylcholinestase and thus the dorsal tegmental pathway into which they project was also potentially mainly monoaminergic and not cholinergic.

There has been no satisfactory histochemical or immunohistochemical technique available for staining acetylcholine in neurons, however, the synthesizing enzyme for acetylcholine, ChAT has recently been localized in the central nervous system by immnohistochemistry (Houser, Crawford, Barber, Salvaterra & Vaughn, 1983; Mesulam, Mufson, Wainer & Levey, 1983). ChAT is a truly specific component of the cholinergic neuron, and it may be found together with acetylcholine in cell bodies and dendrites, all along the axon, in the axoplasm and in the nerve ending. Thus, ChAT affords the advantage of identifying cholinergic neurons with a high degree of specificity as to the transmitter. As for morphological detail, the cell bodies stain intensely, enabling the classification of ChAT-positive cells according to their size and shape, although terminals and fibers do not stain as reliably (Butcher & Woolf, 1984; Houser et al., 1983; Sofroniew, Campbell, Cuello & Eckenstein, 1985). By immunohistochemical staining of ChAT, it has become apparent that the original acetylcholinesterase-positive cells identified by Shute and Lewis (1963), within the region of the cuneiform nucleus do correspond to cholinergic neurons. These cholinergic cells are in fact located in the site which is now commonly termed

the pedunculopontine tegmental nucleus (PPT) and in the laterodorsal tegmental nucleus (LDT) in all species, but are more dispersed in the cat, extending caudally into the parabrachial nucleus and the LC (Jones & Beaudet, 1987a, 1987b; Mesulam et al., 1983).

It has now been confirmed by immunohistochemistry that neurons in the basal forebrain (including the nucleus basalis, substantia innominata, nuclei of the horizontal and vertical limb of the diagonal band of Broca and the septum) provide the main cholinergic innervation to the cortex (Mesulam et al., 1983). Almost all of the cells projecting from the basal forebrain to the cortex are cholinergic (Mesulam, Mufson & Wainer, 1986; Satoh, Armstrong & Fibiger, 1983). The ChAT-positive cells in the dorsolateral ponto-mesencephalic tegmentum within the PPT-LDT nuclei, may also project up to the cortex (Jones & Beaudet, 1987b; Mesulam et al., 1983; Vincent, Satoh, Armstrong & Fibiger, 1983). Thus, while some of the projections from the PPT-LDT neurons terminate in the lateral hypothalamus and subthalamus, others continue to ascend and innervate the prefrontal area of the cortex (Jones & Beaudet, 1987b). Recently some weakly-staining, ChAT-positive cells have been identified in the arcuate nucleus and other regions of the hypothalamus (Tago, McGeer, Bruce & Hersh, 1987) and together with other ChAT-positive neurons from the subthalamus, they may provide projections to the cortex (Paxinos & Butcher, 1985).

Although some of the early pharmacological experiments (Villablanca, 1965a, 1965b, 1966a, 1966b, 1966c) suggested that the cortex might be capable of self-activation and that the endogenous or autochthonous mechanism involved might be cholinergic, initial attempts at staining the cortex for cholinergic neurons yielded results which were controversial, because some investigators failed to find these cells at first (Butcher & Woolf, 1984; Kimura, McGeer, Peng & McGeer, 1981; Sofroniew, Eckenstein, Thoenen & Cuello, 1982). Eventually, however, using an improved antiserum to ChAT, Eckenstein and others (Eckenstein & Baughman, 1984; Eckenstein & Thoenen, 1983; Houser et al., 1983) observed lightly-staining ChAT-positive cholinergic cells in the cortex of the rat.

With the development of the histochemical and immunohistochemical methods, it is now possible to identify cell bodies as well as their projections and their target structures. The main group of cholinergic cells is located in the basal forebrain, which sends the major projection to the cortex. The next largest group is in the brainstem in the dorsolateral pontomesencephalic tegmental area and it projects mainly to the diencephalon and the forebrain. Other smaller numbers of cells are found in the medulla and in the cortex. The visualization of these cell bodies confirms and clarifies results which had been obtained from pharmacological and associated types of studies on the effect of

acetylcholine on cortical activity. At the same time, the possibility is presented for newer and innovative experimental approaches affecting the cholinergic neurons in a more specific manner than had been possible up to this point.

F. Anatomical Overlap and Functional Interaction

After the immunohistochemical identification of putative cholinergic neurons and their projecting fibers it was possible for investigators to direct their experimental lesions and electrical and chemical stimulation at specific brain targets in order to study the effects of the cholinergic system separately from those of the other transmitter systems. It was also possible to correlate the disruption of vigilance states with destruction of brain structures described in earlier animal experimental studies, as well as clinical cases, and to deduce the nature of the transmitter systems which must have been affected in cases in which disruption of the sleep-waking cycle occured.

The influence exerted by cholinergic neurons on wakefulness may depend significantly on their interaction with catecholaminergic as well as with other types of neurons (Jouvet, 1972). Evidence exists from various types of experimental interventions that cholinergic and catecholaminergic neurons may interact in order to regulate the sleep-waking cycle (Karczmar, 1970; Hobson & Steriade, 1986;

Steriade & Hobson, 1976), and indeed, in the pontomesencephalic tegmentum the catecholamine and cholinergic neurons are found intermingled with each other (Jones & Beaudet, 1987a). It is to be noted, however, that the ratio of CHAT-positive to tyrosine hydroxylase (TH) -positive neurons is not the same throughout this region (Jones & Beaudet, 1987a). Cholinergic neurons are found more abundantly at the anterior pole of the pontomesencephalic tegmental region, and mainly within the PPT and LDT nuclei. There are fewer, though still substantial number of such cells in the PB and LC nuclei at the caudal pole of the region. Conversely, the majority of catecholaminergic cells are in the LC and PB nuclei, which are at the caudal pole of the region, but they also extend anteriorly into the LDT and PPT nuclei (Jones & Beaudet, 1987a).

The identification of the overlapping nature of these cells and their differential distribution density, help to explain results obtained from earlier experiments. After reviewing several studies involving cell destruction in and around the LC region, Jouvet (1972) came to the conclusion that cells in the anterior pole of the LC were concerned with wakefulness, because lesions in that region resulted in a decrease in wakefulness, and this area corresponds to the region where acetylcholine cells are more abundant than catecholamine ones. Lesions in the principal and caudal LC, which would have destroyed mainly catecholamine

neurons, have no enduring effect on wakefulness (Jacobs & Jones, 1978; Jouvet, 1972). Jouvet concluded that the effect of these previous lesions upon waking may have been due to destruction of the cholinergic cells which lie intermingled with the catecholaminergic neurons, even though the cholinergic cells are concentrated more rostrally than the catecholaminergic ones.

In summary, the initial attempts at mapping the cholinergic neurons was made by staining cell bodies and then fibers for acetylcholinesterase, which was not a specific indicator of cholinergic structures, but it was useful in helping to delineate the projection system which are now associated with the ARAS. The search continued for a more specific marker for acetylcholine and culminated in the development of immunonohistochemical methods, which enabled the specific identification of cholinergic cell bodies for the first time. Given our current knowledge concerning the localization of the cholinergic system in the brain, it is interesting that many of the early hypotheses regarding the importance of cholinergic neurons in the process of cortical activation and wakefulness may have been correct. The basal forebrain cholinergic neurons provide the major cholinergic innervation to the cortex, except for a small contingent of local cholinergic neurons in the cortex. The cholinergic neurons of the basal forebrain have been shown to be critical to cortical activation, because of the deficits that re-

sult following lesions to those cells (Lo Conte, Casamenti, Bigl, Milaneschi & Pepeu, 1982; Stewart, MacFabe, & Vanderwolf, 1984). The contribution to cortical activation and wakefulness by the cholinergic neurons of the dorsolateral pontine tegmentum, which compose the ascending cholinergic reticular system, remains to be similarly tested.

G. Sleep States and Transmitter Agents.

After the identification of PS and slow wave sleep as two different states, dependent on different anatomical structures, research efforts were concentrated in an attempt to identify the specific chemical neurotransmitters involved in controlling each of the two states. A vast array of these agents have been implicated in the control of these two states. Most of these agents are considered as modulators of of slow wave sleep and PS, in which they are thought to play possibly important but nonessential roles, whereas others retain the original classification as transmitters, because they are considered to be of critical importance in the expression of these states (for reviews see Drucker-Colín, Aguilar-Roblero and Arankowsky-Sandoval, 1985; Krueger, 1985; and Jones, 1988). Of these neurotransmitters, only serotonin, acetylcholine and noradrenaline will be addressed in the following section of this introduction: serotonin because of the wide attention it has commanded due to its influence on the state of slow wave sleep, and acetylcholine

and noradrenaline because these two substances have been implicated in both states of sleep (especially in PS), and because both are of central importance to the present study.

1. Slow Wave Sleep and Serotonin.

Considerable evidence from pharmacological, neurophysiological and neurochemical studies indicate that serotonin plays a role in slow wave sleep (for reviews see Jacobs, 1985; Jacobs & Jones, 1978; Jones, 1988; Jouvet, 1972; Koella, 1985; and Krnjević, 1974). The identification of serotonin in the brain by Twarog and Page (1953) and by Amin, Crawford and Gaddum (1954), was followed shortly thereafter by experiments on its role in sleep. In order to test the influence of serotonin on sleep, its precursor 5-HTP (5-hydroxytryptophan) is often used, because serotonin itself does not cross the blood brain barrier, whereas 5-HTP does (Udenfriend, Weissback & Bogdanski, 1957). Large doses of 5-HTP, injected systemically into cats and rabbits led to a state of cortical synchronization and somnolence (Monnier & Tissot, 1958), and direct injection of serotonin or its deaminated metabolites into the ventricles of the brain, or into neighbouring areas not protected by the blood brain barrier, led to cortical synchronization and apparent slow wave sleep (Jouvet, 1972; Koella, 1985).

The results from experiments prior to the discovery of the state of PS were sometimes difficult to interpret.

Treatment with 5-HTP, which increased brain serotonin and slow wave sleep, also caused long-term suppression of PS (Delorme, 1966). Such results suggested that systems other than those controlling slow wave sleep must have been affected as a result of the 5-HTP treatment. Similar findings were obtained from other pharmacological agents affecting slow wave sleep. For example, reserpine, which is known to decrease serotonin by preventing its storage, leads to the concommitant suppression of slow wave sleep (Jouvet, 1972). Reserpine, however, also decreases the catecholamines by the same mechanism, and suppresses PS for twice as long as it suppresses slow wave sleep (Jouvet, 1969, 1972). The effect of reserpine on the indolamine system was parcelled out from its effect on the catecholamines by administration of the serotonin precursor, 5-HTP. When 5-HTP was injected into reserpinised cats it caused immediate cortical synchronization, whereas DOPA, the catecholamine precursor, when administered to reserpinized cats caused PS to be reestablished. These results led to the suggestion that serotonin was related to slow wave sleep and that the catecholamines were involved in PS (Matsumoto & Jouvet, 1964). Jouvet (1969, 1972) concluded however, that this type of pharmacological treatment produced interference with multiple transmitter systems in the brain, and therefore that its effect does not ideally lend itself to unambiguous interpretation. Therefore, attempts were made to find more

suitable drugs for testing, which included the monoamine oxidase inhibitors (MAOI), which prevent catabolism of the monoamines and cause a consequent increase of monoamine concentration in the brain. Mixed effects were also obtained from these. Some MAOIs increased slow wave sleep in cats (Jouvet, Vimont & Delorme, 1965), but others suppressed it in rats (Mouret, Vilpula, Frachon, & Jouvet, 1968).

Because it is a specific inhibitor of brain serotonin synthesis, Jouvet (1969) believed that (PCPA) was a more ideal drug than the MAOIs for use in sleep research. Administration of this drug caused a reduction of serotonin and of slow wave sleep but did not significantly affect the levels of the catecholamines (Delorme et al., 1966; Jouvet, 1969, 1972; Koe & Weissman, 1966). The duration and amount of slow wave sleep reduction corresponded to the level of 5-HT reduction (Koella, Feldstein & Czicman, 1968). Conversely, after PCPA injection had caused a large decrease of brain serotonin, (up to 85%) and of slow wave sleep, injection of 5-HTP, the precursor of serotonin, reestablished serotonin level. The level of reestablished serotonin corresponded directly to the size of the dose of the 5-HTP injection (Hoyland, Shillito & Vogt, 1970). In fact, very small 5-HTP injections (2-5 mg/kg) were sufficient to restore to normal levels the slow wave sleep that was eliminated by PCPA (Jouvet, 1968; Mouret, Froment, Bobillier & Jouvet, 1967; Pujol, Buguet, Froment, Jones, & Jouvet, 1971). Jouvet (1969, 1972) saw the depletion of 5-HT and

concommitant reduction of slow wave sleep by PCPA, and the immediate return of slow wave sleep with 5-HTP injection as the crucial argument justifying a role for 5-HT in sleep mechanisms. There were, however, some inconclusive or contradictory findings where PCPA administration that caused very low levels of brain serotonin led to only moderately low levels of slow wave sleep, especially following chronic administration over periods longer than one week (Cohen et al., 1970; Rechtschaffen, Lovell, Freedman, Whitehead & Aldrich, 1969). It appeared that although the pharmacological intervention offered strong clues and suggestions as to the role of serotonin in sleep, the evidence was never altogether conclusive, because the results were open to alternative interpretations.

A new approach to the solution of this problem was to be found in the development of the fluorescense histochemical method for identifying monoamines in the brain (Falck, Hillarp, Thieme & Thorp, 1962). Neurons containing 5-HT were then identified in the raphe nuclei, and their projections mapped (Dahlstrom & Fuxe, 1964). Jouvet (1969) interpreted the new developments as making possible the mergence of the fields of neuroanatomy, neuropharmacology and neurophysiology, which would allow a more rigourous way of thinking about and investigating brain mechanisms of sleep.

Fluorescence histochemistry showed that the raphe group of nuclei in the brainstem extend along the midline from the midbrain to the medulla. The majority of the 5-HT neurons are located in the midbrain and pontine raphe nuclei, but a substantial number is also found in the medulla. Altogether, less than half of the neurons located in the raphe nuclei are serotonergic (Törk, 1985), and they innervate virtually the entire central nervous system. The rostrally located nuclei (dorsal and central superior) project mainly rostrally to the forebrain, including the thalamus, hypothalamus, basal forebrain, and all cortical areas. The medullary group (magnus, pallidus and obscurus) mainly projects caudally into the spinal cord (Steinbusch, 1984) but also sends substantial projections to the surrounding area, such as the reticular formation and the nucleus of the solitary tract (Dahlstrom & Fuxe, 1964; Törk, 1985).

After identification of the location of the serotonin neurons and their target areas, it was possible to focus on the influence of the raphe relatively independently of other structures in the region. Jouvet (1969, 1972) realized that lesions of serotonin neurons in the raphe nuclei could cause a decrease of the serotonin levels in the brain without affecting serotonin level in the periphery. Accordingly, Jouvet and his colleagues (Jouvet, Bobillier, Pujol & Renault, 1966; Jouvet & Renault, 1966),

after performing lesions to the raphe found that there was a high and consistent 3-way correlation between the amount of raphe tissue remaining after the lesion, the level of brain serotonin and the amount of daily slow wave sleep. These findings therefore provided strong evidence for the participation of serotonin in slow wave sleep. Confirmatory support also came from other studies, showing that the raphe projected to hypnogenic structures, which when stimulated could lead to sleep (1969, 1972). In addition, injections of serotonin directly into the preoptic area and the central medial nucleus of the thalamus (Yamaguchi, Marczinski & Ling, 1963) and more recently, injections of small amounts of serotonin directly into the ventrolateral part of the posterior hypothalamus, lead to slow wave sleep onset (Sallanon, Buda, Janin & Jouvet, 1985). In the caudal brainstem, injection of the serotonin precursor 5-HTP into several medullary sites in rabbits also lead to cortical synchronization of slow wave sleep (Ledebur & Tissot, 1966). It was also seen that neurons within the nucleus of the solitary tract increased their rate in response to stimulation by serotonin (Key & Metha, 1977), and activity of cells in this nucleus correlates well with the appearance of slow wave sleep (Eguchi & Satoh, 1980a, 1980b). Furthermore, electrical stimulation of the solitary tract may have a synchronizing effect on neurons of the motor cortex (Dell & Padel, 1965; Magnes et al., 1961) and leads

to slow wave sleep, whereas, destruction of serotonin fibers within the solitary tract nuclei is associated with the abolition of cortical synchronization and loss of slow wave sleep (Nosjean, Arluison, & Laguzzi, 1987).

In spite of the positive results obtained from chemical intervention, electrophysiological and lesion studies, there was also evidence which cast doubt on the sleep-inducing function of the raphe neurons. For example, after selective destruction of serotonin neurons with 5,6-DHT and 5,7-DHT (dihydroxytryptamine) in the raphe, there was some short-term decrease of slow wave sleep, which was not longlasting, however (Froment, Petitjean, Bertrand, Cointy, & Jouvet, 1974; Jacobs, 1985; Jacobs & Jones, 1978). It was also shown that raphe neurons actually decrease their rates of firing with the onset of and during slow wave sleep (McGinty & Harper, 1976).

Information derived from electrical stimulation of the raphe has also been inconclusive. Stimulation of the midbrain raphe in rats at low voltage and frequency led to slow wave sleep (Kostowski, Giacalone, Garattini & Valzelli, 1969). However, the range of stimuli parameters within which stimulation was effective was rather narrow, and variations outside the range led to arousal or had no effect. The reports are contrary to those made later by Jacobs, Asher and Dement (1973), who stimulated the dorsal raphe during sleep in cats and caused EEG signs of arousal in the

sleeping animals. Other studies indicate that stimulation of the raphe magnus can lead to EEG and behavioural signs of arousal in rabbits and cats (Jacobs et al., 1973; Polc & Monnier, 1970). Jacobs et al. speculated that the arousal signs seen in the animals in their study were probably due to non-specific effects of the stimulation, and that the variance between their results and those of Kostowski et al. (1969) was probably due to procedural and species differences. Therefore, while it is apparent that stimulation of the medullary raphe leads to arousal, conclusions drawn from the results of electrical stimulation can be only tentative. It is to be noted that some raphe neurons may not be directly related to the sleep-wake cycle at all. In a recent review, Jacobs (1985) has suggested that spontaneous activity in the rostral raphe neurons is strongly related to arousal levels, but that the caudal neurons show only a weak relationship to vigilance states, relative to their rostral counterparts.

Probably because of its complex organization and diverse patterns of activity, inconsistent results are often obtained after experiments performed on the serotonergic raphe system. This situation has led to interesting suggestions on the role of the raphe in sleep. Steriade and Hobson (1976) interpret most of the data on the raphe as suggesting that it may be partially responsible for the "ultradian, within-sleep periodicity" of sleep stage

alterations (Steriade & Hobson, 1976). As such it would be a component of a putative mechanism influencing sleep and waking rhythms, and it is responsive to regularities of environmental influences, by which it participates in setting the circadian and ultradian rhythms relevant to arousal processes. It appears that serotonin may be involved in such functions, because it is the active neurotransmitter involved in regulating the diurnal rhythm of the light-sensitive circadian clock (circadian rhythm of optic nerve impulses) in the eye of the aplysia, a marine gastropod (Nadakavukaren, Lickey & Jordan, 1986), moreover, Jacobs (1985) has noted that the raphe neurons fire at a regular pace both in vivo and in vitro.

Another rhythm-related function of the serotonin neurons suggested by Sallanon et al. (1985) is that they may act to facilitate the accumulation or synthesis of a sleep-promoting factor (SWS2 factor) during waking, when these neurons are most active. This hypnogenic factor appears to have its site of action in the ventrolateral part of the posterior hypothalamus, but its mechanism of action is still unclear (Sallanon et al., 1985). Other suggestions are that the raphe influences memory processes (Siegel & Brownstein, 1975), because when it is damaged in experimental animals it causes a disruption of the ability to habituate to recurrent stimuli (Aghajanian, Rosecrans & Sheard, 1967; Connor, Stock, Barchas & Levine, 1970). The latter

suggestion is consonant with the notion (Jacobs & Jones, 1978) that the insomnia consequent to raphe destruction, or depletion of serotonin by drugs, is due to hyper-responsivity to externally and internally generated sensory-related events, which would thus prevent an animal so treated from falling asleep.

In evaluating the involvement of brainstem systems in slow wave sleep, and the way they interacted with each other in order to control this state, Steriade and Hobson (1976) concluded that the basal forebrain area has an excitatory influence on the nucleus of the solitary tract, which in conjunction with the raphe, exerts inhibitory influences on the reticular formation. Once the reticular formation is inhibited, it allows the thalamo-cortical system to go into action to develop the activity and the cycle of inhibition and excitation necessary for synchronization of the cortical EEG during slow wave sleep.

2. Slow Wave Sleep and Acetylcholine?

After the discovery that the ARAS was deactivated by sleep-inducing structures in the caudal brainstem and rostral hypothalamus (Moruzzi, 1960), interest arose in determining which neurotransmitter might be involved in the deactivating process. Acetylcholine attracted attention because it and its agents were known to have an effect on vigilance states (Rinaldi & Himwich, 1955a, 1955b; Wikler,

1952), but particularly because it evoked sleep when it was administered in brain areas where electrical stimulation had also caused sleep onset in cats (Hernández-Peón, 1962, Hernández-Peón et al., 1963). Another reason why acetylcholine was thought to be a likely candidate was because parenteral injections of atropine prevented sleep produced by stimulation of the preoptic area (Hernández-Peón & Chávez-Ibarra, 1963), and local application of acetylcholine crystals to the preoptic area and the posteromedial hypothalamus, or injected into the brain in the proximity of the hypnogenic centers in several parts of the brainstem and forebrain, led to sleep onset. On the basis of results such as these, the cholinergic sleep system was defined as lying essentially within the limbic forebrain-midbrain circuitry described earlier by Nauta (1946, 1958), and of having an ascending component from the spinal cord. Activity within the limbic forebrain system was thought to bring about the state of sleep by directly inhibiting the ARAS, and injection of cholinergic agents into different parts of the descending component (Hernández-Peón & Chávez-Ibarra, 1963; Velutti & Hernández-Peón, 1963) or into the medullary ascending component (Cordeau, 1962), led to short episodes of slow wave sleep, followed by long episodes of PS. Conversely, lesions within the preoptic area were followed by insomnia (McGinty & Sterman, 1968).

Although Hernández-Peón acknowledged the two states of sleep (Hernández-Peón et al., 1963), he supported the unitary concept of sleep, in which slow wave sleep and PS were seen as being dependent on the same mechanisms. Conversely, the dual nature of the sleep state, and its dependence on different mechanisms, was a central point emphasized by Jouvet (1962, 1965). In addition, Jouvet and his colleagues argued that the different sleep mechanisms were dependent on different neurotransmitters. Thus, shortly after the identification of monoamine neurons in the brain, Jouvet (1969) proposed the monoaminergic theory, which indicated that a monoamine was centrally responsible for each state of vigilance.

Jouvet (1969, 1972, 1975) acknowledged that acetylcholine could play a role as a relay for serotonin in triggering the onset of PS and that it participated in cortical desynchronization of PS as well as that of waking. Opinions therefore differed between Jouvet and Hernández-Peón as to how acetylcholine participated in sleep (Hernández-Peón & Chávez-Ibarra, 1963; Jouvet, 1969, 1972). This difference of opinion of the investigators involved in the research on the role of acetylcholine in sleep states reflected the different points of emphasis in their theoretical standpoints on how sleep was organized (Hernández-Peón & Chávez-Ibarra, 1963; Jouvet, 1969, 1972, 1975).

In recent years, reports linking acetylcholine to slow wave sleep have been sparse or indirect. Szymusiak and McGinty (1986a) showed that kainic acid lesions in the basal forebrain of cats, which presumably destroyed cholinergic cells, effectively reduced the amount of slow wave sleep as well as PS. The injections were made in the horizontal limb of the diagonal band of Broca and in the lateral preoptic-substantia innominata region, where cells fire preferentially during slow wave sleep (Szymusiak & McGinty, 1986b). Less directly, but still to some extent illustrative of the possible role of acetylcholine in slow wave sleep, Stewart et al. (1984) effected electrolytic lesions in the basal forebrain in a group of rats. The lesions caused a reduction of acetylcholinesterase staining in the cortex, indicating a loss of innervation from the lesioned neurons. The lesions also caused a reduction of large, irregular, slow activity (LISA), similar to that seen in slow wave sleep. However, these authors suggest that the effect may have been due to damage to fibers of passage rather than to cholinergic neurons, because lesions with kainic acid in the same area caused an increase rather than a decrease in slow wave activity.

The data supporting a role for acetylcholine in slow wave sleep were often open to alternative explanations. In another similar experiment, decrease of slow wave sleep, from lesions aimed at presumed cholinergic fibers in the

preoptic area (McGinty & Sterman, 1968), was interpreted as being due to damage of serotonergic projections from the raphe nuclei (Jouvet, 1975). Besides, administration of cholinergic agonists invariably precipitated the onset of PS, usually after a very short preliminary slow wave sleep bout (Baxter, 1969; Dren & Domino, 1966; Hernández-Peón et al., 1963).

Jouvet (1972, 1975) suggested several reasons why acetylcholine might not underlie slow wave sleep. For example, although inhibition of cholinergic activity was possible through the administration of the anticholinergic agents hemicholinium-3 and atropine, these drugs do not inhibit behavioural sleep, as would be the case if slow wave sleep were dependent on acetylcholine. Conversely, slow wave sleep is reduced by lesions of the raphe system, even when the cholinergic system is intact.

Hobson and Steriade (1986) also rejected the idea that acetylcholine could play a role in slow wave sleep. They suggest that this state may be passive, in that it occurs during the alternation between waking and PS, and that the timing and duration of slow wave sleep may be regulated by humoral factors that are responsive to circadian and metabolic signals not detectable in neuronal electrical activity. Recently, however, Szymuziak and McGinty (1986a) have shown that neuronal activity in the ventral portion of the basal forebrain anticipates the onset of slow wave

sleep, thus fulfilling a criterion (Hobson, McCarley & Wyzinski, 1975; Sakai, 1985a, 1985b) for establishing if activity in a structure is a determinant of a particular state. In addition, these cells have a low rate of firing in PS and waking. Such evidence shows these cells are involved in slow wave sleep, but no proof was provided that they were cholinergic.

From the preceding studies on the role of acetylcholine in slow wave sleep, it appears that disagreements on this issue were due to the different emphasis on the organization of sleep into two different states. As pointed out by Jouvet (1975), it is evident that acetylcholine participates in PS, but there is little convincing evidence that it also mediates slow wave sleep.

H. Paradoxical Sleep.

As illustrated in an earlier section, the approach to the study of the mechanisms underlying the PS phenomena has traditionally taken two routes: (1) the search for specific mechanisms needed to initiate and maintain the state of PS (the so-called PS center), in terms of coordinating the occurrence of the different variables; and (2) the search for the mechanism underlying the different components of the state. These efforts often progressed simultaneously, although most of the initial experiments were designed to identify the mechanisms involved in the

initiation and maintenance of PS as a whole rather than the individual variables. Neurotransmitters were invoked to explain this phenomenon, from the time it was discovered in animals (Dement, 1958; Jouvet & Michel, 1959). The two transmitters that have received the most attention from investigators have been noradrenaline and acetylcholine, and will be reviewed in the following sections.

1. Paradoxical Sleep and Noradrenaline.

Systemic, intracarotid and intraventricular injections of amines were shown to cause alteration of both sleep and waking (Feldberg & Sheerwood, 1954; Mandell & Spooner, 1968). The results from many such experiments, however, were later judged to be flawed, either because it was discovered that noradrenaline does not cross the blood brain barrier easily (Weill-Malherbe, Axelrod & Tomchick, 1959), or because the noradrenaline injection might have induced some non-specific effect upon non-noradrenergic neurons (Jouvet, 1972). The experiments by Matsumoto and Jouvet (1964), aluded to at the beginning of this section, were of particular interest because they showed that the state of PS and slow wave sleep could be controlled according to the level and type of drug that was administered to the experimental animals. Intraperitoneal injections of reserpine, which depleted catecholamines and 5-HT stores, eliminated slow wave sleep and PS in cats. During the insom-

nia, injections of DOPA, the precursor of catecholamines, restored PS, while injections of 5-HTP, the precursor of serotonin, restored slow wave sleep.

Through the rest of that decade several pharmacological experiments were performed which led Jouvet (1969) to the conclusion that noradrenaline was the executive transmitter involved in PS. Injections of alpha-methyl-dopa, which metabolizes to alpha-methyl-NA, which in turn displaces noradrenaline, suppressed PS in cats (Dusan-Peyrethon, Peyrethon & Jouvet, 1968). It also suppressed PS rebound when given at the end of a PS deprivation period. Drugs able to cross the blood brain barrier and block alpha-adrenergic receptors also caused a suppression of PS (Jouvet, 1967, 1972; Matsumoto & Watanabe, 1967). Other studies showed that administration of AMT, which decreases catecholamines by inhibiting TH, the enzyme mediating the rate-limiting step in catecholamine metabolism, (Nagatsu, Levitt, & Udenfriend, 1964) led to a reduction or inhibition of PS in monkeys (Crowley, Smith & Lewis, 1968), and in rats (Torda, 1968). When MAO was inhibited with nialimide, PS was suppressed (Mouret et al., 1968). Experiments by Jones (1972) suggested that noradrenaline cells of the pontine tegmentum were involved in PS through the release of deaminated metabolites of noradrenaline, because when noradrenaline re-uptake was inhibited with injections of desipramine, or when the activity of the catabolic enzyme MAO was inhibited, PS was sup-

pressed. On the other hand, when catecholamine levels were increased through the inhibition of the catabolic enzyme COMT with tropolone, DOPA administration led to an increase in PS (Jones, 1972).

The pharmacological approach had provided some early indications of the involvement of noradrenaline in PS through the 1960's, but eventually, the limitation to the method had become evident, because it was often not possible to rule out serious alternative explanations of the results. However, by this time the noradrenaline neurons in the brainstem and their projections had been mapped (Dahlstrom & Fuxe, 1964, 1965), opening up the possibility of investigating the function of these neurons in a more direct fashion by using methods such as neurotoxic, mechanical or electrolytic lesions.

Histofluorescence showed that noradrenaline neurons were located in the brainstem, and especially concentrated in the LC. Bilateral electrolytic lesion of the LC nuclei including the nucleus subcoeruleus and a portion of the nucleus RPC, caused a substantial loss of PS and an associated reduction of noradrenaline in the brain (Jouvet & Delorme, 1965; Roussel, Buguet, Bobillier & Jouvet, 1967). These lesions may have also included the medial parabrachial nucleus (Jouvet, 1972), which in cats contains noradrenaline neurons (Jones & Moore, 1974; Maeda, Pin, Salvert, Ligier & Jouvet, 1973).

In order for the lesions to affect the expression of PS, they had to destroy at least the caudal two-thirds of the LC and the nucleus subcoeruleus. Destruction of these nuclei caused a decrease of 30 to 40% of the noradrenaline in the telencephalon and diencephalon, and PS was definitely suppressed (Buguet, Petitjean & Jouvet, 1970; Mouret et al., 1968; Roussel, 1967). By considering the results from the electrolytic lesions of the LC on PS in light of the pharmacological data on PS, Jouvet (1969, 1972) arrived at the conclusion that noradrenaline LC neurons were critical for the control of PS.

In spite of the vast amount of pharmacological and lesion results supporting a role for noradrenaline in PS, reports that conflicted with such a proposed role had also been emerging from time to time. For example, Havlíček (1967) had noted that intraventricular injections of DOPS (dl-3,4-dihydroxyphenylserine), a precursor of noradrenaline which crosses the blood brain barrier and is catabolized to noradrenaline (Blaschko, Burn & Langeman, 1950; Carlsson, 1964), causes a large increase in slow wave sleep, but only a minimal increase in PS. Meanwhile, Marantz and Rechtschaffen (1967) failed to find a consistent, effect of intraperitoneal injection of AMT on PS in rats, despite a 50% drop in whole brain noradrenaline levels. Other studies showed that noradrenaline might be playing an inhibitory role in PS, because when AMT injections caused brain noradrenaline to be

lowered by as much as 70% in cats, it led to an increase in the amount of PS (King & Jewett, 1971). Similarly, Hartman, Bridwell and Schildkraut (1971) caused increased PS in rats after oral administration of AMPT, and Hartman, Chung, Draskoczy & Schildkraut (1971) noted that destruction of catecholamine neurons in rats by intracisternal injections of 6-Hydroxydopamine (6-OHDA), also caused an increase in PS.

