THE EFFECTS OF BRAIN STEM LESIONS ON SLEEP, WAKING AND TONIC IMMOBILITY IN THE RABBIT

by

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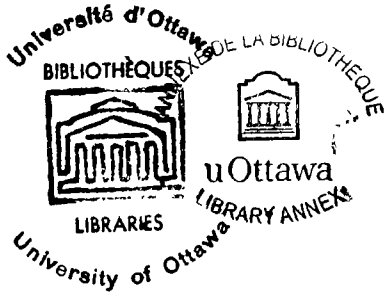
A thesis
submitted to the School of Graduate Studies and Research of the University of Ottawa in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Clinical Psychology.



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Claude M.J. Braun was born in Ottawa, Canada in 1953. He received his primary education in Canada and his secondary education in Canada, France and Belgium. He attended the C.E.G.E.P. of Hull where he received the collegiate degree in Administration. He obtained a B.A. with joint concentrations in Psychology and Philosophy and an M.A. in Clinical Psychology from the University of Ottawa in 1972 and 1975 respectively.

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Abstract

Paradoxical sleep (PS) and tonic immobility (TI) or "animal hypnosis" are two naturally occurring conditions in which muscle tonus has been reported to be inhibited. The brain stem locus coeruleus (LC), has been implicated in processes of tonic muscular control during PS in cats and rats. This nucleus was lesioned in rabbits in an attempt to replicate these findings using another species, and to test the hypothesis that the tonic muscular properties of TI are also dependent upon the LC system. Lesions sparing the LC but involving other reticular nuclei (Reticularis Pontis Oralis and Caudalis: NRPO and NRPC respectively) and non-reticular nuclei (vestibular and cerebellar) were also made. Results replicated the effects of LC lesions on PS observed in other species; namely, the disappearance of PS and the appearance of a new behavior ("PS without atonia") characterized by "phasic activation". In a few LC lesioned cases where prolonged episodes of TI were recorded, similar phasic activation was also observed for brief periods. Lesions of the NRPC resulted in the disappearance of PS and only minimal amounts of "phasic activation". Unlike effects of similar lesions on cats and rats, LC lesions in rabbits did not affect respiration and micturition. Vestibular and cerebellar

lesions did not noticeably affect the polygraphic recordings of sleep and waking. None of the lesions had long lasting effects on induction or duration of TI.

The major conclusions of the study were the following: 1. Lesions of the LC produce a phasic and tonic muscular activation (P) during a state which in other respects resembles PS. 2. Extent of LC destruction correlates positively with the amount of P observed, and negatively with the amount of PS of the type observed in unlesioned animals. 3. Lesions of the LC and of certain parts of the FTG area do not affect the duration or ease of induction of TI.

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Introduction and review of literature

This study analyzes the effects of bilateral pontine tegmental lesions on states of sleep and tonic immobility (TI) in rabbits. The literature review begins by defining and describing the dependent variables, namely the basic sleep states and TI, with special emphasis on these variables in rabbits. Polygraphic, behavioral and neurophysiological parallels are then drawn between paradoxical sleep (PS) and TI, and a rationale is formulated for hypothesizing the involvement of a particular brain stem nucleus (locus coeruleus) in electromyographic aspects of both states. The anatomical, neurophysiological, neurochemical and behavioral literature related to this nucleus are then reviewed. Finally the major research hypotheses of this study are formulated and a brief outline of the organisation of the thesis is given.

Definitions and characteristics of sleep and waking states

The discovery of rapid eye movement sleep (REM-S) in humans by Aserinsky and Kleitman in 1953 has been followed by an explosive progression in the descriptive knowledge of sleep. The identification of two distinct sleep states, slow wave

sleep (SWS) and rapid eye movement sleep in humans (Dement, 1955) was subsequently corroborated in lower mammals (Dement, 1958) and was called paradoxical sleep (PS) (Jouvet, 1961). Three electrographic measures are commonly used to define sleep and waking states, namely, the electroencephalogram (EEG), nuchal electromyogram (EMG) and the electrooculogram (EOG). During SWS, the EEG is characterized by low frequency, high amplitude activity and occasional spindling. The number of eye movements (EOG) is markedly reduced but not absent, and nuchal muscle tone is diminished relative to waking levels. The PS state is characterized by mixed frequency and low amplitude EEG, absence of spindles, increase in cerebral blood flow, isolated eye movements and frequent bursts of rapid eye movements (REMs), nuchal atonia, and marked depression of monosynaptic and polysynaptic spinal reflexes (Jouvet, 1961; Moruzzi, 1963; Giaquinto, Pompeiano & Somogyi, 1964). Waking is characterized by desynchronized low amplitude EEG, behavioral quiescent and active periods, and the presence of eye movements.

Sleep-waking states have also been considered along another dimension, that of tonic-phasic phenomena (Morruzzi, 1963; Pompeiano, 1967; Pivik, 1978). Tonic events (long lasting, relatively stable phenomena) in sleep are interspersed with numerous phasic alterations (sporadic, rapid variations) in several different systems, particularly dur-

ing PS. The slow and regular brain waves and the stable muscular tonus observed during SWS are examples of tonic events. Phasic events, typical of but not exclusive to PS, include twitches in distal musculature (including periorbital areas, limbs, and middle ear muscles), cardiac and respiratory irregularity, etc. The phasic event which has received the greatest attention in sleep neurophysiology is the PGO spike -a monophasic potential (150-200 ms duration, 200-400 mV in amplitude) recorded from the pontine tegmentum, lateral geniculate bodies and the occipital cortex -thus the acronym PGO.

The electrophysiological characteristics of sleep states have been recorded in several species, and it is generally considered that SWS antedates PS in evolution (Allison, 1968). Amphibians exhibit only very short lasting periods of SWS or none at all (Hobson, Goin & Goin, 1976). Reptiles show only SWS (Flanigan, Wilcox & Rechstschaffen, 1973; Walker, Berger & Scott, 1973). Birds show both SWS and PS, but the latter in only a small amount (5%) (Klein, Michel & Jouvet, 1963). Mammals show 3-35% PS. Lower mammals such as the tree shrew and spiny anteater show only minimal amounts of PS. (Berger et al, 1970). It is agreed that PS comprises a larger proportion of total sleep time (TST) in higher species. PS also takes up a larger proportion of TST in premature and normal neonates than after maturation regardless of the species (Ellington, 1972).

Neurophysiological and neurochemical substrates of slow wave sleep

In the last 25 years, transection and lesioning studies led to the conclusion that mechanisms controlling SWS were located in the brain stem reticular formation (Moruzzi, 1963; 1964; 1972). In the 1960s, the mapping out of monoamine, and to a lesser extent cholinergic systems in the central nervous system (CNS), opened the door for neuropharmacological investigation of sleep.

Numerous investigations carried out by Michel Jouvet and colleagues and later in other laboratories implicated a serotonergic-anterior raphe mechanism of control of SWS. The hypothesis was experimentally appealing as well as theoretically plausible because in addition to comprising the vast majority of cell bodies of serotonin (5-HT) containing neurons, the raphe nuclear complex has wide ranging connections within the CNS (Jouvet, 1967; Cooper, Bloom & Roth, 1978). Generally, however, recent studies have failed to support this model.

The initial studies by Jouvet and co-workers of the involvement of 5HT-containing neurons in sleep involved electrolytic lesions of all raphe nuclei except the most caudal. These lesions produced marked insomnia in cats (Jouvet, Bobillier, Pujol & Renault, 1966; Renault, 1967). The above

mentioned lesions were very large however, and extended beyond the boundaries of the raphe complex. A recent study (Mouret & Coindet, 1980) involving discrete well controlled lesions of anterior raphe nuclei, found that these nuclei were not necessary for normal SWS in rats. In an early study, extent of raphe destruction was correlated with sleep loss and 5-HTdepletion and rostral raphe lesions reduced SWS selectively from PS (Jouvet, 1969).