Taken together, the results from the above experiments suggest a role for noradrenaline in some aspect of PS, but a definite conclusion is difficult, due to the nonspecific effects of the pharmacological treatments. All the same, evidence for the relative lack of LC influence on PS also came from unit recording studies, which showed that noradrenaline cells of the LC do not increase their firing with the onset of the state of PS (Aston-Jones & Bloom, 1981). In fact, Hobson et al. (1975) found that these cells ceased firing prior to the onset and for the duration of PS.

A reexamination of the earlier work showed that lesions that had most effectively eliminated PS, had been very large and had often included structures outside the LC. This suggested that even if noradrenaline was involved in the control of PS, other neurons and pathways, which were damaged when the lesions were made, might also be involved in the expression of PS. With more discreet lesions of the noradrenaline neurons of the LC, Henley and Morrison (1974)

reported that the main effect of electrolytic lesions to the LC was a disruption of atonia. Jones, Harper and Halaris (1977) also found that lesions confined to the rostrocaudal extent of the LC were not able to eliminate PS, even though they had reduced the noradrenaline levels in the brain by a significant amount. In these experiments PS returned by the second postoperative day and subsequently recovered to levels not different from baseline. However, muscle atonia did not return and PGO spikes were reduced in frequency during PS. Jones et al. (1977) concluded that their results showed that noradrenaline did not play a central role either in the initiation or maintenance of PS, but might play instead a modulatory role in some of the phasic components.

2. Paradoxical Sleep and Acetylcholine.

As noted earlier, the results from several experiments, in which acetylcholine and its agents had been administered, indicated that acetylcholine was involved in activation of the cortex (Bonnet & Bremer, 1937; Funderburk & Case, 1951; Rinaldi & Himwich, 1955a, 1955b). Shortly after the state of PS was described in the cat (Dement, 1958; Jouvet, 1959), Jouvet and Michel (1960) announced that cholinergic mechanisms must be involved in the control of this state, based on results from the administration of atropine, prostigmine and eserine to cats. Atropine, a cholinergic antagonist, suppressed or shortened the PS

episode, whereas the anticholinesterases, neostigmine and eserine led to an increase in the duration of PS episodes.

a. Systemic Pharmacological Studies.

As already mentioned above, other indications that acetylcholine was involved in PS came from experiments showing that this transmitter was released in large quantities from the cortex in association with cortical activation of PS and wakefulness (Celesia & Jasper, 1966). The level of acetylcholine in perfusates from the ventricles was also highest during PS just before the animal awakened, and was decreased or absent during slow wave sleep (Haranath & Venkatakrishna-Bhatt, 1973).

Many of the problems encountered in interpreting the effect of systemic administration of cholinergic agents on the state of waking were also present when attempts were made to study the effect of these agents on PS using this same method of drug administration. The problems included the strong likelihood that acetylcholine would be catabolized before properly exerting its effect centrally on sleep, as well as the possibility of its central and peripheral effects being confounded.

In order to circumvent these drawbacks, novel methods of applying the drugs were devised, and it was often necessary to use other cholinergic agents that could increase or decrease the amount of endogenous acetylcholine

in the brain, or mimic some of the properties of acetylcholine. Both muscarinic and nicotinic drugs may induce cortical activation of PS, but they may have differential effects on the other variables of this state (Domino & Yamamoto, 1965; Jewett & Norton 1966). In the following paragraphs, the effects of some of these cholinergic agents on PS will be examined.

Intravenous infusion of different levels of the muscarinic agonist arecholine accelerated the onset of PS in humans when administered during the first, second or third slow wave sleep period (Gillin & Sitaram, 1984; Gillin et al, 1985; Sitaram, Moore & Gillin, 1978a; Sitaram et al., 1980), but did not usually affect the duration of the PS episode (Sitaram, Moore & Gillin, 1978b), although it sometimes shortened the second PS period (Sitaram et al., 1978a). After the infusion of arecholine PS onset is much faster in emotionally depressed human subjects and in those who have suffered and recovered from depression, than in normals who have never been pathologically depressed (Sitaram, Gillin & Bunney, 1984). The difference in effects is attributed to a higher level of muscarinic cholinergic receptors in the brains of subjects associated with depression (Shiromani, et al., 1987b). The effect of arecholine is blocked or attenuated by scopolamine, a centrally-acting muscarinic antagonist, depending on the ratio of arecholine to scopolamine (Sitaram et al., 1978a).

Intramuscular injections of scopolamine, caused a decrease of PS time in human subjects, with a compensatory increase in slow wave sleep but no changes in waking (Sagales, Erill & Domino, 1969). There was an inhibition of PS after the initial injection, for approximately 4 1/2 hours, after which time, PS occurred in bursts for the rest of the night. Using some of the same drug doses as had been used by Sagales et al. (1969), Sitaram and his colleagues (1984) found that with daily, morning administration of scopolamine, tolerance to the drug developed, and was evident as a decrease in latency to the onset of PS the second night, but on the third night there was increased arousal, which was interpreted as the result of "muscarinic supersensitivity", developed as a result of continued administration of the drug (Sitaram et al., 1984). Supportive and explanatory evidence for this interpretation was obtained from chronic (1 week), daily scopolamine treatment in rats, which caused an initial decrease in PS a few hours after injection on the first day, with a development of tolerance to the drug during the following days (Sutin, Shiromani, Kelsoe, Storch & Gillin, 1986). Additionally, there was an increase in muscarinic receptor binding in the caudate and hippocampus and a corresponding increase in PS after withdrawal of the drug seven to nine days later. The increase in total PS was

as a result of an increased number of episodes, as there was no effect on their duration.

Intravenous injections of nicotine in sleeping cats led to an increase in PS (Domino & Yamamoto, 1965). After the nicotine infusion there was an initial period of arousal, followed in a few minutes by slow wave sleep, and fifteen to twenty minutes later PS ensued. These authors suggest that the arousal effects were due to a release of arginine vasotocin and epinephrine, which affected peripheral systems, and the effect on PS may have been due, to some extent, to the release of vasopressin. However, they also showed that the main effect of the nicotine was on the central nervous system, because the effect of nicotine was blocked by mecamylamine, a ganglionic blocking agent which when administered in large doses can cross the blood brain barrier (Goth, 1968). On the other hand, the effect was not blocked by trimethidinium, also a ganglionic blocking agent, but which does not cross the blood brain barrier. Jewett and Norton (1966) found that low, subcutaneous doses of nicotine led to an increase in PS, but high doses blocked PS. Slow wave sleep was not affected by any dose, thus showing that the effect of nicotine was not on sleep in general, but that it preferentially affected PS.

Mecamylamine is a nicotinic ganglionic blocker which is able to cross the blood brain barrier and to block cortical activation induced by nicotine (Domino et al.,

1968), but in cataplectic dogs it was able to produce only a slight blocking effect after intraventricular injections in high doses (Delashaw, Foutz, Guilleminault & Dement, 1979). Similar ambiguity surrounds atropine, which is thought to have both muscarinic and nicotinic antagonistic influence in the brain, but the effect of this drug on cortical activation of PS induced by nicotine is somewhat controversial. On one hand, Longo (1966), on the basis of a review of the literature and an earlier experiment (Longo, Von Berger, & Bovet, 1954) concluded that atropine had no effect on cortical activation of PS induced by nicotine. Contrary to this opinion, Domino et al. (1968) reported that pretreatment with atropine blocked the cortical activation of PS and hippocampal theta (a sign of PS) induced by nicotine and DMPP, a nicotinic agonist (Domino et al., 1968; for review see Gillin, Mendelson, Sitaram & Wyatt, 1978b).

Reduction in the level of acetylcholine by means of HC-3, which interferes with choline transport, as noted above, also affects the expression of PS. Four hours after HC-3 administration, PS was absent and subcortical acetylcholine was reduced by 50% (Dren & Domino, 1966). Neocortical slow waves were predominant at the time (Domino et al., 1968). This EEG effect was most likely due to the lowered level of acetylcholine in the brain, which was shown to correspond with lowered amounts of PS (Domino & Stawiski, 1970). In a different study, 5 mg. of HC-3, injected

intraventricularly in dogs, caused amygdala spiking and blocked hippocampal theta activity (a sign of PS), but at the same time, did not begin to affect neocortical activation for four hours. This result demonstrates a different effect of HC-3 on neocortical and limbic PS activating systems (Domino et al., 1968).

Similar suppression of PS was obtained by Hazra (1970) after injecting HC-3 into the fourth ventricle in cats. Wakefulness and behaviour normally associated with waking were not affected, and the decreased time in PS was compensated for by an increase in slow wave sleep time. The cholinergic muscarinic agonists arecholine, pilocarpine, and physostigmine reversed or blocked the influence of HC-3 on PS. However, administration of nicotine could neither reverse nor block the effect of HC-3, as had been done with the muscarinic agonists on PS (Domino et al., 1968).

Eserine (physostigmine) is an inhibitor of cholinesterase which can precipitate the occurrence of PS in cats, rats and rabbits (Karczmar et al., 1970) and in human subjects too, but does not affect the duration of the episode (Gillin et al., 1978a). Following the administration of reserpine (which would lower the levels of endogenous serotonin and catecholamines) in cats, rats or rabbits, eserine injected subcutaneously or intraventricularly can induce all the signs of PS, including cortical EEG desynchrony, partial atonia, hippocampal theta, PGO waves recorded from the occi-

pital cortex and REMs. Intravenous injection of atropine in these reserpinized-eserinized animals caused a return of full synchronization of the EEG and elevated muscle tonus, and the elimination of hippocampal theta, occipital cortex spikes, and REMs. Eserine or reserpine alone did not cause muscle atonia of PS, although reserpine alone led to PGO spiking and an EEG that alternated between synchronization and desynchronization. The control of the expression of these variables of PS, through the combined enhancement of acetylcholine levels and the depletion of monoamine levels, suggests that an active and central role is played by acetylcholine and that an inhibitory and permissive role is played by the monoamines in the expression of this state. It also illustrates that the presence of a state, be it waking, slow wave sleep or PS, may depend strongly on the ratio of available acetylcholine to catecholamines. When acetylcholine is high and catecholamines are low, PS is expressed. When the levels of both acetylcholine and the catecholamines are low, slow wave sleep signs appear, and when both acetylcholine and catecholamines are high, waking signs predominate (for reviews see Hobson & Steriade, 1986; Jacobs & Jones, 1978; Jouvet, 1972; Sitaram et al., 1984; and Steriade & Hobson, 1976). As the experiment by Karczmar et al. (1970) illustrates, endogenous levels of acetylcholine and the catecholamines can be manipulated experimentally to yield valuable information on the influence of these agents

on state control. However, in order to gain a better understanding of the organizational and functional components of the cholinergic mechanism of PS, it was necessary to devise methods to systematically study the specific effect of acetylcholine and its agents on individual areas of the brains involved in PS. Examples where this approach is used are described in the following section.

b. Regional Pharmacological Studies.

As noted earlier in the section on waking, studies had shown that cortical activation was dependent on the ARAS and acetylcholine (Rinaldi & Himwich, 1955b). Based on results of injections of cholinergic agonists and antagonists, and on earlier lesion work, Jouvet (1962) and Jouvet and Michel (1960) speculated that the mechanism controlling the rapid cortical waves of PS was located in the caudal brainstem, but it was believed to be separate from the ARAS.

In some of these early attempts at investigating the role of acetylcholine in sleep-waking states, Cordeau, Moreau, Beaulnes and Lawrin (1963) injected acetylcholine-bromide into several brainstem regions. Only in a few instances were they able to induce PS and slow wave sleep with injections in the pons and medulla. The poor results may have been due to the rapid degradation of the acetylcholine by acetylcholinesterase, before an effect on sleep could be attained, for far more dramatic results were reported by

Hernández-Peón et al. (1963), who applied acetylcholine powder directly to various sites in the midbrain and forebrain along the limbic circuit of Nauta (1946, 1958). The targets for stimulation included the septal fibers forming the roots of the medial forebrain bundle, the septal area rostral to the anterior commissure, the subthalamus, as well as the lateral, dorsal, and posterior hypothalamic periventricular system of fibers. The effect of the acetylcholine was evident within seconds. Electrocortical activation ensued almost immediately, and was followed in a few seconds by slow wave sleep, which lasted only a few minutes before being replaced by PS. The state of PS was characterized by REMs, atonia and cortical activation. In contrast, atropine inhibited PS when injected in the areas where acetylcholine had been most effective in inducing PS (Velluti & Hernández-Peón, 1963.

In similar studies, George, Haslett and Jenden (1964) showed that cortical activation and atonia similar to that occuring in PS could be elicited by injections of oxotremorine, a muscarinic agonist, or carbachol, a mixed muscarinic-nicotinic, long-lasting cholinergic agonist into the caudal mesencephalic tegmentum and the rostral pontine tegmentum (George et al., 1964). Baxter (1969) obtained similar effects from tamping carbachol into the midbrain near the aqueduct at the level of the superior colliculus and the nucleus of the third nerve. Gnadt and Pegram (1986)

also report that in rats, in the region of the rostral pons, near the parabrachial nucleus and at the level of the motor trigeminal nucleus, carbachol injections effectively induced PS.

Carbachol was also used by Mitler and Dement (1974) to identify as precisely as possible, the areas of the brain involved in controlling PS. Injections into the dorsal anterior pontine tegmentum, in the region of the LC, caused a brief, general arousal initially, which was followed by a gradual onset of atonia and appearance of PGO spikes and REMs in the presence of the EEG activation which persisted from waking into the state of PS. Atonia could be reversed by atropine injections. Similar results were reported after injections of carbachol in this region near the LC by Van Dongen, Broekamp and Cools (1978). Additional studies (Baghdoyan et al., 1985), in which microinjections of carbachol were made in the rostral pons, produced a PS-like state with PGO spikes and REMs similar to those occuring in PS, whereas injections in the rostral midbrain or in the medulla inhibited the PS-like sleep state. The effective area for evoking PS was first reported by these workers to be in the FTG region in the pons.

Another drug, bethanecol, a relatively pure muscarinic cholinergic agonist, was injected into several areas of the brainstem reticular formation, including the central tegmental field of the midbrain, the FTG of the

pons, and the lateral tegmental field (FTL) of the medulla (Hobson, Goldberg, Vivaldi & Riew, 1983). Only injections in the pontine FTG were effective in causing an immediate transition from waking to PS. Activation of the EEG was present and atonia came on gradually. There was a sudden onset of PGO waves, and REMs were also evident. It is possible that the central tegmental field and the tegmental reticular nucleus were within the sphere of the injection, but in these cases the latency to onset of PS was much longer than with the FTG injections.

Lesions were sometimes combined with the injection of cholinergic agents in circumscribed regions of the brain to specifically delineate the areas involved in PS. Hernández-Peón et al. (1963) performed electrolytic lesions of the MFB, thus preventing the customary onset of sleep when acetylcholine injections or implantation of acetylcholine crystals were made anterior to the lesion. Sleep could still be induced with injections caudal to the lesion, thereby supporting the idea that sleep was due to a descending cholinergic limbic mechanism, of which the MFB was an important component pathway. The interpeduncular nucleus and the pontomesencephalic limbic area were also found to be important components of the system (Hernández-Peón & Chávez-Ibarra, 1963; Hernández-Peón et al., 1963).

The above studies generally show that cholinergic drug injections in the brainstem, especially in the ponto-mesencephalic tegmentum, are effective in causing the onset of PS. As such, they confirm results from other reports indicating in a broad manner the structures likely to participate in PS. Reports by several researchers (Amatruda, Black, McKenna, McCarley & Hobson, 1975; George et al., 1964; McKenna, McCarley, Amatruda, Black & Hobson, 1974) suggested that the FTG was the most reliable area where PS could be induced by carbachol injections. Other reports on unit recordings made in the pons and medulla, showed that the iontophoretic application of acetylcholine caused a moderate number of these cells (35%) to fire (Bradley, Dhawan & Wolstencroft, 1966). Taken together, these findings provided part of the basis for Hobson et al. (1975) to suggest that cholinergic cells of the FTG were the main group of neurons controlling PS. Other factors influencing this decision included the finding that the cells in the FTG fired in anticipation of the onset of PS (Hobson et al., 1975; McCarley & Hobson, 1975).

The pharmacological, electrophysiological and, to certain extent, the transection studies had indicated that ACh was vital to the control of PS, and that the pons-caudal midbrain area was the location where this effect was likely to be exerted. The vital piece of information needed to confirm these findings, was the histochemical identification

of the cholinergic neurons, which so far had not been accomplished. Therefore, confirmatory tests, such as abolition of PS by destruction of the cholinergic cells supposedly controlling the state, could not be made. Nonetheless, encouraging steps were being taken in identifying the cholinergic mechanism in the attempt to firmly relate the physiological and behavioural observations to neurological structures controlling the expression of state.

c. Histochemical and Neuroanatomical Studies.

As noted earlier, it had been suggested that the ARAS was comprised of the ascending cholinergic reticular system described by Shute and Lewis (1963, 1967). A more detailed study of the acetylcholinesterase cell distribution in the cat and rabbit brainstem was conducted by Papp and Bozsik (1966), who distinguished these cells from those staining for non-specific types of cholinesterases. Amongst other cells, they categorised the cells of the nuclei RPO and RPC, as well as those of the tegmental fields in the pons and medulla, including the FTG and the FTL, as staining intensely for acetylcholinesterase. These attempts to identify the cholinergic systems in the brain served as clues to sleep researchers in their endeavour to specify the cholinergic structures involved in the control of PS. Palkovits and Jacobowitz (1974) also identified cholinesterase-positive cells in the pontine and medullary magnocellular zones.

Based partly on these results by Palkovits and Jacobovits, as well as the pharmacological and electrophysiological studies noted above, Hobson and his colleagues (Hobson et al., 1975; Steriade and Hobson, 1976) conjectured that the FTG cells might be cholinergic, although they were aware that the commonly acceptable criteria for denoting a substance as a neurotransmitter had not been satisfied regarding the FTG cells (for review see Phillis, 1970), nor had satisfactory histochemical methods been developed which could positively identify these cells as cholinergic.

Ironically, the development of immunohistochemical methods for identifying ChAT-positive cells confirmed the doubts regarding the cholinergic nature of the FTG cells. Some of these cells were initially found to have a limited degree of ChAT positivity (Satoh et al., 1983), but the ChAT immunohistochemical methods have failed to reveal that the large FTG cells of the reticular formation are cholinergic, although there are numerous medium-sized ChAT-positive cells in the caudal pontine FTL and in the medullary FTG, as well as the magnocellular tegmental field (FTM) and the FTL (Jones & Beaudet, 1987a, 1987b; Sakai et al., 1986; Satoh et al., 1983). In fact, pontine FTG cells which fire during PS have been found not to be associated with the regions in the pons where cells stain positively for ChAT (Shiromani et al., 1987a).

Destruction of the FTG cells also contradicted the proposal that these cells were the executors of PS. Kainic

acid, an excitatory neurotoxin which destroys nerve cell bodies but not fibers (Campochiaro & Coyle, 1978; Coyle & Schwarcz, 1983; Kohler & Schwarcz, 1983; Schwarcz, Scholz & Coyle, 1978) was injected into the FTG and destroyed the majority of cells in that area, yet had no effect on PS (Drucker-Colín & Bernal-Pedraza, 1983; Sastre, Sakai & Jouvet, 1981). In addition, radiofrequency lesion of the lateral, caudal pontine tegmentum eliminated PS, whereas destruction of the medial, caudal pontine tegmentum, which included the cells of the FTG, did not eliminate PS (Friedman & Jones, 1984a, 1984b). Unit recording studies also provided contradictory evidence to the claims of Hobson et al. (1974a; 1974b) because they showed that the cells of the FTG fire in relation to movement during PS as well in waking (Siegel & McGinty, 1977; Vertes, 1977). During PS, activity in these cells appears to be related to myoclonic twitches, which are controlled by the reticular formation (Vertes, 1984).

In spite of convincing evidence militating against the FTG neurons playing an executive role in the PS process, these cells may still perform some non-essential function in the state of PS, as has been noted in other sections of this thesis. Cells have been found in the FTG which fire tonically in PS, whether there is movement or not, but they do not fire during waking, also regardless of any movement (Vertes, 1979, 1984). These cells may also be related to hippo-

campal theta, a sign of PS, which appears to be controlled by neurons in the nucleus RPO (Vertes, 1980, 1981, 1984). However, this point was not addressed in the studies cited above in which these neurons were destroyed. Nonetheless, the results of cholinergic agonist stimulation of these cells on PS are probably not simply due to diffusion of the drug, for Drucker-Colín and Bernal-Pedraza (1983) were unable to obtain an effect on PS with carbachol injection in the FTG after the cells had been destroyed. Gnadt and Pegram (1986) also suggest that there is only minimal diffusion of the drug for short distances after carbachol injections.

A vast amount of work was devoted to the investigation of the role of the FTG in PS, and with the eventual negative findings disproving a key role in PS control, attention was re-focussed onto the rostral pons and caudal midbrain tegmental region, which the original pharmacological and lesion work had indicated contained structures that were important in PS. From the work of Koelle (1954) and Shute and Lewis (1967) it was also known that amongst the cells in and around the vicinity of the LC there were acetylcholinesterase-containing cells. With the development of the ChAT immunohistochemical methods, it has been shown that this area contains the largest population of cholinergic cells in the brainstem (Jones & Beaudet, 1987a, 1987b; Woolf & Butcher, 1986), and that such cells are intermingled

in this area with the noradrenergic ones (Jones & Beaudet, 1987a, 1987b).

Electrophysiological work had also shown that cells in the dorsolateral pontine tegmentum displayed activity in relation to several of the variables of PS. Neurons in the region of the parabrachial nucleus, the LC alpha and the X-area of Sakai (1985a, 1985b), within the PPT, fire in relation to PGO spikes. Sakai (1985a, 1985b) also suggests that neurons in the region of the LC (peri-LC alpha and LC-alpha) play a command role in the control of muscle atonia in PS. In addition, large electrolytic lesions (Jouvet & Mounier, 1960) and 6-OHDA lesions (Buguet et al., 1970) in this area lead to an elimination of PS, including cortical desynchronization of PS, a loss of atonia and of PGO spiking. Such lesions were aimed at the noradrenaline system, because it was believed to control PS. Even at that time however, it was evident to Jouvet (1972) that these lesions may have damaged the supposed cholinergic system described earlier by Shute and Lewis (1963). With the knowledge of the location of these pontomedullary cholinergic cells, their importance for the control of PS state can now be evaluated directly. The RPO-RPC area, originally considered to be important for the generation and control of PS, is responsive to stimulation by cholinergic agents, and electrophysiological recordings show that many of its cells fire tonically during PS. However, such cells are not cho-

linergic because they do not stain positively for ChAT (Shiromani, et al. 1987a). It appears that these RPO-RPC area cells may play a nonessential role in the generation and maintenance of paradoxical sleep, because this state is unaffected by their destruction. The selective destruction of the nerve cell bodies in the dorsolateral pontomesencephalic tegmental cholinergic cell area remains to be performed in order to determine directly the importance of these neurons in the generation and control of the PS state and its defining variables.

II. SUMMARY and PROPOSAL

Acetylcholine has long been known to influence the state and individual variables of waking by exerting its control through the ARAS. One source of this acetylcholine influence may be the cholinergic dorsolateral pontomesencephalic tegmental neurons thought to project to the basal forebrain, which in turn projects to the cortex. The other major targets of the dorsolateral pontomesencephalic tegmental field neurons are the thalamus and hypothalamus, which in turn project to the cortex as well. Other important targets of the cholinergic neurons of the dorsolateral pontomesencephalon that probably participate in wakefulness include structures in the midbrain, pons and medulla. In addition, the dorsolateral pontomesencephalic tegmentum may send a minor cholinergic projection to the prefrontal cortex. The cortex also produces an endogenous supply of acetylcholine which may aid in the maintenance or promotion of cortical activation, but which may not be enough to maintain cortical activation on its own. The cholinergic neurons of the dorsolateral pontomesencephalic tegmental area may also participate in mechanisms of waking through possible interaction with catecholamine neurons present in the region which may modulate wakefulness. Previous studies employing non-selective electrolytic lesions (Jones et al., 1969) or sub-selective 6-OHDA lesions (Buguet et al., 1970), which

caused a decrease in waking and its associated EEG desynchronization would have damaged both the acetylcholine and catecholamine cells. Therefore, in view of the fact that the cholinergic cells of the dorsolateral pontomesencephalic tegmentum comprise the major cholinergic group in the brainstem area, and because of their connections with other regions related to cortical activation and waking, it is possible that destruction of the majority of its neurons will result in some deficit of the state of waking and of some of its defining variables.

Acetylcholine is also known to play a pivotal role in the development, organization and expression of PS and of its component variables. The evidence reviewed shows that the systems involved in the control of PS are located mainly within the dorsolateral pontomesencephalic tegmental area, but that important sites or structures in the brainstem and forebrain may also exercise an influence over the expression of this state. It is upon these structures that acetylcholine may exert its effect in order to trigger and control PS. It is also possible that the monoamine transmitters, and possibly other agents, participate in a modulatory role in the expression of PS. With the development of immunohistochemical methods to stain the cholinergic and catecholaminergic cells selectively, it is seen that the cholinergic cells are intermingled with the noradrenergic ones (Jones & Beaudet, 1987a, 1987b), and because both transmitters may

influence sleep-waking states (Jouvet, 1972), one of the challenges in studying the influence of the neurons in this area on PS is to dissociate the effect of destroying the cholinergic neurons from that of the catecholaminergic ones. These neurons can now be approached directly by the lesion technique. The lesions will be made by injecting the neurotoxin kainic acid into the dorsolateral pontomesencephalic tegmentum. This neurotoxin has been reported to have minimal effect upon noradrenergic neurons in the LC of the rat (Kohler & Schwarcz, 1983), but to effectively destroy cholinergic neurons in the basal forebrain of this species (Stewart et al., 1984). Therefore, it may be possible to inject this drug into the dorsolateral pontomesencephalic tegmental area of the cat and cause only minimal damage to the catecholaminergic cells of the LC, but to effectively destroy the cholinergic cells.

The main purpose of this study is to investigate the importance of cholinergic neurons of the dorsolateral pontomesencephalic tegmentum in sleep-wakefulness states, including most notably waking and PS and various of their component variables. The study of the effect of destruction of cholinergic neurons in the dorsolateral pontomesencephalic tegmentum on the amplitude of the EEG and EMG and on the rate of PGO spikes during the different states is also of major importance, because neurons in the dorsolateral pontomesencephalic tegmentum are believed to be associated with

the expression of these variables (Jacobs & Jones, 1978; Jones, 1988; Jouvet, 1972; Sakai, 1980, 1985a, 1985b; Vertes, 1984).

One further aim of the study is to investigate the effect of the lesions on the innervation of some brainstem and forebrain areas known to be involved in vigilance states, and which may be innervated by the cholinergic cells of the dorsolateral pontine tegmentum. Such a study should provide information on the magnitude of innervation of such structures from the dorsolateral pontomesencephal, and possibly the nature and relative importance of such a structure or region for a particular aspect of the sleep-waking process.

III. METHODS

A. Animal Care and Preparation.

Thirteen fully conditioned, mongrel, male and female adult cats (2.4-4.4 kg) were aseptically implanted under sodium pentobarbital (Nembutal) anaesthesia (38 mg/kg, ip) with a standard set of electrodes for recording sleep-wakefulness states. Stainless steel screws were implanted over the occipital and parietal cortices for recording the EEG, over the olfactory bulbs for recording olfactory bulb spindles (OBS), and over the orbits for recording the electrooculogram (EOG). Flexible, stainless steel, stranded wire loops were inserted into the neck muscles for the electromyogram (EMG). For recording ponto-geniculo-occipital waves (PGO), a platinum-iridium, tripolar, teflon-coated, depth electrode was implanted stereotaxically into each lateral geniculate body, 6 mm rostral to the antero-posterior (AP) zero plane, 3 mm above the Horsley-Clarke zero plane, and 10 mm lateral to the midline. All leads coming from the cat's head were soldered to points in an Amphenol 24-point connector, which was then cemented to the cat's skull.

The cats were allowed to recover for at least two weeks, following electrode implantation, after which time they were housed permanently in a sound-attenuated recording chamber with food and water available ad libitum for the

duration of the experiment. The recording chamber was equipped with a coaxial (Microdot) cable connected to a cannular slip ring assembly (Airflyte Electronics Co.), which was balanced by a weighted boom. This cable set relayed the bioelectric signals from the connector on the cat's head to a polygraph recorder. The room in which the recordings were done was under controlled lighting (700 h to 1900 h) and temperature (22^{o}C). The cats were given a one-week adaptation period in the chamber, during which time the different gains and other settings on the recording equipment were adjusted in order to obtain the best quality recordings in terms of signal amplitude and ranges. The cats were recorded continuously for 22.5 hours per day during the adaptation period and for the duration of the experiment. The other 1.5 hours were used for calibrating the recording equipment, storing the data and cleaning and feeding the cats. The cat's rectal temperature was also taken during this time both before and after the experimental lesion. Each day, during the adaptation period the polygraphic records were scored to determine the percent of the day spent in PS. Adaptation to the recording situation was considered to be complete when the percentage of PS remained stable from day to day and above 10% of the total recording time. After the cats had adapted to the daily routine of the recording situation and the recording equipment settings had been adjusted, baseline recording was begun and continued

until three complete days of stable polygraphic and cassette tape recordings of digital data were obtained. The animals were then operated a second time for intracerebral injections of kainic acid. After the second operation polygraphic recording was performed continuously for 28 days. Both polygraphic paper records and cassette tape records of data were collected for every cat on postlesion days 7, 14, 21 and 28.

B. Kainic Acid Injections.

The cats were pretreated with atropine sulfate (.02 mg, ip) to control mucus secretion and 30 minutes later anaesthetized with Nembutal (sodium pentobarbital) (30 - 35 mg/kg, ip), after which they were positioned in a stereotaxic apparatus. Kainic acid was dissolved in a phosphate-buffered saline solution (4 mg/ml) and injected with a Hamilton 10-microliter syringe affixed to a micromanipulator. The needle was lowered stereotaxically at a 45 degree angle from the vertical plane, through the cerebellum to the pontomesencephalic tegmentum on each side. Six descents were made so as to inject a total of 4.8 ug of kainic acid into 8 different points at a rate of 0.16 µg/minutes. The injections were aimed at the following stereotaxic coordinates, determined by reference to Berman's atlas (Berman, 1968): 1a) A0.5, L2.0, V-2.5 (0.4 µg kainic acid), 1b) P0.5, L2.0, V-1.5 (0.4 µg kainic acid), 2) P1.0,

L2.0, V-2.5 (0.8 µg kainic acid), 3) P2.0, L2.0, V-3.0 (0.8 µg kainic acid), 4a) A0.5, L3.5, V-3.0 (0.8 µg kainic acid), 4b) P0.5, L3.5, V-2.0 (0.4 µg kainic acid), 5) P1.0, L3.5, V-3.0 (0.8 µg kainic acid), 6) P1.0, L1.0, V-2.0, (0.4 µg kainic acid). After each injection the syringe was left in place at each injection point for five minutes before being removed. Body temperature, heart rate and respiratory rate were monitored during the course of the injections, and after each injection these physiological functions were allowed to stabilize prior to the subsequent injection. The full series of 8 injections were performed first on one side and then repeated on the other side. At the end of the injections the burr hole in the skull was covered with gel foam and sealed with dental cement. The cat's head was washed with Bacitracin-saline (1000 units/ml), an antibiotic solution, and the skin was sutured. The cat was then injected subcutaneously with 40 mls of a 5% dextrose solution (Dextran) to replenish lost fluid, and placed in its cage to recover.

C. Postlesion Care.

Following the kainic acid injections the cats were nursed until they recovered. They were given daily subcutaneous 5% dextrose injections, until they could be given liquid or food by mouth. They were given oral doses or intramuscular injections of the antibiotic tetracycline

(dose 50-250 mg) and their rectal temperature was taken daily until their temperature stabilized. Two cats, KA7 and KA10 developed mild seizure activity after the lesion, so that in addition to the normal care given the other cats, these two cats were given diazepam (oral or i.m., 0.5 - 1.0 mg) on the days they showed epileptic activity in the EEG or if they had seizures. The data from these two cats were not included in the statistical analyses. After recovering the ability to chew and swallow, the cats were given water and regular canned food daily by hand until they were able to feed themselves. The bladder and bowels were voided by massage on a daily basis when urination and defecation did not occur spontaneously. Twice weekly a neurological test was performed to assess tactile, visual, auditory, olfactory and proprioceptive function, as well as sensorimotor integration and nociceptive responses. At this time a record was also made of affective responses such as pupillary dilation, hissing, playing, grooming, etc. At least once a week, behavioural observation was made of all of the cats before and after the injections. Formal behavioural observation was performed on six of the 13 cats that were recorded.

D. Collection, Analysis, Quantification and Representation of Signal Features.

The EEG, EMG, EOG, OBS and PGO activities were filtered, amplified and recorded on a Grass Model 78D polygraph. Filtering for these measurements were as follows: EEG, 1.2-80 Hz.; EMG and PGO waves, 12-80 Hz.; EOG, 0.1-80 Hz.; and OBS, 40-80 Hz. The OBS signal was further filtered by microprocessor before being recorded on the polygraph. Sixty Hz activity was filtered from all channels and the paper speed was 5 mm/sec.

The amplified and filtered signals from the polygraph were passed to a Buxco Data Logging System for quantification (Buxco Electronics Co.). This system was composed of three units: a signal conditioning unit, a data logger and a Texas Instrument 733 ASR computer terminal equipped with a cassette recorder. The average amplitude of the EEG, EMG, EOG and OBS, as well as the rate of the PGO waves were recorded. For the former, the signals (amplitude) were conditioned by being filtered, full-wave rectified and smoothed. The filtering parameters at this stage were: EEG, 0.4-100 Hz.; EMG, 5-350 Hz.; EOG 0.2-3.6 Hz and OBS bandpass filtered was centered at 40 Hz. The detection of PGO waves was made by passing the positive part of the wave from the LGB recording through adjustable time and amplitude windows. The time window was usually set between 30-75 msec. The

amplitude window also varied from cat to cat. The threshold was set high enough to avoid detection of background activity, including most eye movement potentials during waking. The high-amplitude cutoff was set high enough to detect PGO spikes during PS, but low enough to avoid detecting movement artifacts during waking, which are usually of higher amplitude than PGO spikes (Jones, 1971; Webster, 1985).

The data logger converted the data from analog to digital form (10 Hz. sampling rate), averaged the digital values for each signal for each minute, and transferred the averaged data in ASCII character form to the computer unit where it could be printed as digits on the computer terminal and also stored on a cassette tape. The gain settings on the data logger were set to obtain a reasonable range of 0-70 or 0-80 relative amplitude units for the averaged data (EEG, EMG, EOG, and OBS). For the PGO wave channel, the gain was set so that when six calibration spikes were entered from the polygraph, six units would be generated by the data logger in the PGO channel. A 50 μv or 0.5 mv signal was used to calibrate the PGO channel. The half-amplitude high-frequency level of the calibration channel was set at 0.1 kHz. The corresponding rise time constant was 3 msec. The EEG, EMG, EOG, and OBS channels were calibrated with a 100 μv sine wave pulse for conversion of relative amplitude units to microvolts. The quantitative data on the cassette

were synchronized with the polygraphic data by having the data logger send a series of pulses to the polygraph signal marker at the beginning of each minute. At the end of each recording day the 1350 one-minute epochs of data were transferred to a PDP 11/60 computer (Digital Equipment Corporation).

1. Polygraphic Analysis.

Polygraphic records were analyzed according to traditional classification of the states of waking, stages I and II slow wave sleep, and PS. By using all five electrographic variables when scoring the records, three states were classified according to rules developed by Jones (Jones, 1971; Webster, 1985). The two states of slow wave sleep were combined as one, for encoding and presentation of the results. Each minute of one baseline day and of postlesion days 7, 14, 21, and 28 was analyzed and classified for each cat.

For quantitative analysis and graphic display each of the day's 1350 epochs, according to its classification as one of the three states or as artifact, was entered by hand into a computer file. The total number of minutes of each state was then calculated, as were the mean amplitudes of the EEG and EMG variables and the mean PGO rate for each day analyzed. The data were then symbol-coded to denote the three different states. At this point, timeplot graphs up to 10 hours long were made by computer to display the relative

values for the three states and the five variables. In addition, the total number of minutes could be displayed on a trivariate graph according to procedures described in detail by Friedman (1983) and Friedman and Jones (1984a). Briefly, each one-minute epoch was represented as a point in three-dimensional space, according to its value on the three variables: EEG amplitude, EMG amplitude and PGO spike rate. The position of the points relative to each other could be appreciated in three dimensions by rotating the graph either to the right or left and projecting it with perspective onto a two-dimensional plane using the method of Hall (1979).

In order to test the reliability of the scoring procedure the polygraph records for 6 of the cats (KA11, KA12, KA14, KA15, KA17 and KA18) on baseline day and postlesion day 21 were scored by a second experienced scorer (LP) and the results were correlated with those of the author on the same cats. The results showed a correlation of 94.4% (SD 1.6) for baseline day, and 90.3% (SD 1.2) on postlesion day 21. These correlations are comparable to those normally found between two scorers as well as between computer-scored and human-scored data (Friedman, 1983; Friedman & Jones, 1984a, 1984b).

For statistical evaluation the amount of each state was calculated as a percentage of the total number of epochs recorded daily (approximately 1350). Separate single-factor repeated measures design ANOVAs were used to analyze the

changes in the percent states of waking, slow wave sleep and PS on postlesion days 7, 14, 21 and 28 relative to baseline day. Separate, two-factor ANOVAs (states and baseline and postlesion day) were used to evaluate the change in number and duration of episodes of the three different states on postlesion day 21 relative to baseline day. Separate, two-factor, repeated measures design ANOVAs were also used to evaluate the effect of the lesion on the amplitude of the EEG and EMG and on PGO spike rate during the three states on postlesion days 7, 14, 21 and 28 relative to baseline. Newman-Keuls post-hoc tests were conducted on all significant main effects and interactions.

E. Perfusion and Tissue Processing.

The 13 lesioned cats, and 6 without lesion or implant, but of comparable size and sex, whose brains were to be processed as controls, were sacrificed under deep barbiturate anaesthesia (50mg/kg, ip) by intracardial perfusion of a Sorensen buffer rinse for 10-15 seconds, followed by 1000 mls of a fixative solution, containing 4% paraformaldehyde, 0.1% glutaraldehyde and 0.2% picric acid in a 0.1 M Sorensen buffer solution. The flow rate of the solution was controlled by a Varistaltic pump (Fisher Scientific) at 150 ml/minutes. The brains were removed from the skull and postfixed with the same fixative solution for one hour at room temperature (22^0C). The brains were then dissected into 4 blocks (anterior forebrain, posterior forebrain, upper brainstem and lower brainstem) which were left in a Sorensen

buffer with 30% sucrose solution for two to three days in a refrigerator (4^0C). The brains were then frozen in Freon at -50^0C and stored at -80^0C in a deep freezer until they were cut. The brains were sectioned in the coronal plane on a freezing microtome at 20 µm thickness and 5 sections were collected at every 10th section interval. One series of sections was stained for Nissl substance with thionin in order to assess the effect of the kainic acid injections on cells in general in the brainstem and forebrain. A second and third series of sections were processed by PAP (peroxidase antiperoxidase) immunohistochemistry to enable the identification of ChAT-positive and TH-positive cells in the pontomesencephalic tegmental region. Of the remaining two series of sections one was processed for ChAT by radio-immunohistochemistry, and the other for AChE by the copper thiocholine histochemical technique (Koelle, 1954). The latter two series were processed in order to study the cholinergic innervation of the forebrain and brainstem.

ChAT immunohistochemical staining was performed according to a PAP method (Sternberger, 1979). The sections were rinsed in a 0.1 M Tris-saline solution containing Lysine (1.8 gr. Lysine/100ml. Tris-saline) for 30 minutes, incubated for 30 minutes in normal rabbit serum (NRS) (1:30, NRS:Tris-saline), rinsed again in Tris-saline and incubated overnight at room temperature (22^0C) in an anti-ChAT monoclonal antibody from rat-mouse hybridoma (Boehringer,

Mannheim) reconstituted in deionized water. The sections were rinsed again in Tris-saline containing 1% NRS and incubated with rabbit anti-rat immunoglobulin serum (Miles) diluted with Tris-saline-1% NRS for 30 minutes. The sections were rinsed again in Tris-saline-1% NRS and incubated with rat PAP (Sternberger-Meyer) diluted in a Tris-saline-1% NRS solution. For the double bridge procedure (Vacca et al., 1975) the sections were rinsed in Tris-saline-1% NRS and incubated with rabbit anti-rat immunoglobulin serum for 20 minutes, rinsed in Tris-saline-1% NRS again and incubated once more in rat PAP for 20 minutes. For the revelation procedure, the sections were exposed to cobalt chloride (0.5% $CoCl_2$) in Tris-saline for intensification of the peroxidase reaction product (Adams, 1981), incubated in 0.05% diamino-benzedine (DAB) in Tris-water with 0.01% hydrogen peroxide (H_2O_2) for 6 minutes. The sections were rinsed in phosphate buffer and mounted on 2% gelatinized slides out of cold water. The mounted sections were dehydrated through alcohols of different concentrations (85%, 90% and 100%) for ten minutes each, cleared in xylene and coverslipped with permount.

Sections from control and lesioned animals were processed together as pairs in the same experiment for ChAT radio-immunohistochemistry. Experimental sections were incubated overnight in the same primary antibody as that used for the ChAT immunohistochemistry (anti-ChAT monoclonal

antibody from rat-mouse hybridoma). Subsequently they were incubated for 30 minutes with sheep anti-rat (Amershan) iodinated (I^{125}) antibody diluted at a 1:10 ratio in a Tris-saline:1% normal goat serum (NGS) solution. The sections were then rinsed in Tris-saline, placed in buffer and mounted onto gelatinized slides under cold tap water and left to dry. Subsequently the slides were dehydrated through alcohols and cleared in xylene. Some series of slides for paired control and lesioned animals were applied to LKB ultrofilm and kept for 10 days in a refrigerator at 4^0C. Other series of sections were coated with NTB-2 emulsion and maintained in light-proof boxes in a refrigerator at 4^0C for 6 weeks. Following the development of the film with Kodak GBX developer and development of the emulsion with Kodak D19 developer, the sections were lightly stained with thionin and rinsed in alcohols and xylene. In order to test the specificity of the antibody some sections were processed with the primary antibody omitted from the process.

Immunohistochemistry of the TH sections was conducted according to a similar procedure as for ChAT, except for the double bridge procedure. In this case the TH antibody from rabbit (Eugene Tech International) was diluted 1:1000 in a Tris-saline solution which contained 1% NGS. Goat anti-rabbit immunoglobulin serum (Miles) and rabbit PAP (Dimension Laboratories) were subsequently employed.

AChE staining was performed according to the method described originally by Koelle (1954) and adapted by Geneser-Jensen and Blackstad (1971). Sections were collected in a phosphate buffer solution and then rinsed three times in a cold 25% aqueous sodium sulphate solution. The sections were incubated for 75 minutes at 37^{o}C in a medium made up of 4 mM of acetylthiocholine (AThCh, Sigma), 2 mM copper sulphate, 10 mM of glycine, 50 mM acetate buffer at pH 5 and 0.2 mM ethopropazine (Sigma), a selective nonspecific cholinesterase inhibitor. After incubation the sections were rinsed in 7 changes of distilled water and then treated in a solution of 1.25% sodium sulphide in 0.1 N hydrochloric acid, at pH 7.8 for 1 minute. The sections were rinsed again in 7 changes of distilled water and then transferred to a 1% silver nitrate solution for one minute, after which they were returned to a distilled water bath from which they were mounted onto gelatinized slides and dehydrated in alcohols up to 95%, cleared in xylene and coverslipped with permount.

F. Quantification of Histological Data

The extent of kainic acid damage was judged by evidence of obvious cell loss or gliosis in Nissl-stained material. The outline of the area of nerve cell loss was drawn from thionin-stained sections with the aid of a drawing tube attached to a Wild Leitz stereo microscope from sections taken at 1 mm intervals at approximate stereotaxic

levels A3, A2, A1, AP0, P1, P2 ,P3, P4 and P5 (Berman, 1968). The extent of the lesion was determined at each level from the outline of the area of nerve cell loss with a Bioquant Digitizing Morphometry program operated with an Apple IIe computer.

The volume of gliosis in the area normally occupied by ChAT-positive cells was outlined on drawings made from thionin-stained sections. Measurement of these outlines were performed in the same way and at the same levels as was done for the total area of gliosis described above.

The number of ChAT-positive and TH-positive cells was counted on sections at 1 mm intervals [(A3, A2, A1, AP0, P1, P2, P3, P4 and P5 (Berman, 1968)] which corresponded to adjacent thionin-stained sections used in the analysis of gliosis. Preliminary studies in which cells had been counted in sections 20 µm apart showed that counting cells in sections 1 mm apart provided a reliable estimate of the total number of cells. Cells were counted in the PPT, LDT, parabrachial and LC on each side. The cells were counted under a light microscope equipped with camera lucida. The length (long axis) of the ChAT-positive and TH-positive cells in these nuclei was measured by means of the Bioquant Digitizing Morphometry program. The number of cells in each nucleus, at each level, and on each side was calculated using the Abercrombie correction factor (1946).

In order to test the reliability of the quantification procedures, all the above measurements were made by another investigator (BJ or LP) besides the author on 6 of the lesioned cats and on the control cats. The results of the count of ChAT-positive cells correlated highly (r=.99) with those of the author.

Separate one-factor ANOVAs were conducted in order to compare the difference between left and right sides in total volume of gliosis, volume of ChAT-positive cell area invaded by gliosis, the number of ChAT-positive and TH-positive cells, and the length of ChAT-positive and TH-positive cells. ChAT-positive cells from 6 control animals were counted and measured, and TH-positive cells were counted and measured in 3 control animals. Since no difference was found between left and right sides in these measurements, both sides were added together and were used in separate, two-factor ANOVAs to estimate the effect of the lesion on total volume of tissue damaged, the amount of gliosis in the area normally occupied by ChAT-positive cells, the numbers of ChAT-positive and TH-positive cells and the length of ChAT-positive and TH-positive cells. These measurements and counts were made in the sections according to the nuclear divisions (PPT, LDT, parabrachial and LC). In addition, ChAT and TH-positive cell numbers were also measured according to division by the levels A1 to P5.

Post-hoc Newman-Keuls tests were conducted on all the significant main effects and interactions.

Sections stained for AChE and those processed for ChAT by immunoautoradiography were examined by light microscopy for the intensity of staining on left and right sides in structures throughout the forebrain, midbrain and hindbrain. The AChE-stained sections from lesioned animals were then compared simultaneously with those of matched controls by a comparison tube connecting two Wild Dialux 20 microscopes. The level of staining in each nucleus in each section from the lesioned animal was assessed visually and compared with the level of staining in the same nucleus in the control section. The results from these analyses corresponded closely with results from densitometric measures of the same material made by another investigator (BJ). Representative examples from each region were photographed with a WILD MPS 51 camera mounted on a WILD M7S - Stereomicroscope or with a Wild Vario-Orthomat camera on a Leitz Orthoplan microscope.

IV. RESULTS

A. Total Area of Gliosis.

The area of gliosis extended over 8mm from A3 rostrally, to P4 caudally in the cat (KA18) with the most extensive lesion (Figs. 1, 2, 3; Table 1, and Appendix A:Table A1). In most cats, the greatest degree of gliosis was evident between A1 and P3, and the area surrounding and ventromedial to the brachium conjunctivum was maximally involved (Table 1, and Appendix A:Table A1). The lesions were ellipsoid in shape, with the long axis extending from the dorsomedial periaqueductal gray region to the ventrolateral tegmentum (Fig. 3). The lesions were roughly symmetrical in most cats (Fig. 3) and the total volume of the area of gliosis was similar on the two sides (Table 1; Appendix A:Table A1). In most cases, the rostral part of the lesion (A3 and A2) was within the central tegmental field, bordered ventromedially by the red nucleus and ventrolaterally by the retrorubral nucleus or the medial lemniscus. In some cats (KA5, KA6, KA12, KA14 and KA15) the damaged area did not extend as far forward as the A3-A2 level, but occupied a small region of the central tegmental field. Generally, the periaqueductal gray formed the dorsomedial border of the lesions at the A1-AP0 level, however, in four cats the gliosis extended into this area

(KA4, KA5, KA6 and KA15). At the P1-P2 level there was evident gliosis in most cats in the periaqueductal gray. From A1 to P2 the area of gliosis extended into the tegmentum medial to the decussation of the brachium conjunctivum or to the predorsal fasciculus on both sides. Ventrally it extended into the central and paralemniscal tegmental fields just below the brachium, or to the dorsal periphery of the retrorubral nucleus or the rubrospinal tract. The lateral border of the lesion in most cats was formed by the lateral lemniscus (AP0-P2), but in KA5, KA6, KA14 and KA17 the lesion did not extend out as far laterally, though it remained within the outer edges of the central tegmental field. Caudally, at the P3-P5 level, the lesion extended dorsally up to the 4th ventricle and medially, to the medial longitudinal fasciculus. Ventrally, at the P3 level, it descended in most cases midway into the paralemniscal tegmental field, the FTL and the FTG. In other cats, it did not extend much below the level of the medial longitudinal fasciculus and the motor trigeminal nucleus in the dorsal region of the FTG. Laterally, the lesion went midway into the parabrachial nucleus where its border was formed at P3 by the brachium conjunctivum and by the medial parts of the mesencephalic and motor trigeminal nuclei. In most cats the lesions extended back into the periventricular gray and parabrachial nucleus at the P5 level, but in four of the cats (KA6, KA14, KA15 and KA18), the lesion did not

extend so far caudally, but dissipated somewhere between this level and level P4.

B. Cholinergic Cell Area Gliosis.

In the sections stained for Nissl substance, the area normally occupied by ChAT-positive cells consistently showed gliosis and loss of nerve cells in the entire group of cats (Figs. 1, 2, 3; Table 2; Appendix A:Table A2). In general, the gliosis and cell loss located within this region extended from A2 to P4, although in one case (KA18) the gliosis was evident at level A3, and in several of the cats it extended as far caudal as P5 (Appendix A:Table A2). In the group of cats as a whole the area of gliosis was similar on both sides (Table 2). In the rostralmost part of the lesion (A1-P2), the area of gliosis and cell loss sometimes surrounded the brachium conjunctivum to include most of the PPT nucleus, however, in nearly every case, virtually all of the gliotic region was ventral and medial to this structure, with occasional patches of cell-denuded areas visible lateral and dorsal to the brachium conjunctivum. In a few of the cats, at the P1-P2 level, the laterodorsal tegmental nucleus (LDT) exibited gliosis and cell loss in the entire region (Figs. 1, 2 and 3). At the P3-P4 level, which corresponds to the caudal part of the cholinergic cell area, the medial part of the parabrachial nucleus was invaded by gliosis, which ascended dorsally to

encompass almost the entire area of the LC, where partial cell loss was evident.

C. ChAT-Positive Cells.

The number of ChAT-positive cells was significantly reduced (by about 60%) in the cats with kainic acid lesions as could be seen from examination of the sections processed for ChAT immunoreactivity (Figs. 1 and 2; Tables 3 and 4; Appendix B:Tables B1 and B2). The loss ranged from 25% in KA12 to 85% in KA15. When examined according to nuclei, the greatest decrease in ChAT+ cell number (approximately 75%) occurred in the PPT nucleus. The PPT nucleus extends from levels A1 to P2, where the greatest number of cholinergic cells are located in normal animals (Figs. 1, 2, 3; Table 3; Appendix B:Tables B1, B2) and where the greatest number was destroyed by the injections (A0.5 - P1). However, in the PPT nucleus there was a wide range in the decrease across the group, varying from 28% (K12) to 95% (KA18). In the LDT, there was a moderate (45%) mean loss of cells which was statistically significant (Table 3). The decrease ranged from as high as 92% (KA15) to no detectable loss (KA6 and KA19). The LDT extends from levels AP0 to P2, and like the PPT nucleus, contains many cholinergic cells. In the parabrachial nucleus there was a mean decrease (32%). The range of decrease in this nucleus was as high as 68% (KA19) to no detectable loss in several of the cats (KA4,

KA5, KA8, KA11 and KA12). A large proportion (73%) of ChAT-positive cells in the LC were destroyed, with a range from 54% (KA19) to 89% (KA15). When ChAT-positive cell length was compared between the lesioned and control animals, there was essentially no difference in the length between the two groups (Table 5).

D. TH-Positive Cells.

In the nuclei of the dorsolateral pontomesencephalic tegmentum, there was a small but significant decrease (35%) in the total number of TH-positive cells (Figs. 1 and 2; Tables 6 and 7; Appendix B:Tables D3 and D4), ranging from approximately 7% (KA19) to 60% (KA7). The largest mean number of cells was lost in the parabrachial nucleus (approximately 29% of the control level in this nucleus) which ranged from no change (KA8) to a decrease of approximately 59% (KA12) (Table 2). Although the decrease occurred in all nuclei and at all levels of the dorsolateral pontomesencephalic tegmentum, the most significant decrease occurred at the caudal levels of the region from P2 to P4 (Table 7 and Appendix B:Tables B3 and B4). Similar to the ChAT-positive cells, there was no difference in TH-positive cell length between lesioned and control animals (Table 8).

Abbreviations Used in Figures

AMB nucleus ambiguus

AQ aqueduct of Sylvius

bc brachium conjunctivum

bic brachium of the inferior colliculus

bp brachium pontis

CB cerebellar cortex

CD dorsal cochlear nucleus

cll commissure of the lemniscus lateralis of Probst

CLR central linear raphe

CNF cuneiform nucleus

cp cerebral peduncle

crf central reticular fasciculus

CS central superior nucleus

CU cuneate nucleus

CX external cuneate nucleus

dbc decussation of the brachium conjunctivum

dic decussation of the inferior colliculus

DMV dorsal motor nucleus of the vagus

DR dorsal raphe nucleus

dsc dorsal spinocerebellar tract

dtx dorsal tegmental decussation

ea external arcuate fibers

FTC central tegmental field

FTG gigantocellular tegmental field

FTL lateral tegmental field

FTM magnocellular tegmental field

FTP paralemniscal tegmental field

7g genu of the 7th nerve

GR gracile nucleus

IC inferior colliculus

IN nucleus intercalatus

IOD dorsal accessory nucleus of the inferior olive

IOM medial accessory nucleus of the inferior olive

IOP principal nucleus of the inferior olive

IP interpeduncular nucleus

KF Kolliker-Fuse nucleus

LC locus coeruleus nucleus

LDT laterodorsal tegmental nucleus

ll lateral lemniscus

LLD dorsal nucleus of the lateral lemniscus

LLV ventral nucleus of the lateral lemniscus

LR lateral reticular nucleus

lvs lateral vestibulospinal tract (or fibers)

5m tract of the mesencephalic trigeminal nucleus

5M motor trigeminal nucleus

5ME mesencephalic trigeminal nucleus

MG medial geniculate

ml medial lemniscus

mlf medial longitudinal fasciculus

mp mammillary peduncle

mrs	medial reticulospinal tract
5mt	tract of the mesencephalic trigeminal nucleus
3n	3rd nerve
4n	4th nerve
4N	trochlear nucleus
5n	5th nerve
6N	abducens nucleus
6n	6th nerve
7n	7th nerve
7N	facial nucleus
8n	8th nerve
9n	9th nerve
12n	12th nerve
12N	hypoglossal nucleus
oc	olivocerebellar fibers
P	pyramidal tract
5P	principal sensory trigeminal nucleus
PAG	periaqueductal gray
PB	parabrachial nuclei
PBG	parabigeminal nucleus
pf	predorsal fasciculus
PG	pontine gray
PH	nucleus praepositus hypoglossi
pp	pes pedunculi
PPT	pedunculopontine tegmental nucleus
PR	paramedian reticular nucleus

R	red nucleus
rb	restiform body
rc	reticulocerebellar fibers
RM	raphe magnus nucleus
RO	raphe obscurus nucleus
RP	raphe pallidus nucleus
RPo	raphe pontis nucleus
RR	retrorubral nucleus
rs	rubrospinal tract
sad	stria acoustica dorsalis
SC	superior colliculus
SL	lateral nucleus of the solitary tract
SM	medial nucleus of the solitary tract
SNC	substantia nigra pars compacta
SNR	substantia nigra pars reticulata
5SM	alaminar spinal trigeminal nucleus, magnocellular division
SOL	lateral nucleus of the superior olive
SOM	medial nucleus of the superior olive
5SP	alaminar spinal trigeminal nucleus, parvocellular division
5st	spinal trigeminal tract
st	solitary tract
T	nucleus of the trapezoid body
tb	trapezoid body
TD	dorsal tegmental nucleus

TR tegmental reticular nucleus

TV ventral tegmental nucleus

uf uncinate fasciculus

V4 4th ventricle

VIN inferior vestibular nucleus

VLD lateral vestibular nucleus, dorsal division

VLV lateral vestibular nucleus, ventral division

vm vestibulomesencephalic fibers

VMN medial vestibular nucleus

VS superior vestibular nucleus

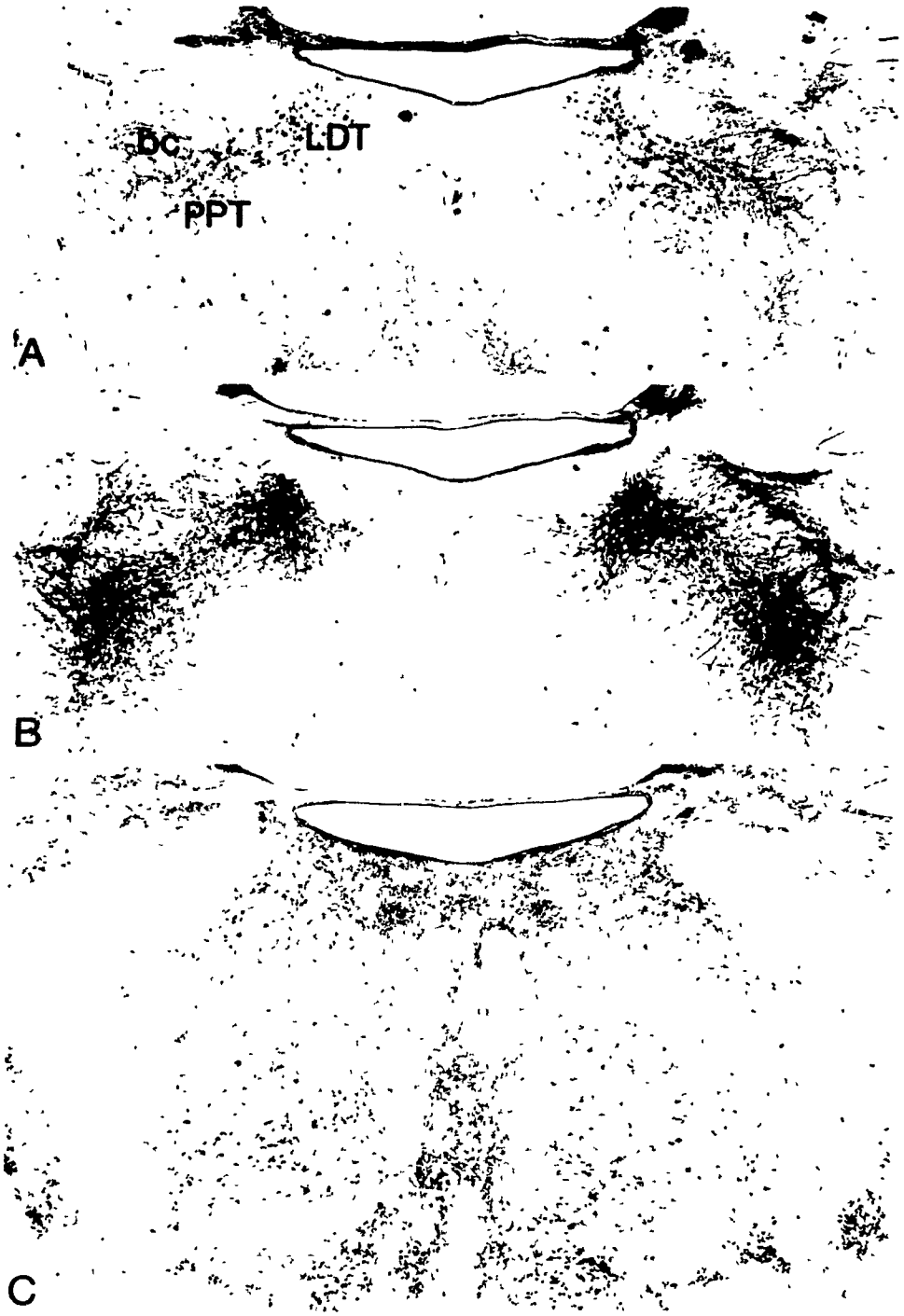
vsc ventral spinocerebellar tract

VTA ventral tegmental area

4x decussation of the 4th nerve

Figs. 1 and 2.

Photomicrographs of adjacent coronal sections through the brainstem of a normal cat (Fig.1) and through the brainstem of a cat (KA18) 28 days after kainic acid injection in the brainstem (Fig. 2). (Approximate level of the section: P2, Berman, 1968). The sections labelled "A" and "B" were processed for immunohistochemistry. Sections labelled "C" were stained for Nissl substance. Figure 1A shows that in the normal cat, ChAT-positive cells are concentrated in the tegmentum medial to the brachium conjunctivum (bc) within the pedunculopontine nucleus (PPT) and in the periaqueductal gray within the laterodorsal tegmental nucleus (LDT). Figure 2A shows that these cells have all but disappeared from these areas after the kainic acid injections in the brainstem. Figures 1B and 2B show that TH-positive cells are also distributed within the same region as the ChAT-positive ones, and that the kainic acid injection produced only a moderate decrease in their number in this area. Figure 1C shows that darkly stained medium-sized neurons corresponding to the ChAT-positive and TH-positive neurons are found intermingled with smaller and more lightly stained neurons in the same area. Figure 2C shows that after the lesion with kainic acid there is a decrease in the number of the medium-size, darkly-stained neurons, corresponding roughly to the number of lost ChAT-positive and TH-positive cells. Figure 2C also shows that there was a loss of the small lightly-stained cells in the region of ChAT-positive and TH-positive cells. Cells were also lost in the pontine tegmentum medial and ventral to the ChAT-positive and TH-positive cell area in the paralemniscal tegmental field, which had been invaded by necrosis. (See Abbreviations for all histological figures on pages 135-139).