Manipulation of 5HT by means other than lesions produced inconsistent results. Pharmacological depletion of SE with para-chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, produced insomnia which was reversed by administration of 5-hydroxytryptophan (5-HTP), the 5HT precursor (Jouvet, 1969). Later studies indicated however that PCPA effects were complex, non-permanent (Dement, Mitler & Henrikson, 1972; Dement, Henrikson, Jacobs & Mitler, 1973) and species-specific (Weitzman, Rapport, McGregor & Jacobs, 1968; Wyatt, Engleman, Kupler, Scott, Sjoerdma & Snyder, 1969; Rechtschaffen, Lovell, Freedman, Whitehead & Aldrich, 1973). Reserpine, which depletes 5HT and noradrenaline (NA) by interfering with vesicular uptake of amines, abolished SWS without affecting PS only when 5-HTP was also injected (Jouvet, 1969). Injection of 5HT directly into the raphe complex induced SWS in one study (Rodriguez, Rojas-Ramirez, Drucker-Colin, 1973) and failed to do so in another (Morgane

& Stern, 1973). The 5HT neurotoxins 5,6 -dihydroxytryptamine (5,6 DHT) and 5,7 DHT depleted SE but not SWS (Froment, Petitjean, Bertrand, Cointy, Jouvet, 1974; Ross, Trulson & Jacobs, 1976).

Electrical stimulation of raphe cells has given equivocal results, increasing SWS in one study (Kostowski, Giacalone, Garattini & Valzelli, 1969) and increasing waking in others (Polc & Monnier, 1970; Jacobs, Asher & Dement, 1973). Studies of spontaneous and evoked activity of raphe neurons have also provided inconsistent and largely negative support for the 5HT model of SWS. Discharge rates of anterior raphe units decreased by as much as 50% during SWS (McGinty, Harper & Fairbanks, 1973; Balzano & Jeannerod, 1970; McGinty & Harper, 1976; Sheu, Nelson & Bloom, 1974).

In conclusion, raphe 5HT-containing neurons, although initially considered the best candidates for mediation of SWS, are apparently not essential for SWS. A modulatory role for these neurons is still indicated however since effects of several of the above manipulations on PGO spikes (Delorme, Froment & Jouvet, 1966; Jacobs, Henrikson & Dement, 1972) and non-executive triggering of PS (Jouvet, 1972) have not been challenged. Other neurotransmitters have not been consistently implicated in the executive control of SWS.

Neurophysiological and neurochemical substrates of paradoxical sleep

In the next 3 sections, a general overview will be given of the PS state. The specific phenomenon of muscle tone during PS, of central importance for the present project, will be reviewed in detail in another section.

After the discovery of REM-S in 1953 the search for the neural structures essential for this state to occur led to the localization of these essential structures to the brain stem reticular formation (Moruzzi, 1972).

Two competing models of PS have since emerged. Jouvet (1969) suggested that a locus coeruleus (LC) noradrenergic mechanism is the executive generator of PS, while McCarley & Hobson (1971) suggested that the gigantocellular tegmental field (FTG) comprising, among other structures, nuclei reticularis pontis oralis (NRPO) and caudalis (NPPC), is a more likely candidate. Despite the fact that it has not been possible to prove the existence of a major role of the FTG in acetylcholine (ACh) synthesis because of technical limitations, Hobson et al postulated that acetylcholine (ACh) is the most important neurotransmitter in their model.

The gigantocellular tegmental field and paradoxical sleep. The FTG appeared to be very promising as a PS center from a neuroanatomical point of view since 2-way connections

had been established between it and other nuclei (raphe, LC) which were shown to be involved in at least secondary or modulatory aspects of PS. Furthermore, the FTG had been shown to be widely connected to lower brain stem and spinal cord as well as to mesencephalic and diencephalic structures (Steriade & Hobson, 1976). Lesions of the caudal NRPO and rostral NRPC selectively abolished all ascending and descending events of PS including nuchal atonia, while SWS and W were less affected (Rossi, Minobe and Candia, 1963; Carli & Zanchetti, 1965; Roussell, Buguet, Bobillier & Jouvet, 1967; Jouvet, 1962). Jones (1979) lesioned the NRPC in cats and abolished the fundamental aspects of PS, tonic and phasic, including muscular inhibition.

In terms of neurochemical properties of the brain stem reticular formation, one of the neurotransmitters which has received the greatest attention in connection with PS is ACh. Hernandez-Peon, Chavez-Ibarra, Morgane and Timo-Iaria (1963) and Hernandez-Peon, O'Flaherty and Mazzuchelli-O'Flaherty (1967) demonstrated that PS could be induced by application of minute crystals of ACh in a highly circumscribed pathway extending from the rostral forebrain to the bulbar tegmentum. This pathway overlaps the cholinergic ventral tegmental pathway. Atropine, a cholinergic receptor blocker prevented PS when applied in these same areas in the same animals (Velluti & Hernandez-Peon, 1963). Drug so-

lutions of carbachol, a cholinomimetic which activates nicotinic and muscarinic receptors, applied to the reticular nuclei NRPO and NRPC produced a form of PS which closely resembled spontaneous PS, more so than observed in the Hernandez-Peon studies (George, Haslett & Jenden, 1964; McCarley & Hobson, 1975; Mitler & Dement, 1974). Though these studies have reported carbachol-induced rapid eye movements, PGO spikes and even muscular atonia, Mitler and Dement (1974) have questioned the isomorphy between this state and normal PS because in their experiment, the cats appeared behaviorally awake despite the presence of numerous characteristics of PS. In a recent study, Silberman, Vivaldi, Garfield, McCarley and Hobson (1980) reduced the volume of each carbachol infusion into cat PTG and obtained PS which was remarkably analogous to the spontaneous state. Carbachol is not the only ACh agonist which enhances PS, nicotine, which activates nicotinic receptors and is short acting, also enhances PS (Domino & Yamamoto, 1965; Jewett & Norton, 1966). The anticholinesterase agent, physostigmine, accordingly produced tonic and phasic phenomena very similar to those observed in spontaneous PS (Matsuzaki, 1968; Magherini, Pompeiano, Thoden, 1972). Anti-cholinergic agents such as the muscarinic blockers, atropine and scopolamine, suppressed PS (Delorme, Riotte, & Jouvet, 1966; Sagales, Erill & Domino, 1969). Finally, hemicholinium, which prevents uptake of choline, injected intraventricularly, suppressed PS (Domino & Stawiski, 1970; Dren & Domino, 1968).

A series of studies were published in the mid 1970s implicating the FTG giant cells in PS. The recording of spontaneous activity of FTG giant cells with extracellular recording techniques consistently indicated that these cells discharged at higher frequencies during PS than SWS and waking. The selectivity of this neuronal group for discharge in PS was also reported to be higher than any other neuronal group previously recorded (Hobson, McCarley, Pivik & Freedman, 1974; Hobson, McCarley & Wyzinsky, 1975; Steriade & Hobson, 1976). The microelectrode evidence for FTG giant cells as generators of PS or any component of PS has been considerably weakened, however, by two key studies. Siegel, McGinty & Breedlove (1977) and Vertes (1977) reported that some FTG giant cells had very high firing rates during active waking in unrestrained cats and rats.

Evidence from electrical stimulation studies indicates that it is possible to enhance PS not only after stimulation of the FTG area (Jouvet, 1962; Frederickson & Hobson, 1970) but also after mesencephalic reticular (Candia, Favale, Guissani & Rossi, 1962; Faure, Bensch & Vincent, 1962) and limbic (Faure, 1965; Passouant & Cadihlac, 1962) stimulation.