DC
LDT
PPT
A
B
C

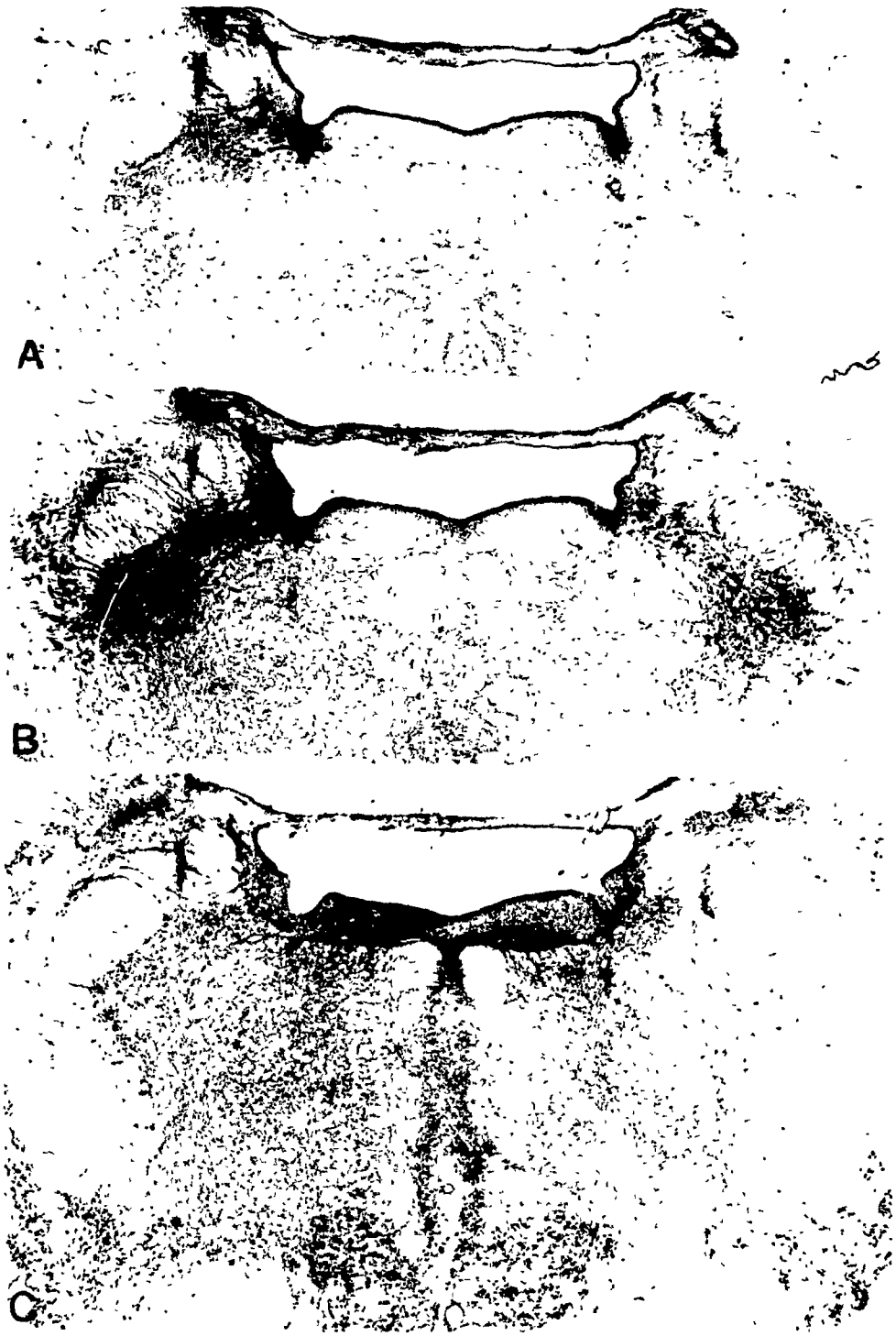

A
B
C

Fig.3.

Schematic drawings of the cat brainstem sections at approximately 1mm intervals showing ChAT+ (closed circles) and TH+ (open circles) neurons (adapted from Jones and Beaudet, 1987). Solid, thick lines encircle areas of gliosis and cell loss seen in Nissl-stained sections of one cat (KA18) 28 days after kainic acid injections in the brainstem tegmentum.

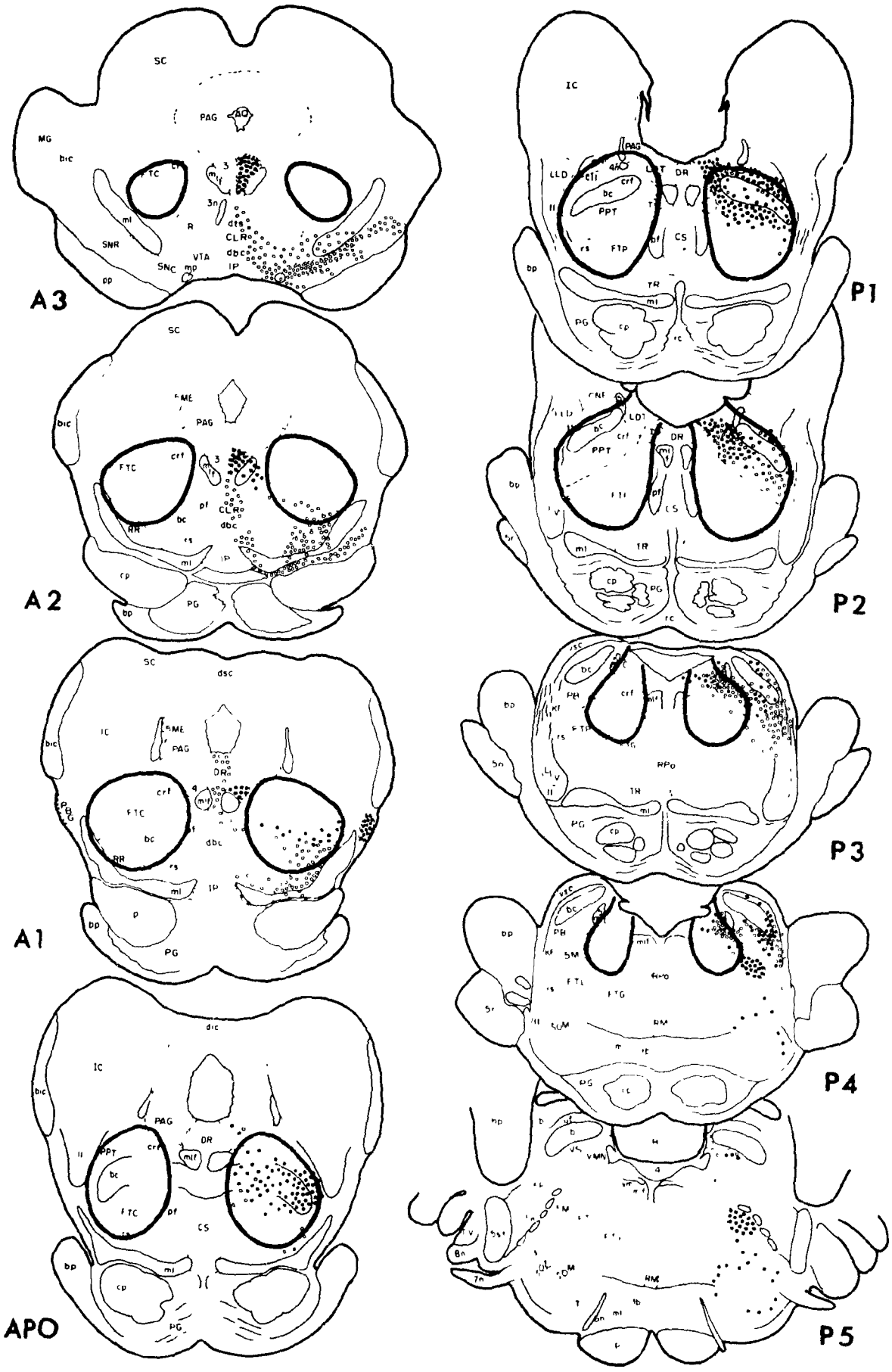

A3
A2
A1
APO
P1
P2
P3
P4
P5

Table 1

Volume of Necrosis Produced by Kainic Acid Injections in the Pontomesencephalic Tegmentum[1]

Level	Left	Right	Sum
A3	0.3	0.3	0.6 (2.1)
A2	3.6	2.3	5.9 (5.5)
A1	8.2	7.7	15.9 (10.3)
AP0	11.0	11.2	22.2 (5.5)
P1	13.0	13.2	26.2 (7.2)
P2	10.5	10.6	21.1 (6.6)
P3	8.0	8.4	16.4 (5.8)
P4	4.8	5.3	10.1 (4.6)
P5	1.7	1.5	3.2 (3.2)
Sum	61.1 (16.1)	60.5 (17.0)	121.6 (32.2)

[1]Mean volume (mm^3) (and standard deviation in brackets) of necrosis/gliosis in the pontomesencephalic tegmentum of 11 cats at stereotaxic levels A1 - P5 (Berman, 1968). In a two factor, repeated measures design analysis of variance (ANOVA), the difference in volume among the 9 levels was significant ($p<.001$). There was no difference between the two sides.

Table 2

Volume of Necrosis Produced by Kainic Acid Injections in the Cholinergic Cell Area of the Pontomesencephalic Tegmentum[1]

Level	Left	Right	Sum
A1	3.2	2.8	6.0 (3.4)
AP0	5.8	6.2	12.0 (2.4)
P1	6.7	7.0	13.7 (3.2)
P2	2.9	2.8	5.7 (2.4)
P3	1.4	1.3	2.7 (2.4)
P4	0.3	0.3	0.6 (0.6)
P5	0.0	0.1	0.1 (0.3)
Sum	20.3 (16.1)	20.5 (17.0)	40.8 (32.2)

[1]Mean volume (mm^3) and standard deviation of necrosis/gliosis within the ChAT-TH cell area of the pontomesencephalic tegmentum for 11 cats at stereotaxic levels A1 - P5 (Berman, 1968). There was a significant difference in volume among levels. There was no difference between the two sides. The approximate total volume of the ChAT-TH cell area was 100 mm^3.

TABLE 3

Mean Number of ChAT Immunoreactive Neurons in the Pontomesencephalic Tegmental Nuclei of Normal and Kainic Acid Injected Brains[1]

Nucleus	Normal		Kainic Acid[2]		%Normal
PPT	17133.6	(7371.7)	4277.4	(3473.8) **	24.9
LDT	8440.2	(2353.5)	4666.3	(2417.0) *	55.3
PB	6128.8	(1714.2)	4144.4	(3003.9)	67.6
LC	1563.4	(855.4)	424.1	(191.4)	27.1
TOTAL	33266.0	(8845.8)	13512.2	(5427.4) ***	40.6

[1] Mean (and standard deviation in brackets) number of ChAT-immunoreactive neurons in the four nuclei (PPT, LDT, PB, LC) of the pontomesencephalic tegmentum in 6 normal cats and 11 cats with kainic acid lesions.

[2] The number of pontomesencephalic ChAT-immunoreactive neurons in the four nuclei (PPT, LDT, PB, LC) were compared between normal and kainic acid injected cats by a two-way (within X between) analysis of variance (ANOVA). The number of ChAT+ neurons was significantly less in the kainic acid injected animals (***, $p<.001$). According to the Newman-Keuls test, the decrease in Chat+ neurons was most significant in the PPT (**, $p<.01$) and LDT (*, $p<.05$) nuclei.

Table 4

Number of ChAT-Immunoreactive Neurons
at Different Levels
in the Pontomesencephalic Tegmentum
of Normal and Kainic Acid Injected Animals[1]

Level	Normal		Kainic Acid		%Normal
A1	1308	(587)	740	(355)	57%
AP0	5228	(1981)	1981	(1459)*	38%
P1	11508	(2843)	3954	(2348)*	35%
P2	7531	(2337)	2268	(2222)*	30%
P3	3405	(1765)	2030	(2378)	60%
P4	3920	(1428)	2367	(2184)	60%
P5	367	(114)	171	(134)	47%
TOTAL	33267	(8846)	13511	(5427)***	41%

[1] Mean number and standard deviation of ChAT-immunoreactive neurons in 6 normal and 11 kainic acid injected cats within the pontomesencephalic tegmentum extending from stereotaxic level (in the coronal plane) A1 to P5 (Berman, 1968). In absence of any difference between the cell numbers on the left and right sides in the control or injected cats, the total number of neurons was employed for analysis. The total number was compared between the two groups and among the seven levels by a two-way analysis of variance (ANOVA) with repeated measures. There was a significant difference in the number of neurons between the two groups (***, $p<.001$) and among the seven levels ($p<.001$), and there was a significant interaction between the group and level factors ($p<.001$). Post-hoc Newman-Keuls multiple comparisons test indicated that the difference in the number of neurons between the two groups was significant at AP0, P1 and P2 (*, $p<.05$).

Table 5

Mean Length of ChAT-Immunoreactive Neurons in the Pontomesencephalic Tegmental Nuclei of Normal and Kainic Acid Injected Brains[1]

	Normal		Kainic Acid[2]	
PPT	20.6	(1.2)	19.4	(2.9)
LDT	18.5	(1.9)	20.3	(2.5)
PB	14.1	(1.7)	15.2	(2.6)
LC	17.5	(1.3)	16.5	(2.7)
Average	17.7	(1.5)	17.9	(2.7)

[1] Mean (and standard deviation in brackets) length (in um) of ChAT-immunoreactive neurons in four nuclei of the pontomesencephalic tegmentum in 6 normal cats and 11 cats with kainic acid lesions.

[2] The length of pontomesencephalic ChAT-immunoreactive neurons in the four nuclei (PPT, LDT, PB, LC) were compared between normal and kainic acid injected cats by a two-way (within X between) analysis of variance (ANOVA). The length of ChAT+ neurons was not significantly different between the normal and the kainic acid injected animals.

TABLE 6

Mean Number of TH Immunoreactive Neurons in the Pontomesencephalic Tegmental Nuclei in Normal and Kainic Acid Injected Brains[1]

Nucleus	Normal		Kainic Acid[2]		%Normal
PPT	5503.0	(1858.9)	3873.9	(1922.9)	70.4
LDT	2885.0	(1571.6)	1706.0	(1046.1)	59.1
PB	9886.6	(1621.3)	7015.5	(1878.3)*	71.0
LC	4301.3	(1353.7)	2438.7	(1029.4)	56.7
TOTAL	22575.9	(3155.0)	15034.1	(3195.7)*	65.1

[1] Mean (and standard deviation in brackets) number of TH-immunoreactive neurons in four nuclei (PPT, LDT, PB, LC) of the pontomesencephalic tegmentum in 3 normal cats and 11 cats with kainic acid lesions.

[2] The number of pontomesencephalic TH-immunoreactive neurons in the four nuclei (PPT, LDT, PB, LC) were compared between normal and kainic-acid injected cats by a two-way (within X between) analysis of variance (ANOVA). The number of TH+ neurons was significantly reduced in the kainic acid injected animals as compared to the controls. Newman-Keuls tests showed that the reduction in the PB nucleus was significantly reduced. (*, $p<.05$).

Table 7
at Various Levels

Number of TH-Immunoreactive Neurons
at Various Levels in the Pontomesencephalic Tegmentum
in Normal and Kainic Acid Injected Animals[1]

Level	Normal		Kainic Acid		%Normal
A1	146	(67)	236	(139)	161%
APO	554	(159)	250	(237)	45%
P1	2025	(555)	930	(619)	46%
P2	5663	(934)	3898	(1214)*	69%
P3	7903	(1184)	5764	(1115)*	73%
P4	4885	(1895)	2661	(1213)*	54%
P5	1400	(466)	956	(591)	68%
TOTAL	22576	(3155)	14696	(3196)**	65%

[1] Mean number (and standard deviation in brackets) of TH-immunoreactive neurons in 6 normal and 11 kainic acid injected cats within the pontomesencephalic tegmentum extending from stereotaxic level A1 to P5 (Berman, 1968). In absence of any difference between the cell numbers on the left and right sides in the control or injected cats, the total number of neurons was employed for analysis. The total number was compared between the two groups and among the seven levels by a two-way analysis of variance (ANOVA) with repeated measures. There was a significant difference in the number of neurons between the two groups (**, $p<.01$) and among the seven levels ($p<.001$), and there was a significant interaction between the group and level factors ($p<.01$). Post-hoc Newman-Keuls multiple comparisons test indicated that the difference in the number of neurons between the two groups was significant at P2, P3 and P4 (*, $p<.05$).

Table 8

Mean Length of TH-Immunoreactive Neurons in the Pontomesencephalic Tegmental Nuclei of Normal and Kainic Acid Injected Brains[1]

	Normal	Kainic Acid[2]
PPT	22.4 (1.2)	22.0 (1.2)
LDT	22.2 (0.6)	22.0 (1.1)
PB	21.7 (1.2)	22.4 (0.9)
LC	20.4 (0.4)	19.4 (1.8)
Average	21.7 (0.9)	21.5 (1.3)

[1] Mean (and standard deviation in brackets) length (in um) of TH-immunoreactive neurons in four nuclei of the pontomesencephalic tegmentum in 3 normal cats and 11 cats with kainic acid lesions.

[2] The length of pontomesencephalic TH-immunoreactive neurons in the four nuclei (PPT, LDT, PB, LC) was compared between normal and kainic acid injected cats by a two-way (within X between) analysis of variance (ANOVA). The length of TH+ neurons was not significantly different between the normal and the kainic acid injected animals.

E. Cholinergic Innervation.

Analysis of the sections processed by AChE staining and of the adjacent sections processed for ChAT immunoautoradiography revealed more staining in control than in experimental animals, and thus of cholinergic innervation in certain forebrain and brainstem areas, as a result of the kainic acid lesion in the pontomesencephalic tegmentum (Figs. 4, 5 and 6). In the normal animals the density of AChE staining in the forebrain and brainstem varied along a wide spectrum between different structures, from a light level in some, such as the ventral medial nucleus of the thalamus (Fig. 4), through to a moderate level of staining in others, such as the dorsal lateral geniculate (Fig. 5), up to a high intensity of staining in others, such as the lateral mediodorsal nucleus of the thalamus (Fig. 4). There was very little apparent difference in the range of staining intensity among control animals, or between corresponding structures on the right and left sides in these animals. ChAT immunoautoradiography also showed a range in density that paralleled the AChE staining.

In the lesioned animals the density of AChE staining also varied along a fairly wide spectrum between different structures, and little difference was seen between corresponding structures on the right and left sides. As compared to controls, however, a decrease in AChE staining was evident in certain structures in the lesioned animals, which were correlated with decreases in ChAT immunoautoradiographic density in the same areas (Figs. 4, 5 and 6).

Fig.4.

Photomicrograph of sections through the thalamus (approximate level: A9.5 Berman & Jones, 1985) of a normal cat (A5, at A and C) and of a cat injected with kainic acid (KA17, at B and D). A and B were processed for AChE and photographed under bright field illumination. C and D were processed for ChAT by radio immunohistochemistry and NTB-2 emulsion and photographed under dark field illumination. A comparable loss of AChE staining and ChAT labelling was found when the two sets of sections were compared in the nuclei identified in the sections and described in the text.

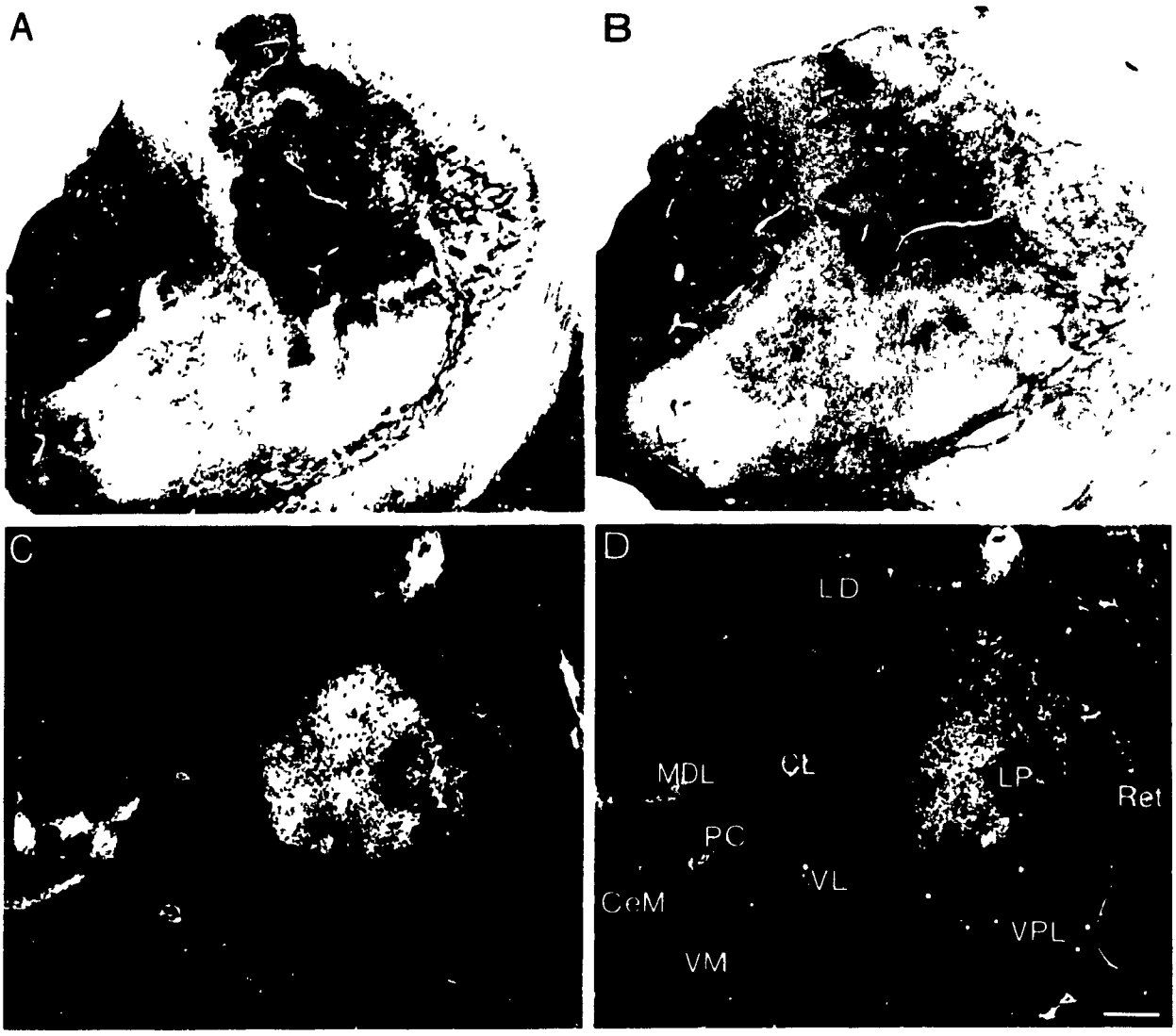

A
B
C
D
LD
MDL
CL
LP
Ret
PC
VL
CeM
VPL
VM

Fig.5.

Bright field illumination photomicrographs of sections through the dorsal lateral geniculate body of the thalamus in a normal cat (Ch 15, at A and C) and of a cat (KA19, at B and D) injected with kainic acid. A and B were processed for AChE staining; C and D were processed for ChAT by radio-immunohistochemistry using tritium-sensitive film. A comparable loss of AChE staining and of ChAT immunoautoradiographic density was evident in animals with the pontomesencephalic lesion, as is described in the text.

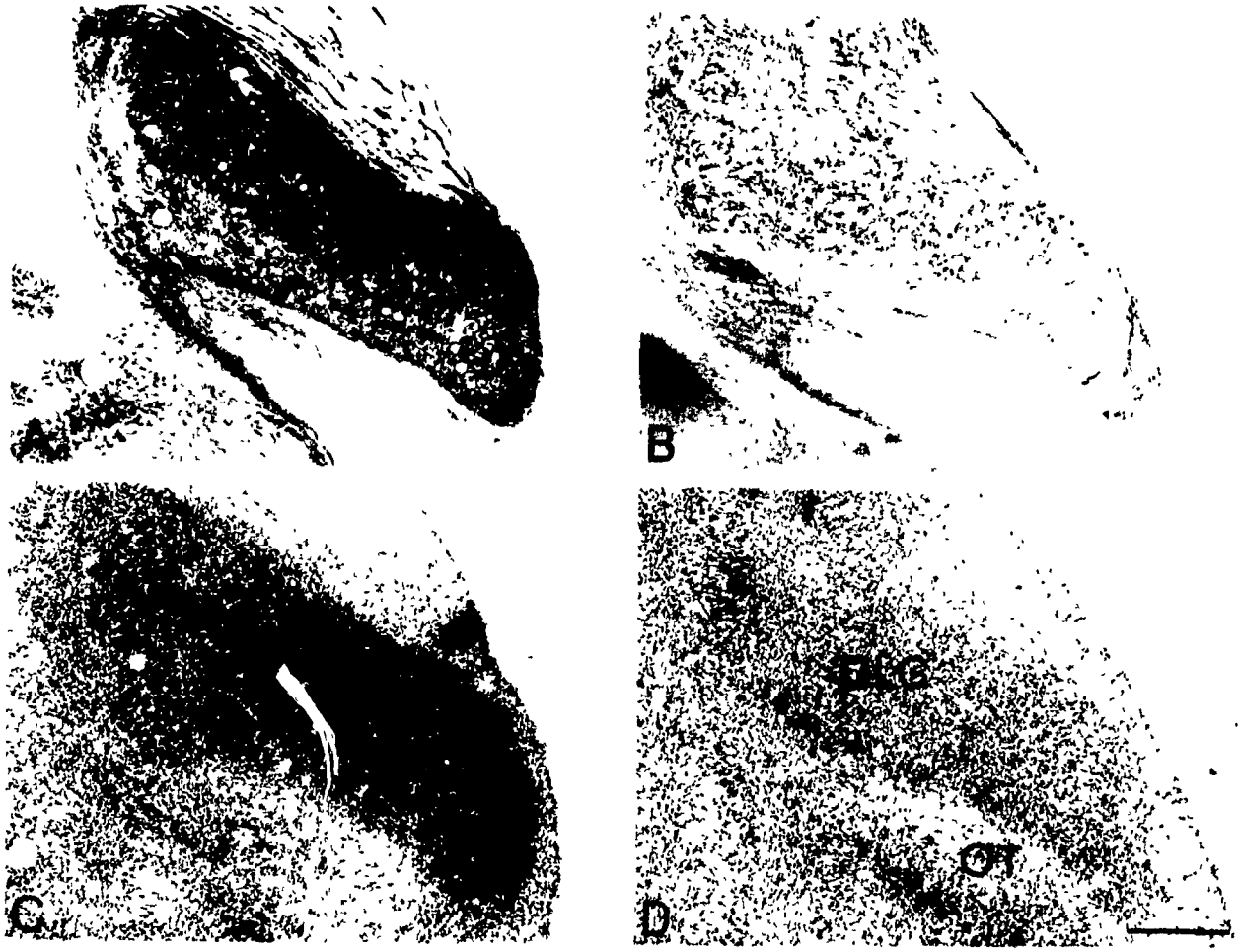
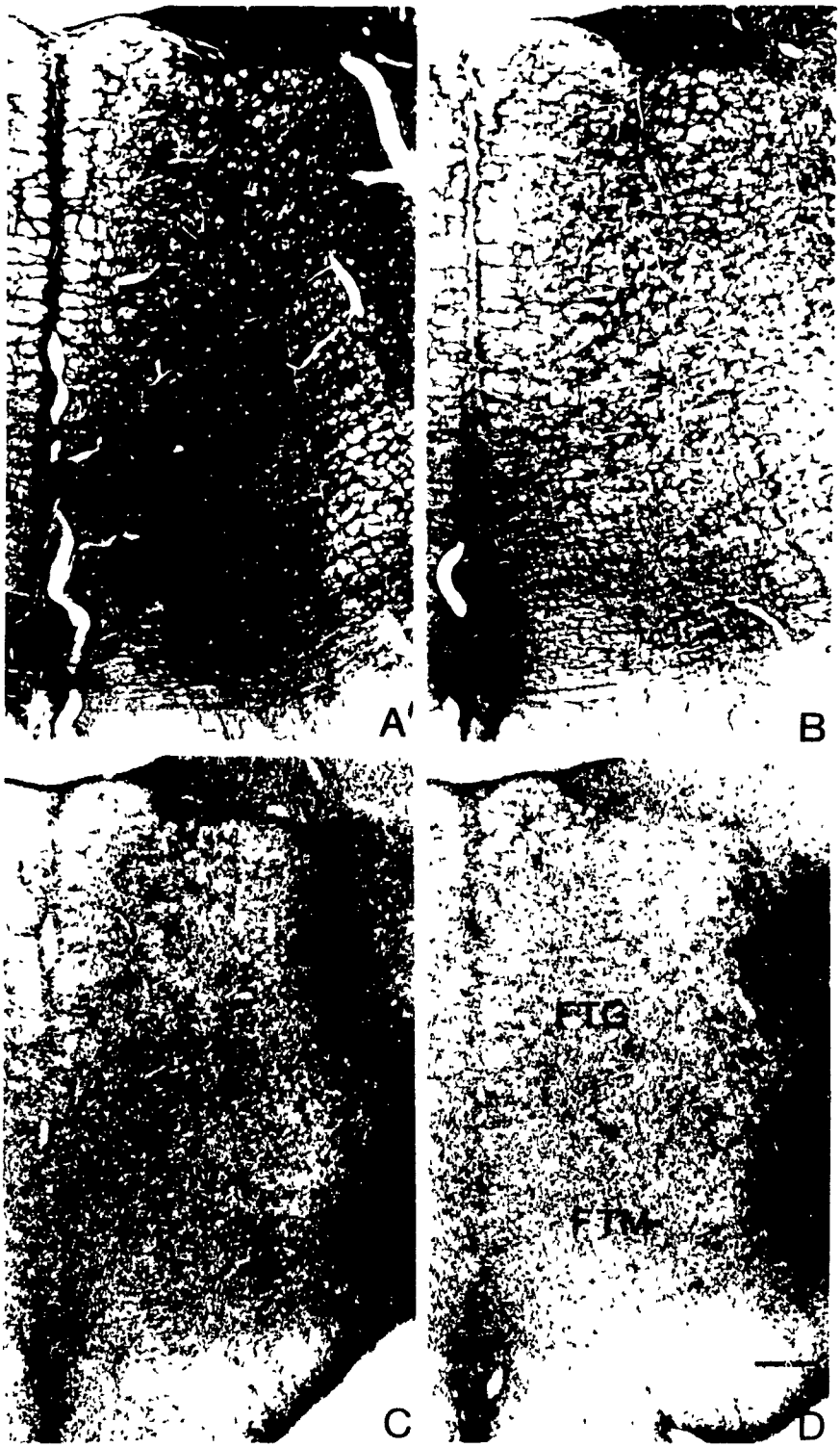

B
C
D
OT

Fig.6.

Bright-field illumination photomicrographs of sections through the reticular formation of the medulla at the caudal level of the facial nucleus (approximately P8.5, Berman, 1968) in a normal cat (A5, at A and C) and of a cat injected with kainic acid (KA18, at B and D). A and B were processed for AChE staining; C and D were processed for ChAT by radio-immunohistochemistry using tritium-sensitive film. A comparable loss of AChE staining and of ChAT immunoautoradiographic density was evident in animals with the pontomesencephalic lesion, as is described in the text.


A
B
C
FTG
FTM
D

Amongst the thalamic nuclei a small but consistent decrease in AChE staining intensity was seen in the centromedian, the parafascicular and the reticular nuclei. A clear though moderate drop in staining intensity was found in the mediodorsal, central medial, paracentral and central lateral nuclei. The most distinct loss of staining in the thalamic nuclei was seen in the lateral mediodorsal, lateral posterior, dorsal lateral geniculate and the pulvinar nuclei (Figs. 4 and 5). No decrease in staining could be discerned in other forebrain structures, including the hypothalamus, subthalamus and basal forebrain.

Within the brainstem, a moderate loss of AChE staining was evident within the intermediate and deep layers of the superior colliculus. A decrease in staining was also evident in the reticular formation, particularly in the FTG and FTM of the medulla (Fig. 6) where the loss was slight but consistent.

F. Clinical and Behavioural Observations

During surgery several physiological functions were altered dramatically as a result of the kainic acid injections. These included heart rate, respiratory rate, body temperature, and reflexes. During or following the first or second injection, heart rate began to decrease from a mean rate of 180 beats per minute (range 150-250) to about

110 beats per minute (range 96-130) by the third injection. Often during the subsequent injections, the heart rate would increase transiently over the preinjection rate for a minute or two, then would return to a lower rate. These bouts of tachycardia, when they occurred, came after the 4th or 5th injection on the first side or during the second or third injection on the second side. The EKG also showed certain irregularities during this period, often increasing greatly in amplitude after the 3rd or 4th injection. In these cases, the injections would be suspended until the heart rate and EKG stabilized. These bouts of irregular activity lasted anywhere from 2 minutes up to 25 minutes. One or two hours after the last injection the rate recovered almost to pre-injection levels (mean 170/minute, range 100-255).

There was a marked fluctuation in respiratory rate throughout the operation. Over the course of the operation before the kainic acid injections were begun, the average respiratory rate under anaesthesia was approximately 22 breaths per minute. There was no change until after the second or third injection when the rate usually increased to about 30 breaths per minute. Inspiration was shorter and shallower than during preinjection under anaesthesia. With subsequent injections, the respiratory rate decreased to about 26 breaths per minute. By the ninth or tenth injection, the rate increased once again to about 36 per

minute. By the end of the operation, the effect varied widely between cats, but in general, as the effect of the anaesthetic began to wear off, the mean respiratory rate was a little higher, but inspiration was shallower than in baseline under anaesthesia.

The effect of the kainic acid injections lowered the core body temperature in the cats. In some cases temperature decreased to as low as 30^{0}C. Temperature began to rise again once the injections were suspended and the effect of the kainic acid and anaesthetic began to wear off. However, by the end of the operation the temperature was always lower than the prelesion level. Simple reflexes such as the deep tendon reflex, pinna reflex and corneal reflex were absent at the beginning of the injections (because of the anaesthetic) and usually did not reappear until several hours after, or sometimes not until the following day. In any case, the return of such reflexes always took longer when the cat was injected with kainic acid than when anaesthesia was induced only for implanting the recording electrodes. Pupils were usually constricted prior to the injections, and after the first or second injection they began to dilate and did not become constricted again until several hours later, or the following day, after the injections.

G. Postlesion Care and Behavioural Observation.

Usually by the first day postlesion, the cats were breathing regularly (15-25 breaths/minute), though slightly below the mean resting rate for normal cats of 26/minute. In two cases (KA5 and KA15), breathing rate was extremely low, but steady (1 and 5 breaths per minute, respectively) but by postlesion day 2 the rate returned to normal. In three cases, breathing was shallow and halting, at a steady rate of 96/minute (KA14, KA18 and KA19) on postlesion day 1. By postlesion day 3, the breathing was within a normal range and smooth, however. The kainic acid injections also affected temperature control only slightly in the long term. On postlesion day 1, the mean temperature (35^0C, range $30-38^0$C) was lower than normal (38.6^0C, range $38.1-39.2^0$C) and over the following few days, it rose slightly above normal in some cats (mean 40^0C, range $38-41^0$C) but by the beginning of week 2 postlesion, it had returned to normal and remained within that range for the duration of the experiment.

On the morning following the operation, the cats were usually unconscious and remained in a prone position. In one case (KA5) the animal remained in this state until the second day postlesion. Usually, on the first postlesion day, reflexes such as the deep tendon, pinna, corneal and pupillary light reflex were still absent in the morning. These reflexes began to reappear again by the early evening

and by the following day, the cat was awake and fully responsive to the simple stimuli evoking these responses.

The cats temporarily lost the ability to feed themselves, to defecate or to urinate. The ability to eat independently returned late in the first or early in the second week. The ability to urinate spontaneously was also variable within the group. KA12, KA14 and KA19 urinated spontaneously during the first week. KA5 and KA11 recovered during the second week and KA8 recovered during the third week, but KA4, KA6, KA15, KA17 and KA18 never recovered the ability to urinate spontaneously. The ability to defecate spontaneously also returned during the first or early in the second week for most cats. There were exceptions, however. One cat (KA8) did not defecate spontaneously until the third week, and another (KA18) did not defecate spontaneously until the fourth week.

Sensory functions were also disrupted in most of the cats for a few days following the lesion. Most of these functions were at least partially recovered during the first week and were fully recovered by the second week (see Appendix C). For example, from early in the first week most of the cats were able to localize and actively avoid nociceptive stimuli, such as tail pinching and pressure on their paws. The response to nociceptive stimuli appeared to be slower than in normal cats. They were also able to perform some reflexive behaviours such as contact placing.

Other more subtle responses, such as that to light pressure, did not recover until the second and third week in some of the cats, and the response to displacement of the hair did not recover in four of the cats by the termination of the experiment. The cats were able to respond to visual, auditory, olfactory, and somatosensory stimuli from the first week. For example, they were able to follow a moving object visually, to locate the source of a sound such as a clap by the experimenter, to locate their food and to respond and localize a part of the body that was touched. These behaviours were all performed efficiently but in what appeared to be a slightly slower and less alert manner than in the normal animal. These mild imperfections in alertness did not fully recover over the course of the experiment.

Motor functions, such as the adoption of waking postures, and common activities such as sitting, standing, walking, climbing and jumping were reestablished during the first week in most of the cats (see Appendix C). In general, the activity level of these lesioned cats during waking was more subdued than in baseline. During waking, there was an increase in postural shifts although spontaneous movements actually decreased. Even though these motor-related functions reappeared soon after the lesion, they were not entirely normal. For example, some of the cats showed a slightly abnormal extension of the hind legs when walking or standing, and as a result they had difficulty balancing and

performing smooth coordinated movements. From behavioural observations there were no noticeable abnormalities in eye movements, but examination of electrographic and computer-analyzed data revealed subtle, but enduring abnormalities.

The last behaviours to reappear were those associated with affective states, such as meowing, playing, exploring, hissing, grooming, etc. Such behaviours usually appeared late in the second or during the third week. Even though it took two or three weeks for these affective behaviours to be expressed spontaneously, from very early in the first week, emotional behaviour could be elicited in these cats, in that they would hiss and growl and struggle vigorously to avoid being fed or given water by hand, or having their bladders and bowels voided.

Sleeping postures and behaviours were abnormal in some of the cats (see Appendix C). For example, there was an increase in movements and postural shifts during sleep, so that sleep appeared to be restless and fragmented. Often the animals drifted into sleep while they were sitting or reclining with their heads still raised, and they would remain in that position over extended periods. Also, while asleep, and often during PS, the cats were often observed to have their eyes open with miotic pupils. In general, the curled posture, normally adopted during PS in normal cats, was assumed less frequently. Instead, during PS these animals remained in a sphinx or outstretched position, more

often than they had done in baseline. The lesioned cats would also twitch during PS more than they had done in baseline. In some cases, these twitching bouts increased in intensity as the cat continued in the state of PS. This twitching often evolved into jerks and caused the animal to change the position of its limbs and body. The animal would frequently awaken as a result of such intense twitching and apparently orient to some nonexistent stimuli in the room or cage. These abnormal behaviours usually appeared during the second week postlesion and continued through to the end of the experiment.

H. Sleep-Waking Data Analysis: Variables and States.

During baseline recording, the three states of waking, slow wave sleep, and PS were distinctly evident in the polygraphic record (Fig.7) and represented on the average 42%, 45% and 13% respectively, of one day's recording (Table 8). These states were also distinctly evident by the coincident variation in the average amplitude of the EOG, OBS, EEG, EMG and in the average PGO spike rate over time (Fig. 8A). The display of data for one baseline day in a trivariate graph, based upon EEG and EMG amplitude and PGO spike rate, also consistently revealed three major clusters of points corresponding to waking, slow wave sleep and PS (Fig 9). Following the kainic acid lesions, waking and slow wave sleep showed minimal changes, whereas PS was

greatly altered (Figs. 7, 8A, 8B, and 9), both amount and characteristics.

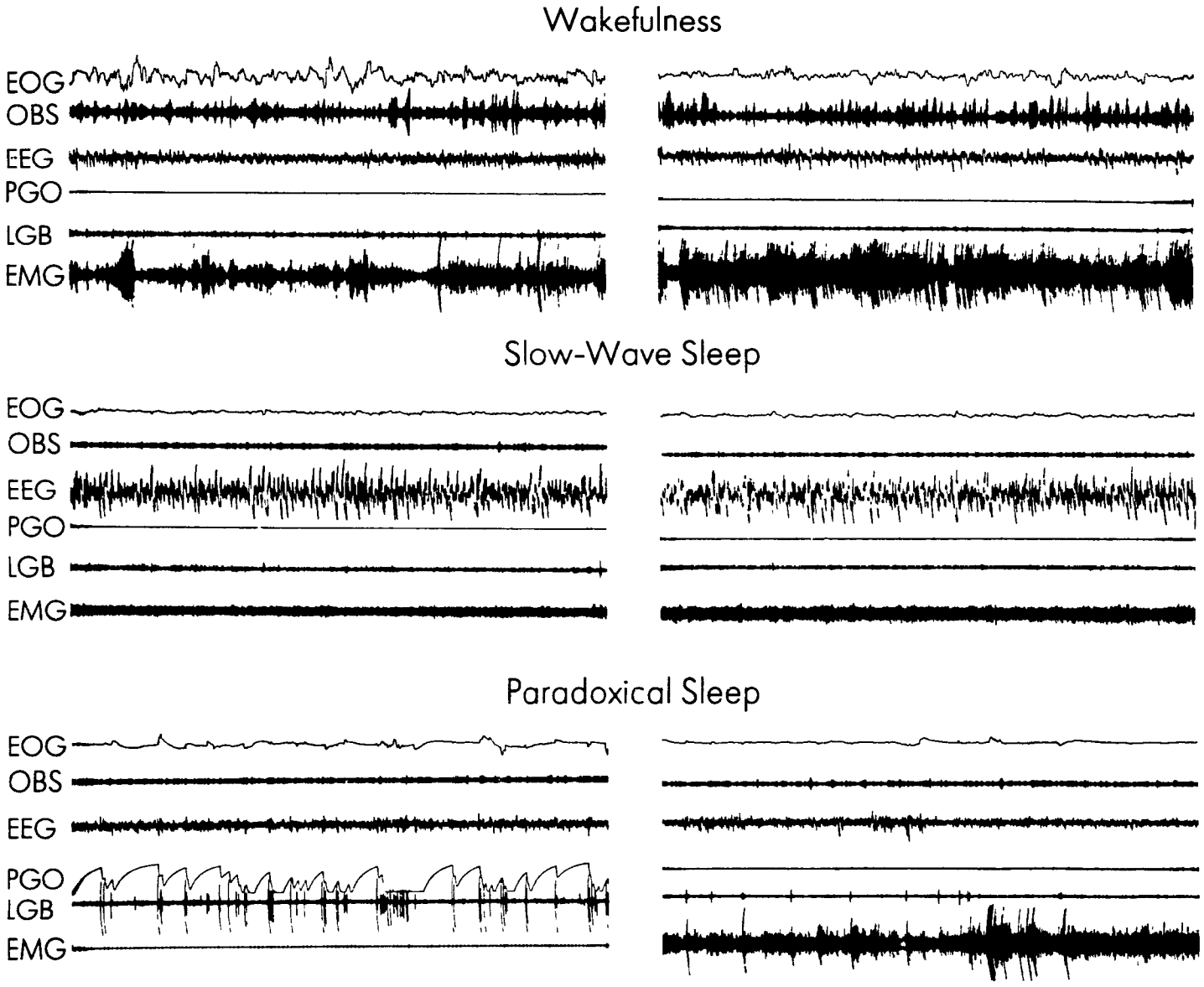
1. Waking.

The group of cats as a whole showed a marked increase (18 - 24%) in the amount of waking over the one month's recording (Table 9). In the trivariate graph (Fig. 9) the cluster of points related to waking on postlesion day 14 appears more dense and compact than in baseline. Here these points represent a 40% increase in the amount of waking over baseline in KA18. In the group as a whole, the increased amount of waking was concomitant with an increased number of waking episodes (Table 10) but not of an increment in the duration of these episodes (Table 11). In fact, there was even a slight decrease in duration of these episodes (Table 11). The increased number of shortened episodes is evident in the timeplot graphs (Figs. 8A and 8B) as a component of the truncated sleep-waking cycles during 10 hours of continuous recording.

The EEG amplitude during waking was significantly decreased for the first week following the lesions (Table 12). In the subsequent three weeks it stabilized at a slightly higher level, but remained below the baseline amplitude.

The EMG during waking did not show a significant change postlesion for the group of cats as a whole (Table

Fig.7. Representative one-minute polygraphic samples of wakefulness, slow wave sleep, and paradoxical sleep before and 22 days after kainic acid injections in the brainstem tegmentum (cat KA18). Note the decrease in amplitude and frequency of REMs, PGO spikes and lack of atonia during PS on postlesion day 22.

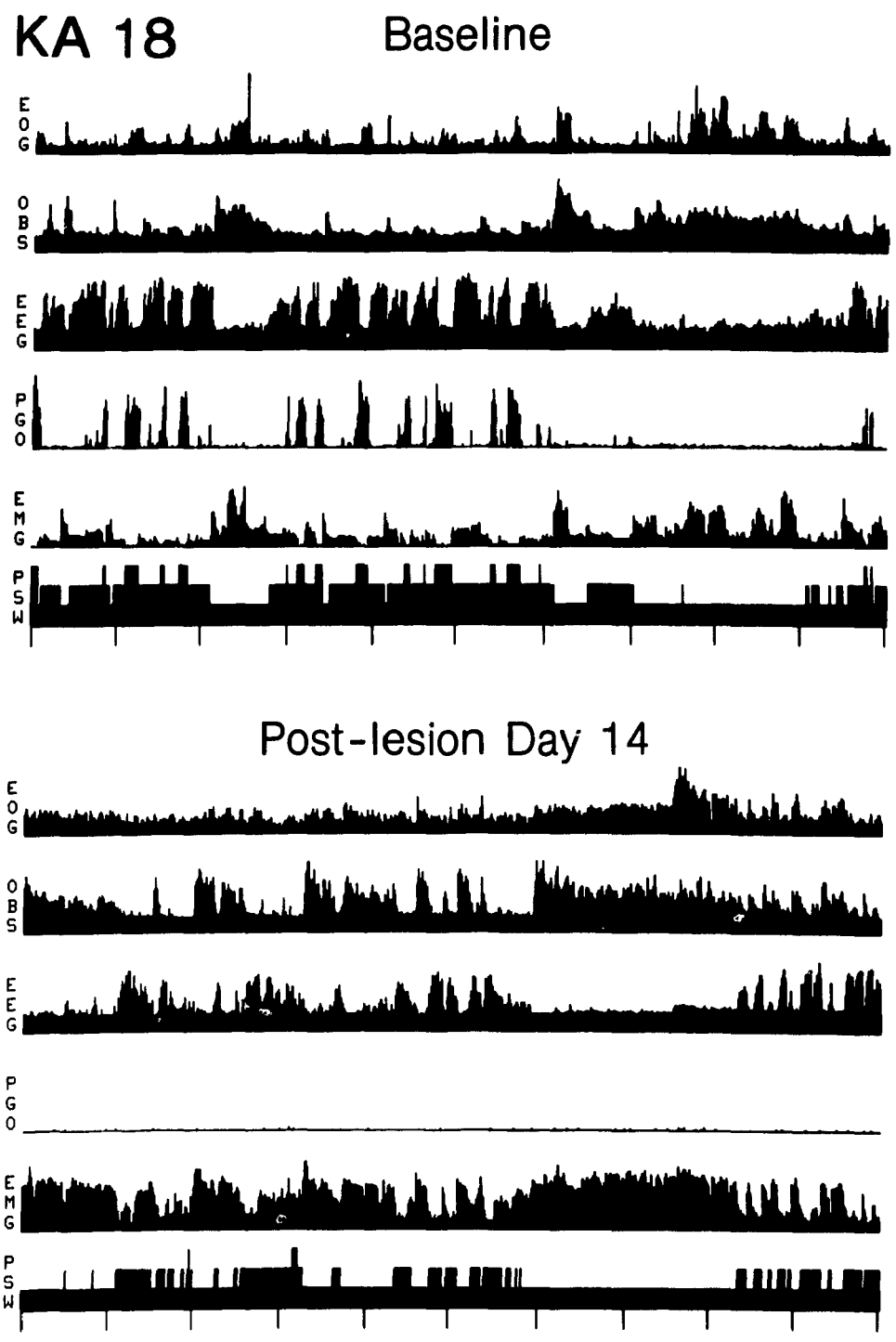


KA18 Baseline Day (1 min.)

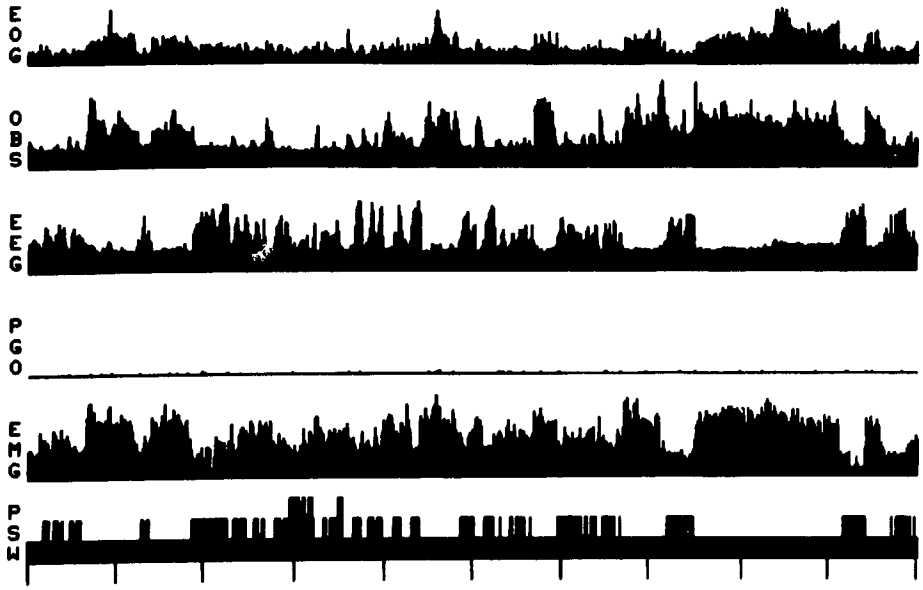
KA18 Post-Lesion Day 22 (1 min.)

Fig.8A and 8B.

Timeplots of quantified polygraphic variables and traditional state classification per minute-epoch for a 10-hour period (note the 1-hour divisions at the bottom of each of the state graphs from 9:00 PM - 7:00 AM) before and 14 (A), 21 and 28 (B) days after kainic acid injections in the pontomesencephalic tegmentum (cat KA18). Abbreviations:EOG, average electro-oculographic amplitude; OBS, average amplitude of olfactory bulb spindles; EEG, average electroencephalographic amplitude; PGO, automatically detected PGO spike rate; EMG, average electromyogram amplitude; P, paradoxical sleep; S, slow wave sleep; W, wakefulness.


KA 18
Baseline
EOG
OBS
EEG
PGO
EMG
PSW
Post-lesion Day 14
EOG
OBS
EEG
PGO
EMG
PSW

KA 18 Post-lesion Day 21



Post-lesion Day 28

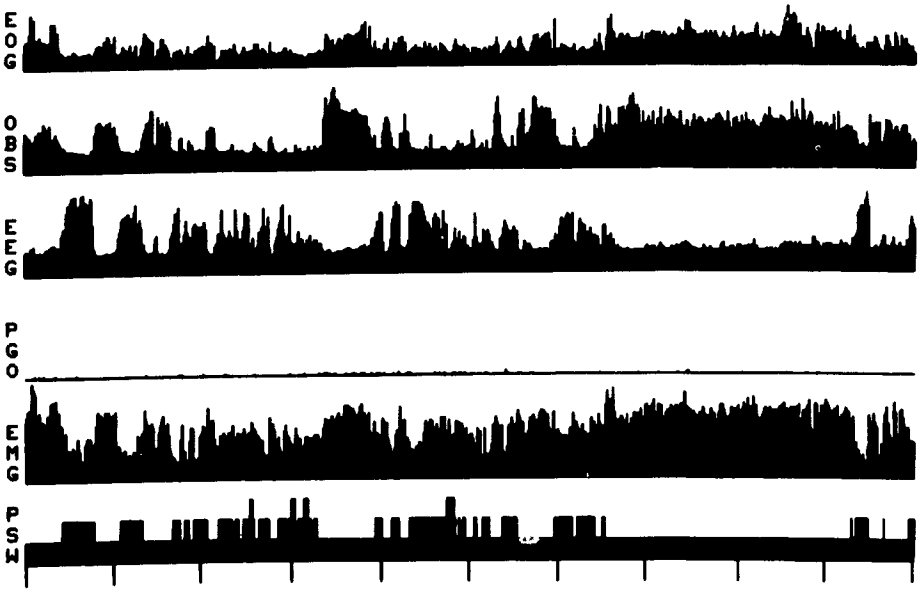
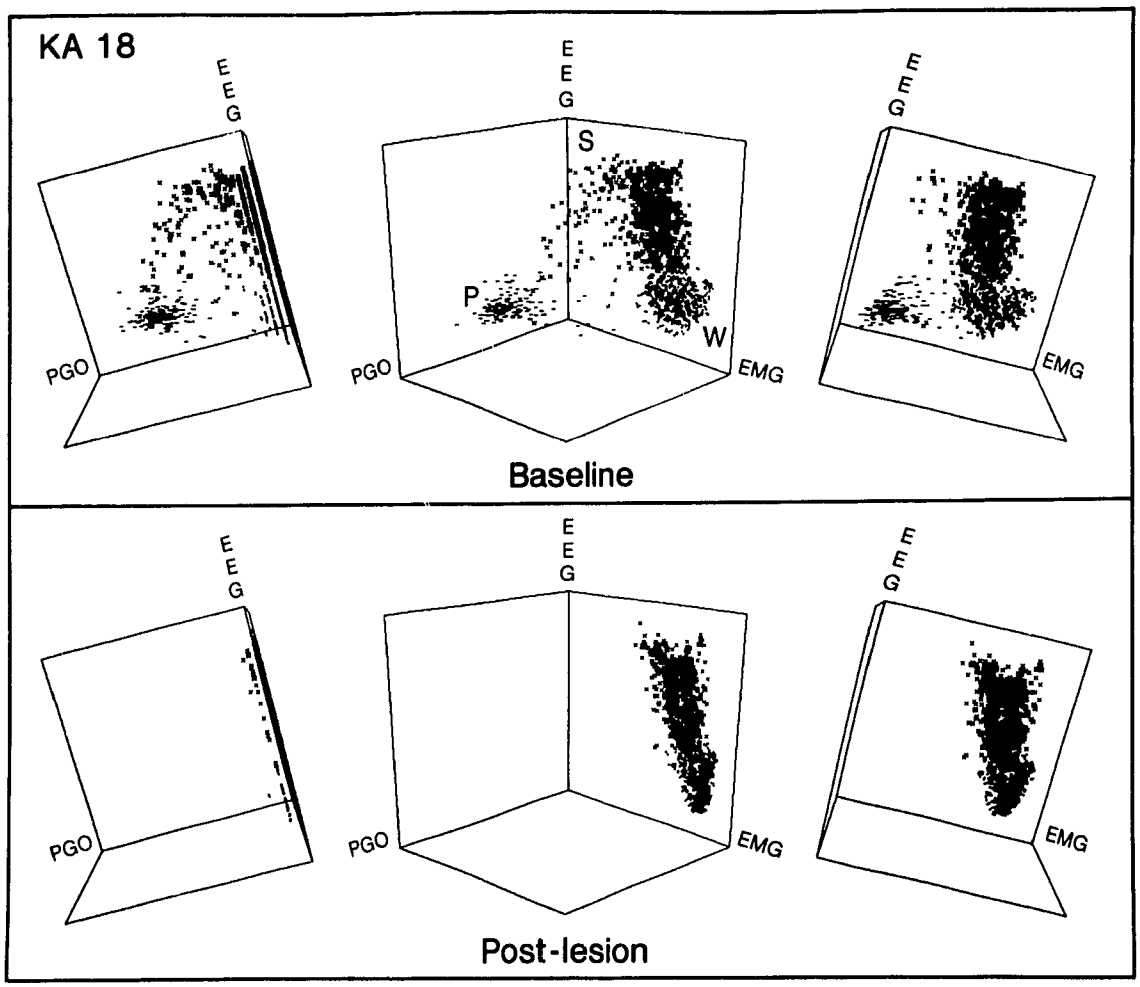


Fig.9.

Projected and rotated 3-dimensional displays on baseline day and on postlesion day 14 after kainic acid injections in the brainstem tegmentum. The symbol-coded clusters are made up of one-minute, handscored epochs which are based on the transformed and scaled average EEG amplitude, EMG amplitude and PGO spike rate per minute-epoch. The data are rotated 45 degrees to the right and to the left to allow for a better appreciation of the 3-dimensional data structure. Note that epochs with similar values on the EEG, EMG, and PGO axes appear grouped as particular states. The handscored states correspond to wakefulness (W, dots), slow wave sleep (S, cross bars) and paradoxical sleep (P, horizontal bars).


KA 18
EEG
PGO
EEG
S
P
W
PGO
EMG
EEG
EMG
Baseline
EEG
PGO
EEG
PGO
EMG
EEG
EMG
Post-lesion

13). There was, however, a gain in amplitude on postlesion day 7 which consistently decreased from week to week for the remainder of the experiment, though it never got to be as low as it had been in baseline. There was no notable change in the normally low rate of spikes (EMPs) recorded from the lateral geniculate bodies in waking (Table 14).

2. Slow Wave Sleep.

The lesion had no significant effect on the amount of slow wave sleep in the group of cats (Table 9). This lack of effect on the amount of slow wave sleep is even evident in the three-dimensional graphs (Fig. 9) which show that the density and shape of the cluster containing the majority of the slow wave sleep epochs is minimally different in the postlesion graph. However, there was a significant increase in the number of slow wave sleep episodes in all the cats (except KA14, which showed a slight decrease of 2%) (Table 10). Conversely, there was a reduction in tne duration of slow wave sleep episodes which was consistent across the group of animals (Table 11). The increased number and concurrent shortening of slow wave sleep episodes (Tables 10 and 11) is also evident as an increase in the number of shorter sleep-waking cycles in the 10-hour long timeplot graphs (Figs. 8A and 8B).

The EEG in slow wave sleep continued to show high amplitude activity very similar tc that found in baseline,

as can be seen in the timeplot graphs (Figs. 8A and 8B). In the polygraphic records it is also evident that full EEG synchronization was attained and EEG waves appear to be very similar to those seen in the baseline records. However, the high-amplitude of these waves was not sustained for extended periods, as they had been in baseline. Rather, they tended to be invaded by instances of high-frequency/low-amplitude waves, typical of waking or PS. This resulted in a slight but consistent reduction in EEG amplitude in the group as a whole during slow wave sleep (Table 12). The loss ranged from about 1% in a few of the cats (KA5 and KA18) to as much as 26% in KA17.

There was a significant increase in EMG amplitude in slow wave sleep during the first week postlesion (Table 13). During the subsequent three weeks the EMG amplitude decreased to the point where it was no longer significantly different from baseline, yet it remained consistently higher than in baseline for the remaining weeks (Table 13).

Even though PGO spikes are not normally evident throughout slow wave sleep, as seen in the baseline sample of the polygraph record (Fig. 7), PGO spikes begin to occur at a high rate just before the onset of PS (Fig. 8A). Following the lesions, there was a slight decrease in the average number of PGO spikes during slow wave sleep, but the decrease was not statistically significant (Table 14).

Table 9. Separate, one-way, repeated measure ANOVAs were performed on each state (W, S, P) comparing baseline day and the four postlesion days. There was a significant effect of the lesion on the amount of time spent in waking ($p<.01$) and in paradoxical sleep ($p<.01$). Posthoc comparison of the means by a Newman-Keuls test showed that waking on all postlesion days was greater than that on baseline day (**, $p<.01$), and that paradoxical sleep on all postlesion days was less than that on baseline day (**, $p<.01$).

Table 9

Percent (% total recording time) of Waking (W), Slow Wave Sleep (S), and Paradoxical Sleep (P) for one Baseline Day (BD) and Postlesion Days (PLD) 7, 14, 21 and 28 After Kainic Acid Injections in the Pontomesencephalic Tegmentum in 11 Cats

		BD	PLD7	PLD14	PLD21	PLD28
	W	44.2	36.0	47.6	50.3	50.0
KA4	S	40.7	54.6	44.7	42.1	42.0
	P	15.1	9.4	7.7	7.6	8.1
	W	47.3	64.3	55.5	55.3	51.3
KA5	S	39.9	32.5	37.0	37.3	40.7
	P	12.8	3.2	7.6	7.4	8.0
	W	42.8	35.0	46.0	40.5	47.6
KA6	S	39.6	55.4	41.3	53.1	42.3
	P	17.7	9.6	12.8	16.4	12.5
	W	35.8	33.9	43.9	40.2	48.1
KA8	S	51.3	55.0	45.4	48.2	43.7
	P	12.9	11.1	10.7	11.6	8.3
	W	50.3	53.3	47.1	46.8	51.6
KA11	S	37.6	38.7	38.1	38.7	36.8
	P	12.1	8.1	14.8	14.5	11.6
	W	40.8	49.4	50.3	48.5	46.8
KA12	S	46.4	38.6	35.7	40.1	40.7
	P	12.8	12.0	14.0	11.4	12.6
	W	42.5	61.5	58.2	62.7	46.4
KA14	S	45.7	38.0	36.5	29.4	43.5
	P	11.8	0.5	5.3	7.9	10.2
	W	42.7	57.3	52.2	51.5	50.4
KA15	S	47.4	42.7	45.1	46.5	47.9
	P	10.0	0.0	2.7	2.0	1.8
	W	40.9	66.9	67.5	70.6	47.8
KA17	S	44.5	32.4	29.4	27.4	43.3
	P	14.6	0.7	3.1	2.0	9.0
	W	44.3	72.6	61.9	66.5	66.8
KA18	S	44.6	27.4	37.5	31.8	29.0
	P	11.1	0.0	0.7	1.7	4.2
	W	35.1	34.1	43.8	42.9	43.4
KA19	S	53.1	60.1	49.7	48.6	49.1
	P	11.8	5.8	6.5	8.6	7.5
	W	42.4 (4.4)	51.3 (14.5) **	52.2 (7.8) **	52.4 (10.4) **	50.0 (6.1) **
X (sd)	S	44.6 (4.9)	43.2 (11.2)	40.0 (5.8)	39.4 (7.4)	41.7 (5.4)
	P	13.0 (2.1)	5.5 (4.8) **	7.8 (4.8) **	8.3 (5.0) **	8.5 (3.3) **

Table 10. A two-way repeated measures analysis of variance was performed on the number of episodes on baseline and postlesion day 21 during waking, slow wave sleep and paradoxical sleep. There was a significant difference between baseline and postlesion ($p<.001$) as well as an interaction with state ($p<.05$). Post-hoc Newman-Keuls tests showed that the number of episodes of waking and slow wave sleep were significantly increased from baseline to postlesion day 21 (**, $p<.01$).

Table 10

Total Number of Episodes of Waking (W), Slow Wave Sleep (S), and Paradoxical Sleep (P) on Baseline Day (BD) and Postlesion Day 21 (PLD21) After Kainic Acid Injections in the Pontomesencephalic Tegmentum in 11 Cats

		BD	PLD21
KA4	W	69	81
	S	61	85
	P	43	41
KA5	W	68	105
	S	71	99
	P	27	41
KA6	W	48	94
	S	61	125
	P	35	32
KA8	W	82	82
	S	92	129
	P	39	67
KA11	W	83	103
	S	82	99
	P	26	42
KA12	W	61	92
	S	69	101
	P	27	49
KA14	W	91	168
	S	92	90
	P	25	25
KA15	W	83	169
	S	84	145
	P	27	10
KA17	W	75	92
	S	84	87
	P	31	13
KA18	W	79	104
	S	82	99
	P	29	8
KA19	W	67	93
	S	88	102
	P	36	38
X (s.d)	W	73.3 (12.2)	107.6 (31.2)**
	S	78.7 (11.4)	105.6 (19.1)**
	P	31.4 (6.0)	33.3 (18.1)

Table 11. A two-way repeated measures analysis of variance was performed on the mean duration of episodes. There was a significant difference between baseline and postlesion ($p<.001$) as well as an interaction with state ($p<.05$). Post-hoc Newman-Keuls tests showed that mean episode duration of both slow wave sleep and paradoxical sleep were significantly decreased from baseline to postlesion day 21 (**, $p<.01$).