The locus coeruleus as a paradoxical sleep center. In the present subsection, studies relevant to the hypothesis

of the LC as an executive generator of the entire PS state including tonic and phasic events (Jouvet, 1969) will be reviewed.

The LC was considered a strong candidate as a possible sleep center since it was known that the LC contains many of the cell bodies of the NA terminals of the CNS, and that it supplies these terminals to wide ranging areas all over the CNS (Jouvet, 1967). In 1965, Jouvet and Delorme reported the first study of the effects of selective LC lesions on sleep and observed what they termed "total and selective abolition of PS", displacement of PGO spikes into SWS, and "hallucinatory-like" behavior during what appeared to be sleep. Subsequent studies involving lesions of the LC reported marked tonic muscular increases during periods of "hallucinatory-like" activation observed following episodes of SWS. However, during these periods of "activation" there was not a disappearance of other properties of PS such as eye movements, cortical desynchronisation, unresponsiveness, pupillary myosis, and increased brain temperature (Henley & Morrison, 1974; Hendricks, Bowker & Morrison, 1977; Jones, Harper & Halaris, 1977). However, lesion studies involving the FTG area produced what appeared to be more complete abolition of PS (see preceding section) than reported following LC lesions. Jouvet (1969) did not abandon his LC model of PS however and interpreted these latter effects as "probable

disruption of LC efferent pathways". The most recent lesioning study reported that LC lesions produced selective abolition of all aspects of PS including eye movements and PGO spikes (Sastre, Sakai & Jouvet, 1979).

Since many of the NA terminals have their cell bodies in the LC, evidence from neuropharmacological manipulations of this neurotransmiter was considered relevant to the LC-NA model of PS. Such studies have generally not been supportive of the hypothesis of an executive role of these neurons in the control of PS. Intraventricular injection of NA has generally not produced paradoxical sleep, but rather stuporous waking (Cordeau, DeChamplain & Jacks, 1971; Hartmann, et al, 1974). Administration of the catecholamine (CA) precursor, L-DOPA resulted in increased waking and dcreased PS (Wyatt, 1972; Delorme, 1966). Injection of NA receptor blockers such as thymoxamine (Oswald, Adam, Allen, Burek, Spence, Thacor, 1974), and phenoxybenzamine (Hartmann, Zwilling, & List, 1973), has enhanced PS. The CA synthesis blocker, alpha-methyl-paratyrosine (AMPT) and the NA neurotoxin 6-hydroxydopamine (6-OHDA) have been repeatedly studied, but each of these two drugs produced enhancing (Hartmann, Chung, Drascoczy, Schidkraut, 1971) and suppressive (Lidbrink & Fuxe, 1973; Iskander & Kaebling, 1970) effects on PS. Most studies involving injections of 6-OHDA and AMPT reported enhancement or no reduction of PS, or had a greater

effect on waking than on PS (Steriade & Hobson, 1976; Jacobs & Jones, 1978). However, although some studies have reported PS suppression following 6-OHDA, a study by Van Dongen, Broekamp & Cools (1978) strongly indicates that 6-OHDA suppression of certain PS phenomena was not mediated by NA neurons (see next section). Still, Jouvet's (1972) thesis of a priming role of certain NA neurons of the LC in the initiation of PS has not been clearly refuted. (For a more detailed review, see Jacobs & Jones, 1978)

Studies of the spontaneous activity of units in the LC area have also given mixed results regarding the LC model of PS. Though anterior and lateral LC cells increased firing during PS (Chu & Bloom, 1973), Hobson, McCarley & Wyzinsky (1975) found that the activity of cells in the posterior-caudal LC and subcoeruleus (LSC) decreased during SWS in two thirds of the samples recorded and decreased further during PS. Other studies have also reported marked decreases in LC unit activity during PS (Foote & Bloom, 1979; Jones, Segal, Foote & Bloom, 1979).