Table 11

Mean Duration in Minutes of Episodes of Waking (W), Slow Wave Sleep (S), and Paradoxical Sleep (P) on Baseline Day (BD) and Postlesion Day 21 (PLD21) After Kainic Acid Injections in the Pontomesencephalic Tegmentum in 11 Cats

		BD	PLD21
KA4	W	8.4	8.3
	S	8.8	8.6
	P	4.6	2.5
KA5	W	9.2	7.1
	S	7.5	5.0
	P	6.3	2.4
KA6	W	12.0	5.8
	S	8.7	4.7
	P	6.8	2.7
KA8	W	5.8	6.6
	S	7.5	5.1
	P	4.4	2.3
KA11	W	8.2	6.0
	S	6.2	5.2
	P	6.3	4.6
KA12	W	8.9	7.1
	S	9.1	5.3
	P	6.4	3.1
KA14	W	6.2	4.3
	S	6.6	3.8
	P	6.2	3.7
KA15	W	6.8	3.9
	S	7.5	4.1
	P	4.9	2.5
KA17	W	7.3	10.3
	S	7.1	4.2
	P	6.4	2.1
KA18	W	7.2	8.5
	S	7.0	4.2
	P	4.9	2.9
KA19	W	7.0	6.1
	S	8.1	6.3
	P	4.4	3.0
X (s.d.)	W	7.9 (1.7)	6.7 (1.9)
	S	7.7 (0.9)	5.0 (0.9) **
	P	5.6 (0.9)	2.9 (0.7) **

Table 12. 1 A two-way, repeated measures analysis of variance (ANOVA) was performed comparing baseline EEG amplitude with that of each postlesion day during each state (W, S, P). There was a significant effect upon EEG amplitude across days ($p<.001$), and there was also a significant interaction between days and states ($p<.01$). Post-hoc, Newman-Keuls tests showed that this effect was mainly due to an increase in EEG amplitude in slow wave sleep (*, $p<.05$; **, $p<.01$).

Table 12

Mean Amplitude (uv) EEG for Baseline (BD) and Postlesion Days (PLD) 7, 14, 21 and 28 After Kainic Acid Injections in the Pontomesencephalic Tegmentum in 11 Cats for Waking (W), Slow Wave Sleep (S) and Paradoxical Sleep (P)[1]

		BD	PLD7	PLD14	PLD21	PLD28
	W	19.1	12.0	15.2	18.5	18.5
KA4	S	53.4	34.6	42.6	46.3	48.0
	P	15.3	11.7	11.6	15.3	15.0
	W	18.3	15.2	19.3	17.8	17.8
KA5	S	25.8	20.9	24.1	24.6	25.4
	P	13.3	14.4	12.8	12.7	12.5
	W	20.4	19.0	18.2	19.9	19.9
KA6	S	42.8	35.5	34.6	36.5	37.8
	P	14.0	13.2	13.7	15.3	15.0
	W	4.8	3.3	3.0	3.5	3.5
KA8	S	10.4	7.5	7.5	8.2	9.6
	P	4.0	2.8	2.9	3.0	3.4
	W	10.7	9.0	10.3	11.0	9.5
KA11	S	23.8	16.8	23.9	23.9	22.1
	P	10.4	8.1	9.8	10.2	8.9
	W	11.8	9.3	9.1	9.8	10.3
KA12	S	25.2	16.0	18.8	19.2	19.4
	P	10.9	7.8	8.6	8.8	9.2
	W	18.1	17.4	14.7	16.6	18.0
KA14	S	36.7	33.0	30.8	31.8	35.3
	P	12.4	13.6	10.7	10.8	11.5
	W	16.8	15.3	15.2	16.6	15.9
KA15	S	31.5	33.4	29.0	27.4	28.5
	P	15.4	14.5	13.6	13.9	15.4
	W	13.0	10.2	13.8	13.4	15.9
KA17	S	29.0	26.7	23.9	21.6	21.6
	P	10.0	8.8	9.5	9.5	10.3
	W	15.4	12.9	14.3	15.3	15.7
KA18	S	32.7	32.7	30.4	29.5	32.4
	P	14.8	15.0	16.5	14.3	17.6
	w	30.9	26.6	26.5	24.6	23.8
KA19	S	42.2	40.4	40.2	38.1	38.1
	P	19.5	16.9	15.7	15.8	15.4
	W	16.3 (6.6)	13.7 (6.2)*	14.5 (6.0)	15.2 (5.6)	15.3 (5.6)
X (s.d.)	S	32.1 (11.5)	27.0 (10.3)**	27.8 (9.9)**	27.9 (10.3)**	28.9 (10.8)*
	P	12.7 (4.0)	11.5 (4.2)	11.4 (3.8)	11.8 (3.8)	12.2 (4.1)

Table 13. A two-way, repeated measures analysis of variance (ANOVA) was performed comparing baseline EMG amplitude with that of each postlesion day during each state (W, S, P). There was a significant effect upon EMG amplitude across days ($p<.001$) and a significant interaction with state ($p<.001$). Post-hoc, Newman-Keuls tests showed that the effect was due mainly to an increase in EMG amplitude in paradoxical sleep (*, $p<.05$; **, $p<.01$).

Table 13

Mean Amplitude (uv) EMG for Baseline (BD) and Postlesion Days (PLD) 7, 14, 21 and 28 After Kainic Acid Injections in the Pontomesencephalic Tegmentum in 11 Cats for Waking (W), Slow Wave Sleep (S) and Paradoxical Sleep (P).

		BD	PLD7	PLD14	PLD21	PLD28
	W	21.2	17.2	24.6	23.9	21.3
KA4	S	2.1	6.6	6.3	7.5	7.3
	P	0.6	7.2	8.2	3.9	3.3
	W	9.4	11.1	9.5	8.8	7.7
KA5	S	4.2	8.1	5.5	2.4	5.8
	P	0.7	5.4	4.2	1.7	1.3
	W	10.2	10.5	10.7	10.8	9.5
KA6	S	2.7	4.8	2.5	2.1	5.7
	P	0.7	2.2	1.2	1.0	0.6
	W	10.3	14.7	13.1	14.3	14.5
KA8	S	4.1	7.5	4.9	4.7	5.2
	P	1.1	6.8	6.4	5.2	4.9
	W	15.6	14.9	18.5	17.7	17.4
KA11	S	7.9	9.4	7.9	8.1	6.5
	P	1.9	7.5	4.1	3.3	4.2
	W	15.7	11.2	15.8	14.3	13.2
KA12	S	5.9	9.6	6.7	5.7	4.9
	P	0.9	14.2	5.4	3.5	3.1
	W	12.3	11.4	13.7	14.6	13.5
KA14	S	4.9	7.5	7.3	9.8	8.9
	P	0.6	4.1	1.8	2.6	1.7
	W	30.7	37.9	24.0	22.8	19.8
KA15	S	9.2	18.3	11.8	11.7	9.0
	P	2.7	12.5	14.2	15.5	9.4
	W	14.0	21.7	19.6	18.7	16.1
KA17	S	7.2	14.7	14.4	12.7	5.6
	P	0.9	4.7	9.4	7.2	3.2
	W	9.4	16.9	17.9	19.8	25.4
KA18	S	4.2	13.3	8.3	11.5	15.7
	P	0.7	11.8	13.0	15.8	18.8
	W	25.6	26.0	23.0	22.3	26.3
KA19	S	6.5	13.4	9.6	7.4	8.2
	P	1.9	12.6	5.4	4.4	4.1
	W	15.9 (7.1)	17.6 (8.3) *	17.3 (5.2)	17.1 (5.0)	16.8 (6.0)
X (s.d.)	S	5.4 (2.2)	10.3 (4.1) **	7.7 (3.3) **	7.6 (3.6) **	7.5 (3.1) **
	P	1.2 (0.7)	8.1 (4.0)	6.7 (4.2)	5.8 (5.1)	5.0 (5.1)

Table 14. A two-way, repeated measures analysis of variance (ANOVA) was performed comparing baseline PGO spike rate with that of each postlesion day during each state (W, S, P). There was a significant effect upon PGO rate across days ($p<.001$) as well as a significant interaction with state. Post-hoc, Newman-Keuls tests showed that this effect was due mainly to a significant decrease (**, $p<.01$) of PGO spikes in paradoxical sleep.

Table 14

Mean Rate of PGO Spikes for Baseline (BD) and Postlesion Days (PLD) 7, 14, 21 and 28 After Kainic Acid Injections in the Pontomesencephalic Tegmentum in 10 Cats for Waking (W)[1], Slow Wave Sleep (S) and Paradoxical Sleep (P)

		BD	PLD7	PLD14	PLD21	PLD28
KA4	W	2.54	1.64	0.49	2.47	1.63
	S	6.23	5.17	5.96	5.46	4.41
	P	52.73	14.45	20.48	17.06	19.22
KA6	W	1.53	1.28	3.64	1.56	1.32
	S	3.65	1.19	0.84	0.33	0.26
	P	36.58	0.73	0.88	0.23	7.54
KA8	W	1.54	0.21	0.18	0.36	1.11
	S	7.21	6.75	6.63	6.77	4.50
	P	70.71	11.37	13.04	13.03	10.36
KA11	W	3.06	0.67	7.44	1.49	1.39
	S	5.59	0.67	9.49	4.10	5.13
	P	23.10	1.17	18.15	30.39	30.52
KA12	W	5.40	2.03	2.53	2.59	2.00
	S	4.93	6.64	5.46	5.08	6.16
	P	64.33	24.72	45.91	46.97	37.80
KA14	W	0.92	0.73	3.26	7.10	3.20
	S	6.10	1.07	0.87	3.21	2.59
	P	13.63	0.57	0.70	1.73	2.58
KA15	W	3.80	0.81	4.65	4.02	3.07
	S	4.71	0.30	0.48	0.52	0.51
	P	28.15	1.47	1.31	1.65	1.65
KA17	W	4.23	1.46	0.91	1.98	1.46
	S	16.77	7.75	1.47	0.57	0.45
	P	38.15	1.70	0.43	0.22	2.78
KA18	W	1.66	0.05	0.07	0.09	0.10
	S	4.30	0.07	0.05	0.04	0.09
	P	56.68	0.88	0.11	0.04	0.07
KA19	W	2.10	4.90	5.44	5.94	2.07
	S	10.12	24.69	20.30	11.52	1.64
	P	39.08	2.95	2.07	3.33	2.36
X (s.d.)	W	2.7 (1.4)	1.4 (1.4)	2.9 (2.5)	2.8 (2.3)	3.4 (4.2)
	S	7.0 (3.9)	5.4 (7.4)**	5.2 (6.2)**	3.8 (3.7)**	2.6 (2.3)**
	P	42.3 (18.4)	6.0 (8.2)	10.3 (14.8)	11.5 (16.0)	11.5 (13.3)

[1] PGO spikes in waking correspond to EMPs (eye movement potentials).

3. Paradoxical Sleep.

The most marked effect of the kainic acid injection was on PS (Table 9). In some cats (KA14, KA15, KA17, and KA18), the state of PS was virtually absent for two or three weeks postlesion (Table 9). The 3-dimensional graphs show that the cluster representing PS was no longer present on postlesion day 14 (Fig. 9), and the timeplot graphs (Figs. 8A and 8B) show that throughout the experiment, PS was distinctly reduced. There was a mean reduction of some 35% in PS in the group as a whole, but the decrease varied from 2% in KA12 to 82% in KA15 on postlesion day 28. A few epochs of PS occurred in KA18 on postlesion day 14 (Table 12). These few epochs are difficult to detect on the 3-dimensional graph (Fig. 9) because PGO spikes are few or even absent, and EMG amplitude is high in these few epochs, causing them to be mixed in with the cluster of waking points. In cases where both of these crucial variables (EMG atonia and PGO spiking) for identifying PS were absent, the OBS proved to be an indispensable variable for distinguishing waking episodes (when OBS are present) from PS (when spindles are absent) (see Fig. 7).

The large decrease in the amount of PS seen in all of the cats (Table 9) is a reflection of the decreased duration of the PS episodes, since the number of such episodes was not decreased in the group as a whole (Tables

10 and 11). However, there was a wide range of effect from animal to animal. For example, KA8 shows an increase of 42% in the number of PS episodes while KA18 shows a decrease of 72% and KA14 shows no change in the number of episodes. There was a consistent effect of decreased duration of PS across the entire group of cats. The range of decrease varied from 32% in KA19 to 67% in KA17 (Table 11). The timeplot graphs show the occurrence of isolated episodes of PS during the sleep-wake cycle. These episodes were too few in number to be distributed throughout the recording day in order to form part of the sleep-wake cycle as had occurred in baseline. The state of PS continued to occur at regular intervals throughout the sleep-wake cycle in cats in which it was not drastically reduced.

During PS, the EEG records (Fig. 7) appeared very similar to that seen in baseline. For the duration of the experiment, individual cats showed only minor decreases or increases in EEG amplitudes during PS, but overall there was no significant effect (Table 12).

Neck muscle atonia was eliminated in all cats for the first two weeks (Table 13). The disruption of atonia persisted for the duration of the experiment, except in one of the cats (KA6) in which atonia during PS was recovered by postlesion day 28 (Table 13). The timeplots also show that during the postlesion days, there was a general increase in the level of the EMG amplitude across states (Table 13).

Often, during the PS episodes, there was no clear difference in EMG amplitude from that seen during waking and slow wave sleep on any of the postlesion days. The polygraphic records also show that there was a consistent increase of the EMG amplitude from baseline to postlesion in PS, and Table 13 shows that the increase was consistent across cats, except for KA6, which showed a recovery of EMG atonia, similar to baseline levels by postlesion day 28.

The rate of PGO spikes during PS was greatly reduced as a result of the lesion (Table 14), in fact, in some of the cats (KA6, KA14, KA17, KA18, and KA19), it was virtually eliminated for the duration of the experiment (Table 14). The timeplots also show that the high rate of PGO spiking seen in PS in the baseline situation was no longer evident postlesion (Figs. 8A and 8B). Instead, throughout the sleep-wake cycle along the PGO graph there are dots of irregular size representing the few isolated spikes occurring throughout the sleep-wake cycle. Often however, during the second and third week postlesion, some very low-amplitude geniculate waves appeared in association with muscle twitches and eye movements during incipient PS episodes, as is evident in the polygraphic record (Fig. 7). However, these waves seldom evolved to the amplitude where they could be detected and counted as PGO spikes by the automatic PGO detector and counter. Table 14 shows that the potent effect on the reduction of PGO spikes was sustained

throughout the experiment in all of the cats except KA11, in which PGO spike levels recovered to within normal levels by the second week postlesion.

E. Correlation of Lesion and Sleep-Wake Data.

Subsequent to the statistical tests which indicated that the kainic acid had caused a substantial destruction of ChAT-positive and TH-positive cells in the brainstem (Tables 2-8) and that the injection had also caused a significant alteration of the state of PS and its component variables (Tables 9-14), Pearson Product Moment correlation tests were performed on the data to determine the relationship between the various histological aspect of the lesion, and its effect upon PS (Table 15). There was a correlation between the total area of gliosis, cell loss, and the amplitude of the EMG; i.e., the larger the area of the pontomesencephalic, lesion the greater was the EMG amplitude during PS. However, there was only a moderate relationship between total area of lesion and the amount of PS, and virtually no relationship with PGO spike rate. The area of gliosis/cell loss in the region normally populated by ChAT-positive cells showed a substantial correlation with the total amount of PS and EMG amplitude. The correlation between the lesions of the ChAT-positive cell area of gliosis and PGO spike rate was substantial, but it was not high enough to be statistically significant (experimental

Table 15

Correlation Between Histological Results (Total Area of Lesion[1], Area of Lesion Normally Occupied by ChAT+ and TH+ Cells[2], Total Number of ChAT+ and TH+ Cells[2]) and Electrographic Results (Amount of Paradoxical Sleep[3], PGO Spike Rate[4] and EMG Amplitude[4]) on Postlesion Day 28.

	Paradoxical Sleep	PGO Spike Rate	EMG Amplitude
Total Area of Lesion	-.53	-.32	.64*
Chat+ & TH+ Area of Lesion	-.72*	-.59	.73*
Chat+ Cells	.69*	.66*	-.41
TH+ Cells	-.18	-.27	.15

Paradoxical sleep was significantly correlated with the area of necrosis, from A1 to P5, normally occupied by ChAT+ and TH+ cells. Paradoxical sleep was also correlated with the number of ChAT+ cells surviving the effect of the kainic acid injection. However, paradoxical sleep was not correlated either with the total area of the lesion or with the remaining number of TH+ cells in the pontomesencephalic area. The mean rate of PGO spikes was correlated only with the number of surviving ChAT+ cells. The mean EMG amplitude (in microvolts) was significantly correlated with total area of the lesion as well as with the area normally occupied by ChAT+ and TH+ cells; however, there was no correlation with either the ChAT+ or the TH+ cells in the pontomesencephalic area.

[1] Levels A3 to P5 inclusive.

[2] Levels A1 to P5 inclusive.

[3] Percent of the total recording time spent in paradoxical sleep.

[4] Mean PGO spike rate and EMG amplitude (uV) during paradoxical sleep.

* $p < .05$

r=.59; criterion r=.60, p=.05). On the other hand, the number of surviving ChAT-positive cells showed a significant correlation with PGO spiking, as well as with the amount of PS. However, it did not show a significant relationship with EMG amplitude. TH-positive cells were not significantly correlated with PS, PGO spike rate or EMG amplitude.

V. DISCUSSION

The purpose of this investigation was to destroy the cholinergic cells in the dorsolateral pontomesencephalic tegmentum with kainic acid in order to study the influence of these cells on the sleep-waking states in general, and on PS and its defining variables in particular. There was a major and significant destruction of ChAT-positive cells, especially in the PPT and LDT nuclei, and a consequent effect of varying degrees occurred on the different sleep-waking states, particularly PS. The defining variables of the states that were measured (EEG and EMG amplitude and PGO spike rate), were also affected to different degrees within each state, but the effect was particularly marked on muscle atonia and PGO spiking during PS. In addition, there was minor but significant destruction of TH-positive cells in the pontomesencephalic tegmental region. Other small, lightly-stained cells in the region were also destroyed, but the chemical neurotransmitter of these small neurons is unknown, and because of their small size, they could not be quantified.

Even though most of the effect of the injections was related to the destruction of the ChAT-positive cells, it is possible that the destruction of noradrenaline cells

and of other unidentified cells may have contributed to some aspects of the deficits that occurred in the expression of the different states. This conjecture is in agreement with the demonstration by Karczmar et al. (1970) that it is the interplay between monoamine transmitters and acetylcholine in the brain that determines the sleep-waking states. In these pharmacological experiments, when acetylcholine and noradrenaline levels were simultaneously elevated, waking ensued, but when acetylcholine was elevated and noradrenaline was low, PS predominated.

The results of the present research may contribute to our understanding of the importance of cholinergic neurons in this potential interaction, for it was mainly the destruction of these neurons, and not of the noradrenergic neurons, that was related to alterations of different aspects of the sleep-waking cycle.

A. Mechanisms of Wakefulness

The ARAS has been identified as a structure essential for the maintenance of wakefulness (Lindsley et al., 1949, 1950), and the data supporting a role for acetylcholine in waking have also been impressive and consistent. Pharmacological research has shown that acetylcholine might participate in both behavioural arousal and cortical activation (DeFeudis, 1974; Gillin et al., 1978b; Rinaldi & Himwich, 1955b) and histochemical studies sugges-

ted that these cholinergic neurons were located in the reticular formation. Because acetylcholine was related to cortical activation, the ARAS was also sometimes called the cholinergic reticular activating system (Lewis & Shute, 1967; Shute & Lewis, 1963, 1967). In the present study, destruction of the cholinergic neurons of the pontomesencephalic tegmentum had very little apparent effect on waking, or on any of the variables defining this state, and electrocortical activity was relatively normal for most of the postlesion period in the waking animal. During the first week the EEG amplitude was lower than in baseline, and although it recovered and remained within the baseline range for the last three weeks of the study, the mean amplitude was always lower than in the prelesion condition. Furthermore, visual analysis of the polygraph record, revealed that during waking the EEG was not significantly affected, except for the lower amplitude.

Behavioural arousal was more or less normal during the postlesion period. The cats were responsive to sensory stimulation and displayed simple spontaneous motor activity from early in the first postlesion week. The destruction of cholinergic neurons in the dorsolateral pontomesencephalic tegmentum did not affect behavioural waking nor electrocortical activation. If such an effect had been observed, it would have been compatible with their role as the ARAS or

the cholinergic reticular activating system as predicted by Shute and Lewis (1963, 1965).

The relative lack of effect on waking on the cats in my study is very different from that reported by Lindsley et al. (1949, 1950). In their study, the lesions destroyed a large portion of the brainstem tegmentum, and the cats were consequently somnolent or comatose and behaviourally unresponsive, and could only be aroused for short periods of time. In contrast to the tendency to desynchronization of the EEG in the present study, the EEG of most of the cats in Lindsley and his colleague's study was almost permanently synchronized, and in the cases where it showed activation in waking, the activated EEG was often invaded by uncharacteristic slow waves.

In the present study, the destruction of cholinergic neurons of the pontomesencephalic tegmentum did not cause a decrease in cortical activation, in fact, there was an increase in the amount of waking, which apparently was an indirect effect of the lesion. The increase in the amount of waking most likely occurred as a result of the decrease in the amount of PS. It was mainly reflected as an increase in the number of waking episodes, caused by the frequent disruptions of the sleep process, however, there was no change in the duration of such episodes.

The results of the present study also diverge from those obtained by electrolytic lesions placed in the

dorsolateral pontomesencephalic region, extending from the anterior pole of the LC into the pontomesencephalic tegmentum, that had led to a decrease in electrocortical waking and an increase in cortical synchronization (Jones, 1969; Jones et al., 1973). The effects on the EEG were attributed at that time to the destruction of noradrenaline neurons or their dorsal ascending pathway. Subsequent, more delimited lesions that destroyed mainly noradrenergic cell bodies, failed to replicate the earlier findings, and cortical desynchronization returned to within normal levels a few days after the lesions, which showed that noradrenaline was not necessary for cortical activation and waking and that the noradrenaline neurons and fibers could not be considered as an integral part of the ARAS (Jones et al., 1977). The discrepancy between these two reports by Jones and her associates was assessed as being due to the destruction of numerous fiber tracts and cell bodies in the pontomesencephalic tegmentum, in addition to the noradrenergic bundle, in the earlier studies. Given that cells in the dorsolateral pontomesencephalic tegmentum are now known to be cholinergic as well, it is likely that cholinergic cells were also destroyed by the electrolytic lesions and that their destruction could have contributed to a decrease in cortical activation.

In the present study, it was expected that destruction of cholinergic neurons in the dorsolateral

pontomesencephalic tegmentum would result in reduced EEG activation because these neurons were assumed to be an important component of the ARAS (Shute & Lewis, 1963, 1967). Recent reports from unit recording studies also indicate that during cortical activation of waking, there is an increase in discharge by neurons in this area of the pontomesencephalic tegmentum (Steriade, Oakson & Ropert, 1982), but instead of a loss of cortical activation in waking, there was an increase in such activation. Therefore, the results fail to confirm that the cholinergic cells in this region form an integral part of the ARAS, or that they are necessary for the promotion of cortical activation of wakefulness (Lewis & Shute, 1967; Lindsley et al. 1950; Shute & Lewis, 1963).

In view of the results from the present study that destruction of pontomesencephalic cholinergic cells did not abolish behavioural waking and cortical activation, it may be deduced that the electrolytic lesions in earlier studies (Jones et al., 1969, 1973; Lindsley et al., 1949, 1950) must have destroyed neurons as well as fiber tracts that were not affected by the kainic acid injections in the present study. At the time of the study by Lindsley et al. (1950), the transmitter nature of the cells that were affected was not known, but some may have been cholinergic, given their location (Jones & Beaudet, 1987a, 1987b; Sakai et al., 1986). In the studies by Jones et al. (1969, 1973) it was

concluded that there was major damage to noradrenaline cells and pathways, because of the observed decrease in brain noradrenaline. From the description of the lesions, however, it is likely that cholinergic cells as well as other unidentified types of cells and fiber systems that contribute fibers to the ARAS were also affected. Damage to such cells and fibers could account for the large increase in cortical synchronization that Jones et al. reported. Only a limited number of TH-positive neurons were destroyed in the present study, but it is unlikely that the remaining TH-positive neurons alone could have accounted for the integrity of the cortical desynchronization, since 60% to 85% of brain noradrenaline may be decreased without affecting waking or cortical desynchronization (Jones et al., 1977). Therefore, the evidence from the present experiment, taken together with that from previous studies (Jones et al., 1969, 1973; 1977; Lindsley et al, 1949, 1950) shows that neither the destruction of the main body of noradrenergic or cholinergic neurons alone can account for elimination of cortical desynchrony, but that destruction of both of these types of neurons, and possibly including fibers of passage, does have such an effect.

The persistence of OBS is also indicative of the viability of the activating system, even after the destruction of cholinergic and other cell bodies in the pontine tegmentum. As noted in the Introduction, OBSs are a sign of

central activation and peripheral arousal. The olfactory bulb and cerebral cortex receive innervation from structures implicated in the control of wakefulness, including noradrenergic innervation from the LC, dopaminergic innervation from the ventral tegmental area and substantia nigra, and cholinergic innervation from the basal forebrain area (Butcher & Woolf, 1986; Switzer, DeOlmos & Heimer, 1985). As the present histological results show, these systems remained largely intact, and this fact may account for the lack of effect of the destruction of acetylcholine neurons of the pontomesencephalic tegmentum the OBS and cortical activation during wakefulness. As noted by Gillin et al. (1978b) and Jacobs and Jones (1978), the monmoamines may play a modulatory role in behavioural and cortical activation related to wakefulness. The same can be said of the influence of acetylcholine in waking, because other transmitters may participate in a similar manner to acetylcholine in wakefulness. For example, stimulation of subcortical structures in the midbrain and forebrain causes not only the release of acetylcholine, but can also cause the release of glutamate, aspartate, glycine and taurine from the activated cortex (Jasper & Koyama, 1967, 1968, 1969).

In summary, it is seen that acetylcholine and the catecholamines, together with several other amines and transmitter substances, may modulate the state of waking

(Hobson & Steriade, 1986; Karczmar et al., 1970), but it is unlikely that any one is critical for the occurrence of the state (Jacobs & Jones, 1978). The present data also suggest that cholinergic cells in the pontomesencephalic tegmentum may not be required for the maintenance of wakefulness, but if they are, that their role in waking must be as part of larger cholinergic, activating system. The main group of neurons of this larger cholinergic system may be the neurons located in the basal forebrain. The pontomesencephalic cholinergic neurons may be involved in cortical activation by way of their influence upon the non-specific, thalamocortical system (Steriade, 1981) to which they have been shown to project in the present study. Thus, the pontomesencephalic tegmental cells appear to project mainly to the dorsal thalamic activating system, whereas other reticular neurons in the area apparently provide the major extrathalamic projection to the hypothalamus and basal forebrain systems.

B. Mechanisms of Slow Wave Sleep

Examination of the data in this study reveals that there was no change in the amount of time spent in slow wave sleep as a result of the kainic acid lesions in the pontomesencephalic tegmentum. Such a lack of effect is not surprising given that there is no strong evidence supporting a role for cholinergic neurons of the pontomesencephalic

tegmentum in slow wave sleep (Hobson and Steriade, 1986; Sakai, 1985a, 1985b). However, there was an increase in the number of slow wave sleep episodes that was compensated for by a decrease in their duration, resulting in no net change in the amount of slow wave sleep time. It is not clear whether the decreased length of episode duration was a result of the increased number of such episodes in an automatic, compensatory, self-regulating operation by the system to maintain a certain amount of slow wave sleep within a set, predetermined range, or if the shortened episodes were a result of disruption of the PS process. Many such awakenings were noted after the animal had been engaged in violent twitches and jerks during the transition from slow wave sleep to PS. Such disruption of the sleep process is also seen after destruction of the tegmentoreticular tract after electrolytic lesions and transections in the caudal pons (Morrison, 1979; Webster et al., 1986).

The disruption of slow wave sleep was especially evident in the EEG amplitude, which was decreased during this state for the duration of the study. However, there was no similar effect of such magnitude on the EEG during PS and waking. It is unlikely that the lesion produced a specific decrease in EEG amplitude during slow wave sleep, but not during any other state. A more parsimonious explanation for most of the decreased EEG amplitude in slow wave sleep would be that it was due to the large number of transitions from

waking to slow wave sleep and vice-versa, as well as from PS to slow wave sleep and vice-versa, during the sleep-wake cycle. Other studies have shown that with large lesions in the FTL and FTG (Friedman, 1983; Webster, 1985), there can be a reduction of EEG amplitude across states, which is probably due to disruption of rostral projections from structures in the medulla that are involved in the synchronization of the EEG (Moruzzi, 1972). It is possible that this loss of innervation to the medulla may have contributed to the reduction in EEG amplitude.

The disruption of slow wave sleep was also evident in the relative lack of movement suppression during sleep, especially during the first week, when there was a higher EMG amplitude in slow wave sleep than during baseline. In the remaining three weeks of the experiment during slow wave sleep, the EMG amplitude was still higher than normal, but by then it was not significantly different from normal and remained at a steady level from week to week. This higher EMG was probably reflecting the increased number of postural adjustments in which the animal engaged during sleep.

A large number of cells within the pontomesencephalic tegmentum have a higher firing rate during slow wave sleep than in waking, and the rate increases further during PS (Huttenlocher, 1961). Such a pattern of firing may correspond to suppression of movement during slow wave sleep as well as during the atonia of PS.

Destruction of neurons such as these may have caused the increase in movement during sleep, and frequent disruption of slow wave sleep in this study.

C. Mechanisms of the Paradoxical Sleep State

The results from the present experiment show that kainic acid lesions of the pontomesencephalic tegmentum caused a lasting decrease in PS in most of the cats and the acute elimination of this state in a few of the others. Moreover, the correlation of the surviving ChAT-positive neurons with the amount of PS was significant. These findings support the results from pharmacological studies indicating that acetylcholine is critically involved in the control of PS (Gillin and Sitaram, 1984; Gillin, et al., 1985).

The demonstration that cells located in the dorsolateral pontomesencephalic tegmentum are critically involved in PS potentially resolves certain differences of opinion regarding the location of the cells necessary for bringing about the state of PS. Although many authors have suggested these cells reside in the dorsolateral region of the rostral pons and caudal midbrain (Jouvet, 1972, 1975; Morrison, 1979; Sakai, 1985a, 1985b), others have found evidence for this control in the pontine reticular formation, including the FTG or the combined RPO and RPC (Baghdoyan et al., 1985; Hobson et al., 1974a; Shiromani et

al., 1987a; Shiromani, Gillin & Henriksen, 1987b; Steriade & Hobson, 1976). However, supporters of both of these positions seem to agree that in order for such neurons to control PS they must fulfill some minimal criteria. For example, 1) such neurons must be active during PS and be capable of activating PS-subsystems (Sakai, 1985b). 2) they must respond to acetylcholine and its agonists and therefore must be cholinoceptive, which perforce indicates that cholinergic neurons must also be involved, a role which might be fulfilled by the cholinoceptive neurons as well (Sakai, 1985a, 1985b; Hobson, et al., 1975; Steriade and Hobson, 1976) or by a different population of cholinergic neurons (Gillin, 1978; Shiromani, et al., 1987a, 1987b). Finally, 3) the destruction of such neurons should eliminate or significantly disrupt PS.

The first criterion is fulfilled by cells in the FTG, RPO-RPC region. As noted earlier, reports suggesting the pontine reticular formation is the location of the PS-on cells have shown that cells in the FTG fire phasically and tonically while those in the LC are silent (Hobson et al., 1975; Shiromani & Fishbein, 1986). The second criterion is also fulfilled by the FTG, RPO-RPC neurons, for in pharmacological studies it has been shown consistently that local injection of carbachol and other cholinergic agents into tne pontine reticular formation (FTG, RPO, RPC) that the cholinoceptive cells of this pontine area participate in

the state of PS, including atonia, PGO spikes, REMs and other variables of the state (Baghdoyan et al., 1985; George et al., 1964; Gnadt & Pegram, 1986; Shiromani et al., 1987a). It is not clear by what means the cells of the FTG region participate in the expression of PS or how they influence this state, although several possibilities have been proposed (Hobson & Steriade, 1986; Shiromani et al., 1987a; 1987b; Steriade & Hobson, 1976). One of such suggestions is that cells of the FTG region participate in PS probably by being activated through the innervation they receive from cholinergic cells, including those of the pontomesencephalic tegmentum and maybe even those of the basal forebrain (Shiromani et al., 1987a; 1987b).

The results from the present experiment do provide evidence of some loss of cholinergic innervation in the pontine FTG area after lesions of cholinergic neurons of the pontomesencephalic tegmental area. This loss is is very slight, however, compared to that seen in the medulla or thalamic areas. Other studies have also provided histological evidence that projections from the pontomesencephalic tegmental area to the FTG area exist (Jones & Yang, 1985; Ohta, Mori & Kimura, 1988), although these studies do not indicate the type of cells in the pontomesencephalic tegmentum providing the innervation to the FTG. The third criterion is not fulfilled by the cells of the FTG area, for as already noted in the Introduction,

extensive destruction of these cells does not affect the occurrence of PS (Drucker-Colín & Bernal-Pedraza, 1983; Friedman & Jones, 1984b; Sakai, 1985a, 1985b; Sastre et al., 1981).

The cells of the pontomesencephalic tegmental area fulfill the first criterion. Based on electrophysiological recordings, Sakai (1985a, 1985b) found that cells located in the area where cholinergic cells were identified by acetylcholinesterase stain and Chat immunohistochemistry, fire tonically in relation to PS. Sakai calls these the PS-on cells. On the other hand, the PS-off cells, also located in the pontomesencephalic tegmental area and raphe, and which are probably catecholaminergic and serotonergic, fire tonically in relation to waking, and stop firing during PS. According to this theory, in order for PS to ensue, all PS-on cells must be firing, and all PS-off cells must be silent (Sakai, 1985a). This theory is the electrophysiological equivalent of the neuropharmacological model suggested by Karczmar and his colleagues (1970) and later also by others (Hobson & Steriade, 1986).

The cells of the pontomesencephalic tegmental area also fulfill the second criterion, for there is some indication that injection of cholinergic agents into this region elicits PS (Baxter, 1969; Katayama, DeWitt, Becker & Hayes, 1984; Mitler & Dement, 1974). However, ideas regarding the cholinoceptive nature of these cells is not free of

controversy, since there is also evidence that perfusion of acetylcholine into this area promotes slow wave sleep and reduces PS (Masserano & King, 1982). In contrast, the cholinergic nature of cells in the dorsolateral pontomesencephalic tegmentum is clearly established (Jones & Beaudet, 1987a, 1987b; Sakai et al., 1986), and the findings in the present study show that these cells are critically involved in the control of PS, and that their projections may affect other parts of the brainstem controlling the state as well as subsystems or variables of PS.

The cells of the pontomesencephalic tegmentum, in contrast to those of the FTG, also satisfy the third criterion, since electrolytic lesions in this area abolish or diminish PS (Jouvet, 1969, 1972; Jouvet & Delorme, 1965; Sastre et al., 1981). These reports are confirmed by conclusions drawn from the present study, which show that selective nerve cell body lesions that destroy the majority of the cholinergic cells in the pontomesencephalic tegmental area, result in a marked deficit in PS.

The findings in this study do not agree entirely with the description by Sakai (1985a, 1985b) of the distribution of cells in the pontomesencephalic tegmentum that are involved in the state of PS. Sakai (1985a, 1985b) indicates that such cells are located mainly in the medial part of the LC-alpha and the peri LC-alpha, which are the same areas that he proposes contain cells that are important

for the control of atonia. The results from the present study do not support Sakai's delimitation of the cells controlling the state of PS to such a circumscribed area. Rather, the present results show a high correlation between the entire population of cholinergic cells in the area of ChAT-positive and TH-positive cells and PS.

Based on unit recording studies, Sakai also maintains that the reciprocal connections between the cholinergic cells of the LC-alpha and peri LC-alpha and those of the cholinergic magnocellular neurons of the reticular formation of the ventromedial medulla are essential for the development of PS. Retrograde labelling studies also confirm the presence of connections between cells in these two areas (Sakai, 1980), though such studies do not indicate the type of transmitter used or produced by these cells. The combined histochemical and electrophysiological results from the present study support the idea of an interaction between these two cell groups, and furthermore indicates that some of these projections are from cholinergic neurons, insofar as there was a loss of cholinergic innervation in the magnocellular and FTG region of the medulla, due to the destruction of the cholinergic neurons in the pontomesencephalic tegmentum. Presumably, in addition to the destruction of the cholinergic cells in the pontomesencephalic tegmentum, the kainic acid lesions would have deprived the ascending, medullary, reticulo-tegmental pro-

jections of their target, an area which is required for the completion of the circuit made up of the connections between these two cholinergic cell groups. The integrity of such circuitry may be necessary for the control of some aspects of PS. Additional support for this idea can be deduced from the effect of transections at the pontomedullary junction (Webster et al., 1986). These transections also could have caused a disruption of the reciprocal connection between cholinergic cells in the pontomesencephalic tegmental area and the magnocellular region in the medulla and the consequent disruption, reduction or elimination of PS. Siegel, Tomaszewski & Nienhuis, (1986) have concluded from results of transections at the pontomedullary junction that the interaction of the medullary generator of ultradian periodicity with pontine mechanisms controls the triggering of PS.

The duration of the PS episodes in the present study was substantially reduced after the lesion. The number of such episodes was not changed for the group of cats as a whole, although it was reduced in a few. This common reduction in duration does not parallel exactly the effects of pharmacological blockade of cholinergic transmission after systemic administration of atropine and scopolamine, which decrease the number, but not duration, of PS periods in human subjects (Sitaram et al., 1978a, 1978b). From their pharmacological manipulations these authors (Sitaram et al.,

1978a, 1978b) suggest that the timing mechanism of PS is under the excitatory control of brain acetylcholine, and that this mechanism may be dissociable from the factors controlling the duration of PS. Similarly, in the study by Sutin, et al. (1986) cited in the Introduction, prolonged scopolamine treatment also led to alterations in the number, but not duration of PS episodes in rats. However, the results from the present study suggest that duration of PS episodes may also be controlled by brain cholinergic mechanisms. Besides, the present results complement those from an earlier report by Jouvet and Michel (1960) that reduction of acetylcholine levels in cats by atropine administration led to a decrease in the duration of PS episodes, whereas enhancement of acetylcholine levels by the administration of physostigmine caused an increase in the duration of PS episodes, and that in neither case was the number of such episodes affected. Evidently, altering the level of acetylcholine in humans in acute studies as Sitaram and his collaborators did with pharmacological agents did not necessarily affect the PS cholinergic timing mechanism and duration of episodes in a directly parallel way to the results of the present study in cats, in which the destruction of cholinergic neurons in the pontomesencephalic area presumably decreased the level of acetylcholine available to the PS mechanism. In any event, even though the results obtained in cats are different from those in human

subjects and rats, the point of common interest is that the PS timing mechanism may be cholinergic. The discrepancies may be due as much to the different methods as to the different species involved in the studies, for it is not inconceivable that the PS cholinergic timing mechanism operates on a different basis in cats and rats and humans.

In summary, from the results of the present experiments it is concluded that the entire population of cholinergic neurons of the pontomesencephalic tegmentum are importantly involved in the control and generation of the state of PS. Such a conclusion is based on the fact that there was a decrease in the amount of PS which correlated well, not only with the number of ChAT-positive cells, but also with the area that normally contains noradrenaline and acetylcholine cells. This finding suggests that cholinergic cells in this area are important for the state of PS, and that these neurons are found in a more extensive area than the LC-alpha and peri LC-alpha, which Sakai (1985a, 1985b) had suggested were important for the expression of PS. The pontomesencephalic tegmental region may also be one of the areas supplying innervation to cholinoceptive cells within the FTG area, which might explain the participation of the FTG in PS. The prediction by Sakai (1985a, 1985b) that the medulla participates in the control of PS is also supported by the results in the present study, since there was a

substantial loss of cholinergic innervation to this structure as a result of the lesion.

1. EEG Desynchronization During Paradoxical Sleep.

Acetylcholine and its agents are known to exert a powerful control over the EEG of PS. The early experiments by Hernández-Peón (1965) and his contemporaries have shown that cortical EEG of PS may be elicited by acetylcholine and its agonists. The antagonists may also have a powerful effect on cortical desynchrony during PS as was demonstrated by Henriksen, Jacobs and Dement (1972), who injected atropine sufate intraperitoneally in cats deprived of PS for five days. Even though other variables of PS were in evidence after the deprivation period, the EEG desynchony was eliminated and replaced by high-amplitude waves during the PS-rebound period. In contrast to those reports that are based on the administration of pharmacological agents, the results from the present study show that destruction of the majority of cholinergic neurons in the pontomesencephalic tegmentum with kainic acid has no effect on EEG desynchronization during PS, not even an acute effect at the beginning of the experiment.

These results would not have been predicted, given that numerous studies have associated the pontomesencephalic tegmental with the control of EEG desynchronization during PS (Jouvet, 1969, 1972, 1975; Sakai, 1985a, 1985b). The

caudal two-thirds of the LC was thought to be important for the various aspects of PS, including cortical desynchrony, and electrolytic and 6-OHDA lesions of the LC disrupted PS and cortical activation. However, these lesions were very large and also destroyed other structures in the vicinity of the LC, including the non-noradrenergic neurons of the pontine reticular formation. In addition, subsequent studies showed that decreases in noradrenaline or lesions confined to the LC-noradrenaline neurons had no significant, lasting effect on the amount of PS or cortical activation in this state (Jones et al., 1977). Rather, as has been indicated previously in this thesis, the noradrenaline system is most likely associated with modulating cortical desynchrony during waking (Hobson & Steriade, 1986; Jacobs & Jones, 1978; Jones et al., 1977; Karczmar et al., 1970; Sakai, 1985a, 1985b).

In the present study, to the loss of cholinergic neurons in the pontomesencephalic tegmentum, was accompanied by a loss of cholinergic innervation at different levels of the forebrain and brainstem, including the thalamus and medulla, which are relevant to the desynchronization process in PS (Sakai, 1985a, 1985b; Steriade & Hobson, 1976). In the diencephalon, there was a loss of innervation to the neurons of the thalamus, including the intralaminar and reticularis nuclei. However, as noted above, extensive destruction is needed in both the thalamus and hypothalamus in order for

cortical desynchronization to be affected (Jouvet, 1962). Evidently, neither the loss of cholinergic cells in the pontomesencephalic tegmentum nor the loss of innervation to other brainstem and forebrain structures had any effect on cortical desynchrony of PS in the present study.

In retrospect, however, the lack of effect on EEG desynchrony by destruction of these neurons is not entirely surprising, since it has been indicated by several authors that at the level of the pons and midbrain the mechanism of cortical desynchrony is diffuse, thus making it difficult to disrupt the process (Candia et al., 1967; Friedman, 1983; Hobson, 1965; Sakai, 1985a, 1985b). Contributions to the maintenance of cortical desynchrony may have come from the influence of other subcortical structures not damaged by the lesion. As outlined by Sakai (1985b), these structures could include the midbrain reticular formation, the RPO and RPC, raphe magnus and the nucleus reticularis magnocellularis of the medulla. These areas provide fibers that course to the thalamus and hypothalamus, which in turn activate the cortical EEG by means of thalamocortical and hypothalamocortical projections. Therefore, in the present study, even though cells from the pontomesencephalic tegmental area that contribute fibers to the structures involved in cortical activation were destroyed by the kainic acid injections, their absence would not significantly affect the flow of activating influences to the cortex. The regular innervation

from other activating sources in the brainstem, together with surviving innervation from the pontomesencephalic tegmentum, were apparently sufficient for maintaining cortical desynchrony during PS.

Among the systems which helped to preserve cortical activation during PS would be the one that controls cortical activation during waking. In addition to the evidence that has been reviewed in the Introduction regarding the similarity between these two systems, it has also been shown that cortical activation of PS and waking may be dependent on the same mechanism (Steriade, 1981; Steriade & Deschenes, 1984). Neurons of the midbrain reticular formation with high firing rates in PS and waking, and low rates in slow wave sleep, project to the intralaminar nuclei of the thalamus and also to the zona incerta. Neurons in these two latter structures in turn project to many areas of the cortex, and there is further evidence indicating that transmission is enhanced in the thalamocortical system during PS and waking relative to slow wave sleep (Steriade & Deschenes, 1984). The increased enhancement is probably due to lifting of the inhibition that reticularis thalami neurons exert on transmission in thalamocortical ones during slow wave sleep (Steriade, Domich & Oakson, 1986).

As noted above, neurons in the FTG area may represent another source of activation of the cortical EEG during PS (Steriade and Hobson, 1976), but these reports are

contradicted by others which show that destruction of FTG cells with kainic acid does not affect any aspect of PS (Drucker-Colín & Bernal-Pedraza, 1983; Sastre et al., 1981). In the present study there was no disruption of the cells in this area, except for the slight damage to neurons in the anterior pole of this region, due to diffusion of the kainic acid ventrally. There was also a slight loss of innervation to the FTG area by cholinergic neurons of the pontomesencephalic tegmentum. It is therefore possible that the PS-related cholinoceptive cells of the FTG region (Baghdoyan et al., 1985; George et al., 1964; Shiromani & McGinty, 1983) that fire specifically both phasically and tonically during PS, may have been involved in preserving cortical activation through their interaction with other systems in the brainstem, important in controlling EEG activation during PS, as has been suggested by Shiromani et al., (1987a; 1987b).

In the caudal pons, lesions in the FTL, rather than the FTG (Friedman, 1983; Friedman & Jones, 1984a, 1984b) also caused a disruption of cortical desynchronization of PS, which was periodically invaded by slow waves of higher amplitude, even though there was no such loss of cortical desynchrony during waking. In contrast to the present findings, Friedman and Jones report that there was a decrease in EEG amplitude in all three states. The overall effect was said to be due to the disruption of a widespread

system of neurons and fibers in the caudal pontine tegmentum which project rostrally and are involved in cortical synchronization. Friedman and Jones (1984b) also showed that destruction of the lateral portions of the RPC or the FTL was responsible for the loss of cortical desynchrony and PS, which supported the original theory of Jouvet (1962) that this region was crucial for PS.

The magnocellular region of the medulla, which also showed loss of innervation as a result of the kainic acid lesion in the pontomesencephalic tegmentum, may participate in PS and cortical desynchrony through its reciprocal connections with the pontomesencephalic tegmentum and with the thalamus and hypothalamus (Sakai, 1985a, 1985b). If it is true that these neurons participate in PS, enough of their reciprocal connections must have survived so as to preserve the occurrence of cortical EEG desynchronization during PS.

The magnocellular neurons of the medulla oblongata project via a ventral route to the caudal hypothalamus, or via a dorsal route to the medial and intralaminar thalamus and share reciprocal connections with the pontomesencephalic tegmentum (Sakai, 1985a, 1985b; Steriade, Sakai & Jouvet, 1984), with which they may interact as well in order to bring about cortical desynchronization in PS. Sakai (1985a, 1985b) suggests that these projections are spared with kainic acid lesions in the FTG, and this explains why

destruction of neurons in this area with kainic acid do not affect EEG desynchrony (Drucker-Colín & Bernal-Pedraza, 1983; Sastre et al., 1981), and why after electrolytic and thermolytic lesions in the FTL and FTG area, where these fibers may originate and through which they course, cortical desynchrony may be disrupted (Friedman & Jones, 1984b; Jones, 1979; Sastre et al., 1981). Further supporting evidence favoring such an interpretation is provided by the transection studies of Siegel et al. (1981) and of Webster et al. (1986).

Electrophysiological evidence suggests that the medullary neurons participate in cortical desynchronization during PS. A small population of medullary reticular cells in the FTG and FTL discharged tonically in relation specifically to the onset of cortical activation during PS (Netick, Orem & Dement, 1977). In another study, PS-related cells were found by Kanamori, Sakai and Jouvet, (1980) in the medullary magnocellular tegmental field. These cells were notably quiet during other states. Because the cells in this region project rostrally to the medial and intralaminar nuclei of the thalamus (Steriade et al., 1984) and fire tonically with respect to PS, it is believed such activity may be indicative of their control of PS variables, especially cortical activation of PS (Sakai, 1985b).

Finally, the possibility should be considered that cell destruction alone in the pontomesencephalic tegmental

area is not sufficient to disrupt cortical desynchrony and PS (Jouvet & Delorme, 1965) in the same way that extensive electrolytic lesions do. The latter lesions are able to disrupt cortical desynchrony probably because, in addition to cell bodies, they also destroy fiber tracts passing through the pontomesencephalic tegmentum (Jones et al, 1977; Lindsley et al., 1950).

To summarize, in the present study, the destruction of cholinergic cells in the pontomesencephalic tegmentum did not affect the EEG of PS in isolation from the effect on the the state of PS as a whole. The amplitude of the EEG was not affected in any way during PS episodes. The results were different from previous studies involving large lesions and in which the EEG of PS was disrupted. Those large lesions can be considered as affecting the EEG desynchronizing systems in a global manner, thus demonstrating the diffuse nature of the EEG mechanism at the pontine and midbrain level (Candia et al., 1967; Friedman, 1983; Henriksen, et al., 1972; Hernández-Peón & Chávez-Ibarra, 1963; Hobson, 1965). In contrast, the lesions made with kainic acid in the present study had no apparent selective effect on the EEG desynchronizing mechanism. Compared to the large lesions in the studies referred to above, the kainic acid lesion in the present study had a more restricted effect that was confined principally to PS. This effect was very potent and enduring and it influenced the PS mechanism as a whole.

2. EMG Atonia During Paradoxical Sleep.

The abolition of EMG atonia as a result of the kainic acid lesions, and its replacement by an elevated EMG during PS were evident for the duration of the experiment. There was no increase in tonus during waking, but in slow wave sleep during the first postlesion week, there was a slight increase in tonus, which was not statistically significant. These results indicate that cells in the dorsolateral pontomesencephalic tegmentum, especially in the region where ChAT-positive, TH-positive cells, and other histochemically unidentified cells are located, are of critical importance for the full expression of atonia. This finding is similar to that reported by Jouvet and Delorme (1965), that lesions in the area of the LC, which is located within the dorsolateral pontomesencephalic tegmental region, may abolish atonia. However, the present study is the first demonstration that destruction of cells and not fibers in the pontomesencephalic region produces the effect.

After reviewing the results of lesions and neuropharmacological studies within his laboratory, and taking into account reports that the LC was composed mainly of noradrenergic neurons, which provided most of the noradrenergic innervation to the brain (Dahlstrom & Fuxe, 1964), Jouvet and Delorme (1965) theorized that the caudal third of that nucleus was the site responsible for the control of muscle atonia, and that lesions in the caudal

third of the LC would deprive the atonia mechanism of its brainstem controlling influence, which would then allow the motor systems of the spinal cord to go into operation unopposed. They speculated that the fiber tract involved in inducing atonia might be the lateral tegmentoreticular tract, which had been described earlier by Russel (1955) as originating in the LC and projecting to the medullary reticular formation. Jouvet and Delorme (1965) assumed that the tegmentoreticular tract projected to the inhibitory area of the bulbar reticular formation. They rejected the possibility that either the medial, lateral or ventral reticulospinal tract (as described by Papez, 1926) was involved, since those tracts decussate and the medial lesions in the brainstem had no effect on atonia.

In later studies, Roussel (1967) reported that total bilateral lesion of the LC abolished atonia and other variables of PS for two to three weeks. However, the lesions made by Roussel (1967) were very large and extended far into the tegmentum ventrally, possibly damaging other fiber tracts and nerve cells. The abolition of atonia took place probably because such lesions involved the destruction of both ChAT-positive and TH-positive cells, as well as of fiber tracts, located ventral to the LC. Shortly after this report, Jones et al. (1977) also found that lesions confined to the LC eliminated atonia, and caused a reduction of PGO spikes during PS, but had only minimal effect on the amount

of PS, which had returned to levels not significantly different from normal by the second week postlesion.

The relevance of the noradrenergic LC system to atonia was called into question again when it was reported that small lesions in the dorsolateral pontine tegmentum, ventrolateral to and not including the LC, eliminated atonia during PS much more effectively than lesions involving direct destruction of the LC (Henley & Morrison, 1974), leading Morrison (1979) to conclude that there was no convincing evidence that brainstem noradrenergic neurons are involved in spinal inhibition. Furthermore, as elaborated above, pharmacological studies indicated that inhibition of the catecholamines with AMPT did not alter muscle atonia or PS in rats (Marantz & Rechtschaffen, 1967; for reviews see Jacobs & Jones, 1978; and Ramm, 1979).

There are indications that some cells in the LC may be active during PS (Chu & Bloom, 1974; Hobson et al., 1974a; 1974b; Morrison, 1979). However, most electrophysiological recording studies show that the majority of cells of the LC actually cease firing during PS, and thus cannot be assumed to play an active role in events of PS (Hobson et al., 1975). The results from the present study are in agreement with such a conclusion, since there was no correlation between the small number of TH-positive cells which were destroyed and the loss of atonia. In addition, such a conclusion is supported by Sakai's finding that the

neurochemical nature of the executive neurons is not monoaminergic since neurons that fire in relation to atonia do not label for TH, nor do cells that contribute fibers to the lateral tegmentoreticular tract. On the other hand, non-catecholaminergic cells in the pontomesencephalic tegmentum were found to fire tonically during PS (Hobson et al., 1975; Sakai, 1980).

The FTG region of the pons had been proposed as a possible site having influence on atonia (Jouvet, 1962, 1965) and subsequently was extensively investigated by the Hobson-McCarley team and their associates (Amatruda, et al., 1975; McCarley and Hobson, 1975), however, as noted above, these neurons may be implicated in the control of PS variables, including atonia, although their influence is not critical to these events. This conclusion is supported by the finding that thermolytic lesions of the FTL, rather than of the FTM or pontine FTG, eliminate atonia during PS (Friedman & Jones, 1984b).

These findings show that the location and type of cells in the brainstem controlling atonia cannot be narrowly defined, but may include several types of cells over a broad region. Contrary to the idea of a relatively diffuse system controlling atonia, Sakai and his associates (Kanamori et al., 1980; Sakai, 1985a, 1985b) have recently presented a rather specific model of atonia based on electrophysiological and anatomical data identifying two supraspinal struc-

tures as being responsible for the induction of atonia. One structure, located in the dorsolateral pontine area, comprises the LC-alpha and peri LC-alpha and the other comprises the nucleus reticularis magnocellularis of the ventromedial medulla, which corresponds in part to the Magoun and Rhines (1946) inhibitory area. In this scheme, pontine neurons play the role of command neurons and the medullary neurons play the role of relay neurons. The pontine neurons supposedly exert an excitatory effect on the medullary neurons via the lateral tegmentoreticular tract. These latter, in turn, via the ventrolateral reticulospinal tract, exert an excitatory influence on the interneurons of the spinal cord. These spinal interneurons then inhibit the spinal motoneurons. There are also reports that lesions of the LC-alpha and peri LC-alpha neurons by local injections of kainic acid result in abolition of muscle atonia (Sakai, 1980, 1985a, 1985b; Sakai et al., 1981), while lesions outside these areas, regardless of whether they are still within the general dorsolateral pontine tegmental area, have no effect on atonia. The lesions in Sakai's report are not clearly analysed or presented, and there is no indication of the extent of damage in terms of quantitative or histological evidence, and only one cat with these lesions was examined. In addition, no reference is made to the nonspecificity of damage to the catecholamine and other neuronal types.

Evidence suggesting support for Sakai's (1985a, 1985b) specific model comes from studies where stimulation of the nucleus RPO, which overlaps with the LC-alpha, depolarizes medullary reticular formation neurons and produces a long-latency hyperpolarization of lumbar motoneurons during PS (Chase, Enomoto, Murakami, Nakamura & Taira, 1981; Fung, Boxer, Morales & Chase, 1982). Morrison (1979) and Sakai (1980) separately proposed that in order for atonia to ensue, the peri LC-alpha and the LC-alpha had to exert a tonic, excitatory influence on neurons in the nucleus magnocellularis of the medulla. However, Morrison (1979) and Sakai (1985a, 1985b) differ regarding the nature of the influence exerted by the lateral reticulospinal tract originating in the medulla. Sakai (1985a, 1985b) suggests that the magnocellularis neurons exerted an excitatory influence via this tract on inhibitory interneurons of the spinal cord, which in turn inhibit the alpha and gamma motoneurons during PS. Morrison (1979), on the other hand, suggests that the magnocellular neurons exert an inhibitory influence directly on the spinal motoneurons.

Kanamori et al., (1980) also propose that neurons in the magnocellular nucleus of the medulla, which project to the spinal cord, and are probably involved with control of muscle atonia during PS. These neurons also have a slower conduction velocity than that of the medullary gigantocellular neurons, are probably cholinergic cells.

Additionally, these authors reported that neurons in the ventromedial medulla in this area fire specifically in relation to the state of PS, and results from injections of quisqualic acid into the medullary area show that destruction of these neurons disrupt neck muscle atonia for an extended period of time (Holmes, Webster, Zikman & Jones, 1988).

The fact that lesions of the pontine tegmentum can disrupt atonia, suggests that the medulla is not able to control atonia on its own. Apparently, interaction with other brain structures is critical for the medulla to perform its inhibitory function. In fact, Siegel et al. (1986) suggest that any such control that these medullary cells may exert on their own upon atonia is likely to be very limited, since it appears that medullary units are similarly active during PS and waking (Siegel, Wheeler & McGinty, 1979). In addition, stimulation of the Magoun and Rhines inhibitory area fails to induce atonia when disconnected from the pons, a finding which led Siegel et al. (1986) to the same conclusion as Sakai (1985a, 1985b), that the medullary area had to interact with the dorsolateral pontine tegmentum in order to effect atonia. In addition, Webster et al. (1986) have shown that transections at the ventral pontomedullary junction, where the tegmentoreticular fibers course, eliminate atonia, whereas transections in the dorsal part of the region have no effect.

Nonetheless, these dorsal fibers may participate in the control of atonia, since this condition is most effectively eliminated by transections involving both the ventral and dorsal fibers at the pontomedullary junction.

In the present experiment, the significant loss of cholinergic innervation in the FTG and FTM of the medulla, which correspond in part to the inhibitory region of Magoun and Rhines (1946), as well as the loss of other chemically unidentified sources of innervation from the pontomesencephalic tegmentum to the area of Magoun and Rhines, may have contributed to the severe loss of atonia during PS.

The conjecture (Sakai, 1985a, 1985b) regarding the atonia-inducing function of cholinergic agents on medullary neurons is based upon data not yet clear of controversy, because injections of carbachol into this area may cause either increased (Baghdoyan et al., 1985) or decreased muscle tonus (Shiromani & Fishbein, 1986) during PS. Shiromani and Fishbein (1986) failed to present any polygraphic samples or statistical data regarding this variable, which was recorded in their study. Therefore it is not known what the magnitude of their effect may have been.

Besides the areas in the brainstem which have been discussed, and which appear to participate directly in the atonia phenomenon, there are other areas whose influence may only become evident under special experimental conditions.

For example, the work by Chase and his colleagues (Chase, 1980; Chase & Wills, 1979; Wills & Chase, 1979) indicate that the system controlling atonia may be diffuse and extensive throughout the brain and functionally state-dependent, since electrical stimulation of several sites within the reticular formation was able to inhibit the jaw muscle reflex in cats during PS. In contrast, these same sites were also found to facilitate the same reflex during waking, thus showing that activity in mechanisms underlying atonia may be highly state-determined.

In summary, results from the present study indicate that the neurons of the pontomesencephalic tegmentum are involved in the induction of muscle atonia. The data show that even though there was a significant destruction of the cholinergic neurons in this area, muscle tonus was not correlated with the number of remaining cholinergic cells. Neither was there any direct relationship between tonus and the existing TH-positive cells. Instead, there was a strong relationship between tonus and the volume of the area occupied by both classes of neurons, as well as between the amount of tonus and total volume of the area destroyed in the pontomesencephalic tegmentum. These results suggest that neither noradrenaline nor acetylcholine alone may be responsible for muscle atonia during PS, but that an interaction between these transmitters may take place in order to bring about the event, and such an assessment is in

agreement with the proposal put forward by Karczmar et al. (1970). In addition, cells in the pontomesencephalic tegmentum that contain as yet unidentified transmitters, may also take part in the induction of atonia. As demonstrated in the present study, the pontomesencephalic cholinergic cells project to the medullary reticular formation, where cells had been shown from early experiments (Magoun & Rhines, 1946) to participate in atonia of PS. These pontomesencephalic cholinergic neurons, as well as other yet chemically unidentified pontomesencephalic neurons with descending medullary projections, may promote atonia by influencing activity in the ventromedial medullary reticular formation during PS.

3.0 PGO Spikes of PS.

The results from the present study show that the cholinergic neurons of the dorsolateral pontomesencephalic tegmental region are involved in the control of PGO spiking recorded in the LGN. This finding is in agreement with previous studies showing that cooling of this area (Laurent, Cespuglio & Jouvet, 1974; Laurent & Guerrero, 1975) caused the number of PGO spikes to decrease in frequency. Sakai (1980) has also reported that bilateral electrolytic lesions of the dorsolateral pontomesencephalic tegmental region eliminate PGO spikes from the lateral geniculate nucleus as well as from the occipital cortex. From examination of the

polygraphic records, there was also a noticeable decrease of REMs during the state of PS, which agrees with earlier reports that this phasic variable is also dependent on pontine cholinergic neurons, since enhancement of acetylcholine levels by intravenous injections of eserine elicts REMs readily (Pompeiano, 1980).

The cholinergic neurons of the pontomesencephalic tegmental region, which were destroyed by the kainic acid injections in the present study, appear to comprise part of the anatomical network over which PGO spikes may be generated and conveyed. This region has well-established connections to the lateral geniculate bodies in the cat (Ahlsen & Lo, 1982; Laurent et al., 1974; Maeda et al., 1973). These neurons are found within all the nuclei of the dorsolateral pontomesencephalic tegmentum, including the PPT, LDT, parabrachial and LC nuclei. The PPT encompasses or includes the region that Sakai (1980) calls the X-area, where neurons have been recorded that fire in association with PGO spikes from the LGN (Sakai, 1980, 1985a, 1985b; Sakai et al., 1986). On the bases of HRP tracing and lesioning and electrophysiological work, Sakai (1980) concluded that all the ascending information concerning PGO spiking was generated within the X-area or relayed via this area to the lateral geniculate body.

Cells of this X-area stain positively for ChAT (Armstrong, Saper, Levey, Wainer & Terry, 1983; Jones &

Beaudet, 1987a, 1987b; Kimura et al., 1981; Sakai et al., 1986), which taken together with the foregoing information on this PGO system, suggests that the production of PGO spikes is dependent on cholinergic neurons. The results in the present study confirm this indication, since they show that there is 1) a correlation between PGO spikes and the number of surviving ChAT-positive cells in the pontomesencephalic tegmentum, and 2) loss of cholinergic innervation in the dorsal lateral geniculate following lesions of the dorsolateral pontomesencephalic tegmentum. As noted in the Introduction, such connections are of importance in recording those spikes in the LGN. The present findings therefore support Sakai's assessment regarding the importance of the cholinergic cells in this area for the production of PGO spikes. In particular, the importance of cells in the so-called X-area is supported, since the rate of PGO spikes is most highly correlated with the number of surviving ChAT-positive cells in the PPT nucleus (r=.78), a higher correlation than between the rate of PGO spikes and the number of cholinergic cells in the total pontomesencephalic tegmentum.

Pharmacological studies have provided further evidence regarding the cholinergic nature of the PGO production system. Increased activation of the chol nergic mechanism by pharmacologic means, such as administration of cholinergic agonists (Baghdoyan et al., 1985; Baghdoyan et

al., 1984; Vivaldi et al., 1980) increases PS, including the release of PGO spikes, whereas pharmacologic inhibition of the cholinergic system with anticholinergic agents such as atropine (Jouvet, 1961, 1962, 1975) may lead to a decrease of PGO spiking.

Even though the data from the present study and examples from previous studies (Jones et al., 1977; Jouvet, 1972, 1975; Laurent et al., 1974; Sakai, 1980; Sakai, 1985a, 1985b) show that the pontomesencephalic tegmental area controls PGO spiking in the LGN, it is possible that other locations contain neurons which function as PGO-on cells (Amatruda et al, 1975; Magherini, Pompeiano & Thoden, 1971; Steriade & Hobson, 1976; Steriade et al., 1984). In experiments designed to isolate major regions of the brain involved in the control of PGO spiking and of PS in general, Jouvet (1962, 1965) showed that total transection of the brainstem rostral to the pons or through the caudal midbrain did not eliminate PGO spikes in the brainstem caudal to the transection, which indicates that in the brainstem caudal to the pontomesencephalic tegmentum, there may be mechanisms actively involved in PGO spike generation. Another explanation might be that this transection by Jouvet (1962) may have left enough pontine tegmental cholinergic cells intact and in contact with pontine (FTG-FTL) cells via descending projections. Furthermore, after electrolytic lesions aimed at destruction of the LC area in the

dorsolateral pontine tegmentum, in an area somewhat more caudal than was affected in the present study, PGO spike rate was severely reduced, though it was not eliminated (Jones et al., 1977). Similar results were obtained in the work mentioned above by Laurent and Guerrero (1975) after cooling the area around the brachium conjunctivum. They found that spikes were reduced, but not eliminated. Therefore, the operations in these studies which resulted in partially damaged, destroyed or temporarily inactivated cells of the dorsolateral pontine tegmentum, (many of which were probably cholinergic), led to the decrease, though not elimination of PGO spikes. A further possibility is that the cells that were spared may have helped to account for the continued appearance of PGO spikes, through their projections caudally to pontine sites involved in generating PGO spikes.