Studies in which the LC region was electrically stimu-

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[1]Since the focus of the present thesis is on relationships between sleep-waking and tonic immobility states, an exhaustive review of vegetative and behavioral effects of LC lesions did not seem to be indicated. However, three classical neurophysiological effects of LC lesions require brief mention. Integrity of the LC is required for normal uro-genital function in rats (Amaral & Foss, 1975; Osumi, Oishi, Fujiwara & Takaori, 1975; Roussel, Pujol & Jouvet,

lated have not included sleep as a dependent variable[1] (Strahlenhorf, Strahlenhorf, Kingsley, Gintautas & Barnes, 1980; Johnson & Russell, 1952; Baxter & Olzewski, 1955; Ngai & Wang, 1957; Satoh, Eguchi, Watabe, 1979; Redmond, Huang, Snyder & Maas, 1976). None of these authors mentioned a sleep inducing effect of electrical stimulation of the LC.

The evidence for LC determination of the PS state is inconsistent. However, a role for this nucleus in control of tonic muscular activity during PS has been indicated, and the evidence for this relationship will now be considered.

The locus coeruleus and tonic muscular activity during paradoxical sleep

Although several studies have reported that LC lesions are followed by a PS-like state with elevated muscle tonus (Jouvet & Delorme, 1965; Mouret & Delorme, 1967; Henley & Morrison, 1974; Jones, Harper & Halaris, 1977), the impor-

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1976), micturition in cats and rats (Barrington, 1925; Tang & Ruch, 1956; Jouvet & Delorme, 1965; Jones, Harper & Halaris, 1977; Osumi, Oishi, Fujiwara & Takaori, 1975), and respiratory inspiration in cats (Johnson & Russel, 1952; Ngai & Wang, 1957). Stimulation studies have supported the latter two observations (Wang & Ranson, 1939; Johnson & Russel, 1952; Baxter & Olzewski, 1955; Ngai & Wang, 1957). In addition, there is inconsistent evidence of jumping behavior in rats (Arbuthnott, 1973), and forelimb rigidity and hindlimb hypotonia in cats (Jones, Harper & Halaris, 1977) and high experimental mortality in both species (Kuru & Yamamoto, 1964; Amaral & Foss, 1975) following LC lesions.

tance of the LC in this phenomenon has been questioned. Hendricks & Bowker (1977) and Morrison (1979) have stated that partial integrity of the LC is required for the elevated EMG effect during this PS-like state to be observed. Hendricks, Morrison and Mann (1980) have shown that different classes of abnormal muscular activity may result from discrete lesions in different brain stem areas in cats. Not all lesion-induced elevations in PS muscular tonus were accompanied by dramatic "hallucinatory-like" behavior. They described a group displaying "PS without atonia without behavior" as a result of medial LC lesions and lesions dorsal to the LC -centered in the periventricular grey area. Lesions more ventral and rostral to these produced co-ordinated behavior during PS resembling orienting, staring, and searching. A third group with lesions extending even more rostrally, into the midbrain, manifested violent "phasic" attack behavior. Morrison, Mann and Hendricks (1980) have recently interpreted PS atonia-abolishing lesions as disruption of

1. Pontine excitation of medullary inhibitory areas.

2. Indirect pontine inhibition of a brain stem locomotor center.

Contrary to past hypotheses, however, these authors believe that descending muscular inhibition during PS in unlesioned animals is not produced by the LC but by FTG or "other neu-

rons" (Morrison, 1979). Furthermore, it is suggested that selective LC lesions interrupt excitatory LC input to a locomotor center having nothing to do with "oneiric activity", and in indirect support of this notion, increases in motor activity during PS have been noted after LC lesions (Henley & Morrison, 1974; Jones, Harper & Halaris, 1977). These authors hypothesize that this locomotor center is a response center to sudden novel stimuli which requires massive inhibition during the "hyperalert brain state" of PS. "PS without atonia but with explosive behavior" is said to be caused not by LC lesions but by removal of inhibitory input to the LC by destruction of such inputs in more rostral areas of the brain stem. These centers, and not the LC, are hypothesized to activate lower medullary centers responsible for the muscular inhibition of PS.