Steriade and Hobson (1976) have noted that in the pontine FTG, which is to a large extent within the RPC, neurons begin firing and reach their peak rate just prior to the onset of spiking in the lateral geniculate body. During PS, discharges in these two neuronal populations become phase-locked. PGO spikes were hypothesized to be generated by the cells of the pontine FTG because of a) their engagement in the tight phase-locking; b) the temporal precedence in firing of the cells of the FTG over others; and c) the rhythmic discharge of the FTG cells at the same

fundamental frequency as that of the PGO spiking activity (Hobson et al., 1975; Steriade & Hobson, 1976). As was discussed above, however, the destruction of the FTG neurons with kainic acid failed to affect any aspect of PS, including the occurrence of PGO spikes in the LGN, thus indicating that the FTG region is not critical for the occurrence of these waves (Drucker-Colin & Bernal-Pedraza, 1983; Sastre et al., 1981), even though the cells in this region may participate in the event.

According to Friedman and Jones (1984b), PGO spikes are probably an expression of the phasic activation and massive recruitment of ascending reticular pathways that originate in the pontine reticular formation, and where no single population of cells can be considered as being solely responsible for the production of PGO waves. For example, destruction of the neurons of the FTG by large radiofrequency lesions (Friedman & Jones 1984b; Jones 1979) caused a drastic reduction, but not elimination of PGO spikes. Accordingly, these authors suggested that the system that is critical for controlling PGO spikes is located within the pontine tegmentum between the abducens nucleus at P6 and the trochlear nucleus at A1. Shortly after, Siegel, (1985) on the basis of complete transections through the brainstem at several different levels, located a region near that of Friedman and Jones (1984b), between the abducens and the LC, which he thought was "sufficient" to generate the

signs of PS. However, he also thought that the most caudal regions of the pons were necessary for the "appearance" of PGO spikes during EEG desynchrony.

In this respect, structures in several different areas of the brain may influence various aspects of PGO spiking. For example, the firing pattern of LGN spikes may be controlled by the cortex, for in a pontine or a decorticate cat the firing pattern is different from the normal cat, in that bursts of 4-5 spikes are separated by 4-5 seconds intervals (Gadea-Ciria, 1972; Jouvet, 1972). It is also seen that discharge in multiple units recorded from the parabrachial nucleus and the midbrain reticular formation precede the onset of PGO spikes from the lateral geniculate bodies during PS (Ogawa, 1985). Apparently, PGO spikes can be elicited, at least by means of carbachol injection, from a variety of sites within the pontine tegmentum, including the LC (Mitler & Dement, 1974) and the caudal part of the pontine FTG (Baghdoyan et al., 1985). Furthermore, reticulo-reticular connections, as well as connections between the pons and medulla may be required for the normal expression of these waves, because if cats are transected at the pontomedullary junction, which disrupts several different kinds of fibers, possibly including fibers from cholinergic and noradrenergic cells in the medulla, the PGO spiking pattern is disrupted and the number of spikes is reduced (Siegel, 1985; Webster et al., 1986). The effect

ranges from mild suppression to total elimination of these waves during PS, depending on the extent of the damage, and the type of fibers transected (Webster et al., 1986). With the transection, fibers originating in cholinergic and catecholaminergic neurons, and descending from the dorsolateral pontine area, including the LC, and fibers ascending from the medullary magnocellular reticular formation, the FTG and FTL, and from the raphe as well, were severed. In the present study, there was an appreciable loss of cholinergic projections to the FTG and FTM in the medullary area. Disruption of the connection between these structures and the pontomesencephalic tegmentum may have a long-lasting effect on the production of PGO spikes.

The role played by the noradrenergic cells in the generation of PGO spikes cannot be fully assessed from the data analyzed in this study. Reports of the participation of these neurons in PGO production is often controversial. As noted in the Introduction, there is evidence linking these cells to the production of PGO spikes (Jouvet, 1969, 1972), or to the inhibition of spikes (Hobson, 1973; Jacobs & Jones, 1978; Steriade & Hobson, 1976) or as performing a passive gating function in controlling the release of the spikes (King & Jewett, 1971). However, in the present study, the lack of correlation between noradrenaline cells and PGO spikes indicates that these cells are certainly less

important than the cholinergic neurons for bringing about the event of PGO spikes.

In summary therefore, even though various structures of the brain and several neurotransmitters or modulators may participate in the expression of PGO spikes, the results from the present studies indicate that the cholinergic neurons of the pontomesencephalic tegmentum are critical for their generation and production. Electrophysiological studies show that these spikes may be recorded in multiple areas of the brainstem and forebrain in the normal animal. They may also be enhanced or inhibited after local administration of cholinergic agents in several areas of the brainstem and forebrain. Lesion studies show that the rate of these spikes may be reduced after partial destruction of the dorsolateral pontomesencephalic tegmentum, or of the FTG-FTL regions by means of electrolytic and thermolytic lesions, or after transections in the brainstem. However, destruction of cells in the FTG area by local injection of the neurotoxic agent kainic acid into the pontine FTG area has no effect on PGO spike rate. In contrast, results in the present study show that destruction of the cholinergic cells in the dorsolateral pontine tegmentum by local injections of kainic acid may either eliminate these spikes for a period of time or reduce their rate in direct relation to the number of cells destroyed. In contrast, there is no correlation between the number of intact TH-positive cells

and PGO spikes, suggesting neither a strong nor direct influence of noradrenaline on these spikes. Such results support the argument that the cholinergic cells of the pontomesencephalic tegmental area play a critical role in the generation and expression of PGO spikes during PS, and they corroborate Sakai's unit recording findings that the cells of the X-area are especially important in the phenomenon of PGO spike generation.

SUMMARY and CONCLUSION

From analysis of the results of the present study, it is evident that destruction of cholinergic neurons of the dorsolateral pontomesencephalic tegmentum had differential effects on the three states in the sleep-wake cycle, as well as on the variables defining these states. Following the lesion there was an increase in the amount of waking, which must have been a secondary effect to the severe reduction of the amount of PS, for there was only a minimal decrease in the state of slow wave sleep. These results contrast with those obtained after large electrolytic lesions in this region (Jones et al, 1969; Lindsley et al., 1950), which caused a drastic reduction in the amount of waking and an increase in cortical synchronization and slow wave sleep-like or comatose behaviour.

The results of the kainic acid lesions on waking in the present study were also contrary to what was expected in view of the results obtained in studies in which pharmacologic agents have been used to enhance cortical activation of wakefulness (Domino et al., 1968; Gillin et al., 1978b). In such studies cholinergic agonists had an activating effect on the EEG, in waking as well as during PS, and antagonists had a blocking effect on cortical activation. Destruction of the cholinergic cells of the pontomesencephalic tegmentum in the present study did not

abolish cortical activation nor replace it with higher amplitude waves. It was generally believed that cholinergic cells in the area of the pontomesencephalic tegmentum were the source of the fibers of the ARAS, which maintained cortical activation (Rinaldi & Himwich, 1955b; Shute & Lewis, 19673, 1967). The results from the present study suggests the cholinergic cells of the pontomesencephalic tegmentum are not critical for the maintenance of the state of wakefulness. In view of the apparent lack of effect of destruction of the pontomesencephalic tegmental cholinergic neurons upon wakefulness, it is possible that the integrity of this state is maintained through the continued influence of the cholinergic cells of the basal forebrain on the cortex, as well as the influence of cholinergic cells of the cortex itself, and maybe even the surviving cholinergic cells in the pontomesencephalic tegmentum as well. In addition, non-cholinergic cells of the pontomesencephalic tegmentum and fibers coursing through the area from caudal pontine to medullary reticular neurons may also contribute to the maintenance of cortical wakefulness. These cells and fibers were probably destroyed by the large electrolytic lesions of Jones et al. (1969) and Lindsley et al. (1950), which may have accounted for the severe disruption of waking noted in those studies.

There was no change in the amount of slow wave sleep as a result of the lesion to the dorsolateral pontine

tegmentum. This outcome was not unexpected, since there is no strong, consistent evidence linking this state to the influence of acetylcholine. The increased number and decreased duration of the slow wave sleep episodes was probably a secondary effect of the disruption of the state of PS by the lesions.

The most potent effect of the lesion was on the state of PS. There was a permanent reduction of the amount of PS and a consistent decrease in the duration of the episodes, which were correlated with the number of ChAT-positive cells. This effect on the reduction of the amount of PS was in accordance with expectations, for as already mentioned above, numerous pharmacological studies have indicated that acetylcholine is essential to the state of PS (Domino et al., 1968; Gillin & Sitaram, 1984; Gillin et al., 1985). In terms of the anatomical location of the effective lesion for eliminating PS, the present results confirm those of earlier studies. On the basis of lesions it had been determined that the cells in the dorsolateral pontine tegmental area are essential for the state of PS (Jouvet, 1962, 1972, 1975), even though at the time it was believed that acetylcholine played only a minor role in the expression of PS, and that the main effect was as a result of the influence of noradrenaline. The present results also confirm Sakai's (1985a, 1985b) prediction that non-monoaminergic cells in this area are critical for PS.

However, these cells which were identified in the present study as cholinergic, and which correlate with PS, are not confined to the LC-alpha and peri LC-alpha as predicted by Sakai. Rather, the cells of the entire ChAT-TH area are apparently involved in controlling the expression of the state of PS.

Results from the present study indicate that destruction of cholinergic neurons in the pontomesencephalic tegmentum caused a loss of cholinergic innervation in the brainstem areas. The anatomical studies reviewed indicate that these cholinergic cells in the pontomesencephalic tegmental area send out fibers which project heavily to the thalamus as well as to structures in the pontine and medullary regions. The rostral projection is commonly identified with the ARAS. By means of these projections the cholinergic cells of the pontomesencephalic tegmentum may control the mechanisms which constitute the bases for the state as well as individual variables of PS. The destruction of these dorsolateral pontomesencephalic tegmentum cells may have a selective effect on certain variables of PS. For example, cortical desynchronization of PS was not selectively affected by the kainic acid lesions in the pontomesencephalic tegmental area, whereas PGO spikes were chronically suppressed or virtually eliminated for periods of several weeks. EMG atonia during PS was also chronically absent after the destruction of cells in the dorsolateral

pontine tegmentum and surrounding area. However, neither the surviving cholinergic nor noradrenergic cells on their own correlated with EMG amplitude. Instead, the volume of tissue destroyed in the area of the ChAT-TH cells in the pontomedullary tegmentum as well as the total volume of affected tissue, correlated very well with EMG amplitude. Such correlations are interpreted as showing that atonia may be dependent on the interaction between neurons of different transmitter types, including noradrenaline, in the dorsolateral pontine tegmentum.

The conclusion to be derived from these results is that the cholinergic cells of the pontomesencephalic tegmental area are critically involved in the expression of the state of PS, and most particularly, in PGO spike generation during PS. These pontomesencephalic tegmental cholinergic neurons are also important for the control of muscle atonia, but appear to share this task with other cells within the dorsolateral pontomesencephalic tegmentum.

Appendix A - Table A1

Total Area of Necrosis From Levels A3 to P5 on Left and Right Sides of the Brainstem Tegmentum of 11 Cats After Kainic Acid Lesions

	KA4			KA5			KA6		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A2	9.2	6.3	15.5	3.1	3.7	6.8	4.2	0.0	4.2
A1	12.5	12.1	24.6	6.3	4.4	10.7	9.2	8.2	17.4
AP0	11.6	13.6	25.2	7.7	6.7	14.4	13.2	9.3	22.5
P1	13.2	13.1	26.3	7.1	6.5	13.6	11.6	7.1	18.7
P2	13.4	13.4	26.8	4.4	4.2	8.6	8.0	8.8	16.8
P3	5.3	8.0	13.3	5.2	3.8	9.0	10.7	8.6	19.3
P4	3.5	10.3	13.8	1.9	2.3	4.2	6.1	6.6	12.7
P5	2.0	3.6	5.6	1.0	.8	1.8	0.0	0.0	0.0
SUM	70.7	80.4	151.1	36.7	32.4	69.1	63.0	48.6	111.6

	KA8			KA11			KA12		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A2	4.5	4.0	8.5	2.7	1.6	4.3	0.0	0.0	0.0
A1	10.2	10.9	21.1	12.4	13.0	25.4	0.0	0.0	0.0
AP0	10.8	9.7	20.5	10.5	8.5	19.0	6.5	10.0	16.5
P1	12.8	14.8	27.6	9.9	12.6	22.5	10.8	12.9	23.7
P2	9.0	7.7	16.7	11.4	12.5	23.9	9.3	8.9	18.2
P3	7.3	7.6	14.9	8.2	11.0	19.2	8.1	9.6	17.7
P4	6.7	3.3	10.0	4.9	5.7	10.6	3.1	2.1	5.2
P5	4.7	0.0	4.7	1.9	2.5	4.4	3.4	3.9	7.3
SUM	66.0	58.0	124.0	61.9	67.4	129.3	41.2	47.4	88.6

	KA14			KA15			KA17		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A2	0.0	0.0	0.0	0.0	0.0	0.0	4.7	0.0	4.7
A1	0.0	0.0	0.0	6.5	7.8	14.3	4.3	3.2	7.5
AP0	9.9	10.4	20.3	11.8	14.1	25.9	8.8	9.2	18.0
P1	10.4	12.3	22.7	18.5	19.7	38.2	17.0	17.4	34.4
P2	8.4	8.9	17.3	12.3	12.6	24.9	10.1	8.5	18.6
P3	5.1	5.5	10.6	14.3	14.9	29.2	11.9	10.1	22.0
P4	1.9	1.6	3.5	4.5	4.4	8.9	8.8	8.9	17.7
P5	0.0	0.0	0.0	0.0	0.0	0.0	4.3	4.4	8.7
SUM	35.7	38.7	74.4	67.9	73.5	141.4	69.9	61.7	131.6

	KA18			KA19			AVERAGE		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A3	3.7	3.2	6.9	0.0	0.0	0.0	.3	.3	.6
A2	7.9	7.7	15.6	3.3	2.3	5.6	3.6	2.3	5.9
A1	14.8	14.5	29.3	13.9	11.0	24.9	8.2	7.7	15.9
AP0	15.2	14.9	30.1	14.8	17.0	31.8	11.0	11.2	22.2
P1	16.8	16.2	33.0	15.2	12.1	27.3	13.0	13.2	26.2
P2	14.4	15.0	29.4	14.3	16.1	30.4	10.5	10.6	21.1
P3	6.4	6.7	13.1	5.8	6.5	12.3	8.0	8.1	16.4
P4	4.5	4.7	9.2	6.6	8.6	15.2	4.8	5.3	10.1
P5	0.0	0.0	0.0	1.4	1.1	2.5	1.7	1.5	3.2
SUM	83.7	82.9	166.6	75.3	74.7	150.0	61.1	60.5	121.6

Footnote: Values are area in square mm. Sum corresponds to square mm. area, but also to cubic mm. volume, since area measurements are at 1 mm. intervals (Volume=Surface Area X Depth). This footnote also applies to Table A2 of Appendix A.

Appendix A - Table A2

Total Area of Necrosis on Left and Right Sides of the Brainstem Tegmentum of 11 Cats After Kainic Acid Injections in the Region Normally Occupied by ChAT+ and TH+ Cells.

	KA4			KA5			KA6		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	4.0	3.3	7.3	2.5	2.7	5.2	3.8	3.9	7.7
AP0	5.7	6.3	12.0	6.0	5.6	11.6	6.0	5.2	11.2
P1	4.9	5.5	10.4	6.8	6.1	12.9	8.5	4.9	13.4
P2	4.2	4.6	8.8	4.0	3.3	7.3	1.5	1.6	3.1
P3	.7	.6	1.0	0.0	0.0	1.0	1.9	1.5	1.0
P4	0.0	0.0	0.0	0.0	0.0	0.0	.7	0.0	.7
P5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SUM	19.5	20.3	39.8	19.3	17.7	37.0	22.4	17.1	39.5

	KA8			KA11			KA12		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	5.0	5.8	10.8	4.6	3.9	8.5	0.0	0.0	0.0
AP0	6.3	5.3	11.6	2.8	4.1	6.9	6.0	8.3	14.3
P1	6.7	5.9	12.6	2.4	4.9	7.3	7.1	7.0	14.1
P2	2.8	2.7	5.5	2.5	2.6	5.1	1.7	1.6	3.3
P3	.7	.8	1.5	1.7	2.0	3.7	.3	.5	.8
P4	.7	0.0	.7	.2	.4	.6	0.0	0.0	0.0
P5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SUM	22.2	20.5	42.7	14.2	17.9	32.1	15.1	17.4	32.5

	KA14			KA15			KA17		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	0.0	0.0	0.0	3.7	2.0	5.7	4.2	3.3	7.0
AP0	6.2	6.7	12.9	6.0	6.3	12.3	4.5	5.3	9.8
P1	4.7	8.4	13.1	10.1	9.5	19.6	7.2	8.3	15.5
P2	1.8	1.1	2.9	3.1	2.3	5.4	2.8	3.1	5.9
P3	.1	.1	.2	3.1	2.5	5.6	4.5	3.5	8.0
P4	0.0	0.0	0.0	0.0	0.0	0.0	.6	1.3	1.9
P5	0.0	0.0	0.0	0.0	0.0	0.0	.4	.5	.9
SUM	12.8	16.3	29.1	26.0	22.6	48.6	24.2	25.3	49.5

	KA18			KA19			AVERAGE		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	4.5	3.8	8.3	2.8	2.2	5.0	3.2	2.8	6.0
AP0	8.2	8.1	16.3	5.6	6.5	12.1	5.8	6.2	11.9
P1	8.4	8.7	17.1	6.6	7.8	14.4	6.7	7.0	13.7
P2	5.0	5.5	10.5	2.2	2.1	4.3	2.9	2.8	5.6
P3	.5	1.5	2.0	1.9	1.5	3.4	1.4	1.3	2.7
P4	.4	.6	1.0	.2	.5	.7	.3	.3	.5
P5	0.0	0.0	0.0	.1	.1	.2	.0	.1	.1
SUM	27.0	28.2	55.2	19.4	20.7	40.1	20.2	20.4	40.6

See footnote in Appendix A - Table A1.

Appendix B - Table B1

Total Number of ChAT+ Cells in Four Nuclei (PPT, LDT, PB and LC on the Right and Left Sides of the Brainstem Tegmentum From Level A1 to P5 in 6 Normal Cats.

	CHAT2			CHAT3			CHAT4		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	541	439	980	363	319	682	891	1268	2158
AP0	961	1817	2778	487	439	926	3470	4345	7815
P1	4524	4488	9013	5042	4534	9576	8748	8073	16820
P2	1760	2756	4516	2409	2341	4750	4878	3654	8532
P3	986	1139	2125	1053	1510	2563	1596	1158	2754
P4	532	2725	3257	753	2152	2906	1762	1926	3688
P5	187	205	392	184	209	394	93	115	208
SUM	9493	13569	23062	10292	11505	21797	21438	20537	41976

	CHAT5			CHAT14			CHAT15		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	519	496	1015	451	645	1096	1010	907	1917
AP0	1857	2206	4064	3053	2620	5673	5219	4890	10109
P1	4251	7266	11517	4952	5151	10103	5978	6040	12017
P2	3646	4624	8270	4285	4664	8950	5267	4898	10165
P3	1221	5405	6627	1872	2388	4260	1038	1067	2104
P4	2086	578	2664	3296	3230	6526	2344	2136	4480
P5	138	114	252	237	266	503	230	221	451
SUM	13719	20690	34409	18146	18964	37110	21086	20158	41243

	AVERAGE		
LEVEL	LEFT	RIGHT	SUM
A1	629	679	1308
AP0	2508	2720	5228
P1	5583	5925	11508
P2	3708	3823	7531
P3	1294	2111	3405
P4	1796	2124	3920
P5	178	188	367
SUM	15695	17571	33266

The values have been rounded off to the first significant digit, but the sums in the columns and rows are based upon the values up to the first decimal place. This footnote applies to the other tables in Appendix B.

Appendix B - Table B2

Total Numbers of ChAT+ Cells in Four Nuclei (PPT, LDT, PB and LC) From Level A1 to P5 on the Right and Left Sides of the Brainstem Tegmentum of 11 Cats Injected With Kainic Acid.

	KACH4			KACH5			KACH6		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	224	130	355	345	246	591	346	144	490
AP0	1058	802	1861	559	294	853	150	358	507
P1	3195	3017	6212	1066	2317	3383	275	1315	1590
P2	2757	2951	5708	559	1098	1657	252	795	1047
P3	1294	956	2250	366	729	1094	4358	4649	9007
P4	206	704	911	84	236	320	327	946	1273
P5	30	0	30	85	62	146	62	59	120
SUM	8764	8562	17326	3063	4982	8045	5769	8265	14034

	KACH8			KACH11			KACH12		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	286	214	500	578	309	887	259	760	1020
AP0	311	450	761	1391	1427	2818	2129	2068	4198
P1	3182	1768	4950	2080	3147	5227	3433	4027	7460
P2	129	691	821	425	1241	1666	4384	2841	7225
P3	452	180	632	532	870	1402	1037	660	1697
P4	595	2097	2692	348	1458	1806	3074	158	3231
P5	314	182	497	26	0	26	112	0	112
SUM	5270	5583	10853	5381	8451	13832	14428	10514	24942

	KACH14			KACH15			KACH17		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	323	350	673	483	651	1134	645	781	1426
AP0	1043	847	1890	427	414	840	2515	2460	4975
P1	1837	1102	2939	1201	937	2138	3235	3891	7126
P2	488	678	1166	288	239	528	1662	1488	3150
P3	1100	608	1709	76	317	393	285	795	1080
P4	348	3069	3417	106	181	287	63	219	283
P5	54	162	216	51	72	123	95	64	159
SUM	5194	6817	12010	2632	2811	5442	8501	9698	18199

	KACH18			KACH19			AVERAGE		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	254	0	254	354	462	816	373	368	740
AP0	682	655	1338	696	1056	1752	996	985	1981
P1	259	804	1063	690	721	1411	1859	2095	3954
P2	300	272	572	413	993	1406	1060	1208	2268
P3	438	706	1144	656	1268	1923	963	1067	2030
P4	2182	2286	4468	3854	3495	7349	1017	1350	2367
P5	82	56	138	198	119	317	101	71	171
SUM	4196	4780	8976	6860	8113	14973	6369	7143	13512

See footnotes in Appendix B, Table B1.

Appendix B - Table B3

Total Numbers of TH+ Cells in Four Nuclei (PPT, LDT, PB, and LC) on the Right and Left Sides of the Brainstem Tegmentum of 3 Normal Cats From Level A1 to P5.

	TH2			TH3			TH14		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	93	57	150	0	78	78	165	47	211
AP0	299	380	679	117	490	607	189	187	376
P1	877	949	1826	1225	1426	2652	852	745	1597
P2	2558	2889	5447	2447	2408	4855	3333	3352	6686
P3	3362	3986	7348	3557	3541	7098	4343	4920	9262
P4	3574	2993	6567	1250	1582	2832	2354	2902	5256
P5	953	808	1761	51	823	874	613	951	1564
SUM	11716	12061	23777	8648	10348	18996	11850	13104	24954

	AVERAGE		
LEVEL	LEFT	RIGHT	SUM
A1	86	61	146
AP0	202	352	554
P1	985	1040	2025
P2	2779	2883	5663
P3	3754	4149	7903
P4	2392	2492	4885
P5	539	861	1400
SUM	10738	11838	22576

See footnotes in Appendix B, Table B1.

Appendix B - Table B4

Total Numbers of TH+ Cells in Four Nuclei (PPT, LDT, PB and LC) From Level A1 to P5 on the Right and Left Sides of the Brainstem Tegmentum of 11 Cats Injected With Kainic Acid.

	KATH4			KATH5			KATH6		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	0	114	114	142	158	301	50	0	50
AP0	191	114	305	47	45	93	23	120	143
P1	1243	911	2154	144	299	443	536	421	956
P2	2983	1940	4922	1080	608	1688	1947	1916	3863
P3	3194	3342	6536	2786	2814	5600	1562	2907	4469
P4	558	918	1475	1298	1864	3163	618	788	1405
P5	756	1201	1957	368	218	586	164	69	233
SUM	8925	8539	17464	5866	6008	11874	4900	6220	11119

	KATH8			KATH11			KATH12		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	0	98	98	152	175	328	213	191	404
AP0	50	24	74	19	39	58	329	248	577
P1	641	420	1061	992	972	1964	174	515	690
P2	2090	2772	4861	1735	1999	3734	1687	2800	4488
P3	3652	3748	7400	2364	2728	5092	2272	2228	4500
P4	1510	1963	3472	694	855	1548	698	495	1193
P5	675	350	1025	51	663	714	51	57	108
SUM	8617	9374	17992	6007	7431	13438	5424	6534	11958

	KATH14			KATH15			KATH17		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	72	188	260	289	49	338	182	207	389
AP0	24	24	47	217	341	558	47	116	164
P1	454	510	964	72	47	119	371	280	651
P2	1644	1161	2805	912	1278	2190	2030	1913	3944
P3	2122	3460	5582	2039	2174	4213	2954	3168	6123
P4	1814	1974	3788	1781	1313	3094	943	1810	2753
P5	240	884	1124	389	373	762	409	442	851
SUM	6369	8201	14570	5699	5574	11273	6937	7938	14875

	KATH18			KATH19			AVERAGE		
LEVEL	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM	LEFT	RIGHT	SUM
A1	0	23	23	173	122	295	116	121	236
AP0	70	0	70	296	367	663	119	131	250
P1	304	232	536	306	387	693	476	454	930
P2	2012	3072	5085	3002	2300	5302	1920	1978	3898
P3	3208	3562	6770	3544	3570	7113	2700	3064	5764
P4	1145	1163	2308	2576	2499	5074	1239	1422	2661
P5	581	684	1265	998	894	1891	426	530	956
SUM	7322	8736	16058	10893	10139	21032	6996	7699	14696

See footnotes in Appendix B, Table B1.

Appendix C (Results).

CLINICAL NEUROLOGICAL TESTS

Cat Number	4	5	6	8	11	12	14	15	17	18	19
Tactile Function	Week in Which Function was Noted[1]										
evasion reflex	1	1	1	1	1	1	1	1	1	2	1
contact placing	1	1	1	2	1	1	1	1	1	2	1
light pressure response	2	1	1	3	1	1	1	1	3	2	1
displacement hair response	N	N	1	N	1	1	2	1	3	N	2
Visual Function											
threat blinking	1	1	1	2	1	1	1	1	1	1	1
surface placing	1	1	1	2	1	1	1	1	1	2	1
orientation	1	1	1	2	1	1	1	1	1	2	2
following	1	1	1	2	1	1	1	1	1	3	2
Auditory Function											
noise startle	1	1	2	2	1	1	1	1	1	2	2
orientation	1	1	2	2	1	1	1	1	1	3	2
Olfactory Function											
sniffing	1	1	1	2	1	1	1	1	1	2	1
orientation	1	1	1	2	1	1	1	1	1	2	1
Proprioceptive Function											
resistance	1	1	1	1	1	1	1	1	1	2	1
flexibility	1	1	1	1	1	1	1	1	1	2	1
adjustment	1	1	1	1	1	1	1	1	1	2	1
hopping and placing	1	1	1	1	1	1	1	1	1	2	1
fixation in rotation	2	1	1	1	1	1	1	1	1	1	1
free-fall righting	2	1	2	2	1	1	1	1	2	2	2
tactile placing	1	1	1	2	1	1	1	1	1	2	2
Sensory Motor Integration											
sleeping sphinx	1	1	2	4	1	1	1	1	2	2	2
sleeping curled	1	1	1	2	1	1	1	1	3	2	2
waking sitting	1	1	1	2	1	1	1	1	1	2	2
waking standing	1	1	1	2	1	1	1	1	1	2	1
walking	1	1	1	2	1	1	1	1	1	2	1
climbing	1	2	2	2	1	1	1	1	2	3	2
jumping	1	2	2	2	1	1	1	1	2	3	2
Nociceptive Response											
reflex	1	1	1	1	1	1	1	1	1	1	1
localization	1	1	1	1	1	1	1	1	1	3	2

[1] 1,2,3,4: postlesion week in which a function was noted. N: the function did not reappear. See definition of terms on the following two pages.

Appendix C (Continued): Definitions.

CLINICAL NEUROLOGICAL TESTS

Tactile Function

Evasion reflex: touch the feet of the cat and note if it moves them to evade the pressure.

Contact placing: holding the cat in the air, move it towards a surface to note if once it contacts the surface it places its feet on the surface and extends its claws to hold onto the surface. The cat must not see the surface, and the test is made with the forelimbs.

Light pressure response: apply light pressure to respective parts of the cat's body (tail, feet, ears, eyes, mouth, vibrissae) and note if it responds by moving its skin or orienting in some manner to the stimulus.

Displacement hair response: touch the hair of the cat lightly and note if it responds by moving its skin or orienting in some manner to the stimulus.

Visual Function

Threat blinking: move your hand rapidly towards the cat's eyes without touching its vibrissae and note if it blinks.

Surface placing:holding the cat in air, move it towards some surface without touching the surface and note if it moves its forelimbs towards the surface and extends its paws. Here the cat must see the surface.

Orientation: move your hand rapidly to the side of the cat's head and note if it orientates to the stimulus by turning or starting.

Appendix C (Continued): Definitions.

Adjustment: push the cat laterally so as to cause it to lose its balance and note if it adjusts its balance or footing to compensate.

Hopping and placing: pick up one (and then two) of the cat's limbs while causing it to move forward and note if it tries to compensate by hopping and placing with the other limbs.

Fixation in rotation: holding the cat in the air turn it in horizontal circles slowly and note if its eyes and head fixate on the wall as it turns.

Free-fall righting: if the cat is strong and healthy enough, drop it from increasing heights (1 - 4 feet) and note if it extends its limbs and fans its toes in landing.

Tactile placing: holding the cat in the air, with its eyes covered, touch the front of its feet to the edge of a firm surface and note if it raises its feet to place on the touched surface.

Sensory Motor Integration

Sleeping sphinx: note if the cat sleeps in a sphinx position (crouched vertical with paws curled underneath body).

Sleeping curled: note if cat sleeps in curled position (on its side with paws folded).

Appendix C (Continued): Definitions.

Following: move your hand, some food, or some other object back and forth in front of cat and note if it follows the object with its head and eyes.

Auditory Function

Noise startle: clap your hands or make some other sudden, sharp sound at the back of the cat and note if it shows a startle response.

Orientation: note if the cat shows movement of its pinnae, head or body in response to the noise causing it to startle.

Olfactory Function

Sniffing: bring food close to the nose of the cat and note if it sniffs the food.

Orientation: place food in the vicinity of cat and note if it turns towards the food while sniffing.

Proprioceptive Function

Resistance: with the cat in a horizontal position push its fore- and hindlimbs towards and away from its body and note if it shows some resistance (tonus) in response to this movement.

Flexibility: with the cat in a horizontal position pull its fore- and hindlimbs towards and away from its body and note if it shows some resistance in response to this movement.

Appendix C (Continued): Definitions.

Waking sitting: note if the cat sits up (on hind end balanced on extended forepaws) when awake.

Waking standing: note if the cat stands on all fours when awake.

Walking: when placed on the floor, note if the cat is able to walk at least 5 steps.

Climbing: when placed in a room with different level platforms, note if the cat is able to climb up at least 30 cm.

Jumping: when placed on a platform (30 - 120 feet high) note if the cat jumps spontaneously to the ground.

Nociceptive Responses

Reflex: pinch the cat's tail, paw or ear and note if it responds with a withdrawal reflex (flexion).

Localization: pinch any area of the cat's body and note if it orientates to the area.

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