Since most studies involving manipulations of NA neurons, including those using 6-OHDA, failed to alter the tonic muscular aspects of PS it seems unlikely that this neurotransmitter is crucial in the control of tonic muscular aspects of PS. However, Morrison (1979) recently reviewed studies on walking behavior elicited in decerebrate cats by stimulation of brain stem areas including the LC, and injection of NA enhancing drugs (Grillner, 1976; Wetzel & Stuart, 1976; Steeves, Jordan & Lake, 1975), and suggested that this NA system provides a tonic facilitory role in locomotion. The

midbrain and pontine neural systems involved in the locomotor effects are hypothesized to act as "command fibers" which set in motion the "spinal motor machinery".

A different picture emerges when ACh is evaluated as a candidate for mediation of LC control of tonic muscular aspects of PS, particularly following direct chemical manipulations of the LC. It has been established that there is massive presence of acetylcholinesterase (AChE) throughout the LC area, including within NA-containing LC neurons (Knight, 1970; Jacobowitz, 1978; Albanese & Butcher, 1980). This finding is important because Koelle showed in 1954 that AChE is present in ACh neurons in the brain stem. The existence of cholinoceptive LC neurons has been indicated by reports from Holmes & Wolstencroft (1964), Ramon-Molinar (1974), It is interesting that cholinergic manipulations of the LC area have indicated a specific role of the LC in muscle atonia of PS, not of the whole constellation of phasic and tonic events of PS. This indication corresponds to the results obtained following LC lesions. Van Dongen, Broekamp & Cools (1977) reported that carbachol, injected into the LC-LSC region in cats, produced atonia which was reversed by reserpine, an agent which depletes SE and NA.

The same carbachol-induced atonia was not reversible by alpha and beta-NA receptor blocking agents. Carbachol,

injected into an LC whose NA-containing cells were destroyed by previous injection of 6-OHDA, was still effective in producing atonia similar to that observed in PS.

The evidence reviewed above indicates a specific role of the LC in tonic motor aspects of PS although this role appears to involve mediation by ACh rather than NA.

Sleep in the rabbit

The New Zealand White rabbit was chosen as the experimental subject for the present thesis project because it exhibits both PS and SWS, is highly susceptible to experimental induction of TI, and is well established as a research species thus easily available. Accordingly, sleep characteristics of this species will be examined in detail. The sleep cycle of New Zealand White rabbits has been reported to have a period of 20 to 60 minutes (Khazan & Sawyer, 1963; Kawakami & Sawyer 1965). Like most lower mammals, the rabbit spends a relatively small amount of time in PS. Weiss and Roldan (1964) reported that in New Zealand White rabbits, 7.4% of 24 hours consisted of a waking state (W), 77.5% consisted of SWS and 15.1% was PS. Based on 24 hour data obtained from 38 ovariectomized female New Zealand White rabbits, Kawakami & Sawyer (1965) reported that 60.1% of 24 hours consisted of W, 36.7% of SWS, and only 2.7% PS. Narebski, Tymicz and Lewosz (1969) reported results quite similar to those of Kawakami and Sawyer. The great variability of baseline findings on

rabbit sleep is thought to reflect the sensitivity of the species, and it is now generally suggested that long adaptation periods, reduced lighting, good ventilation, silence, non-alien litter, food and water in the recording cage, and 68 degrees F cage temperature be used for obtaining reliable spontaneous sleep in this species.

In the aroused state, rabbits exhibit 8-9 Hz theta activity in cortical and hippocampal leads (Narebsky et al, 1969). Electrodes implanted in the vicinity of the olfactory bulb show a 2 Hz high amplitude rhythm which disappears in PS. In SWS, cortical activity increases in amplitude, slows to delta or slow theta frequencies, and exhibits 9-10 Hz spindles. Although during PS, the EOG demonstrates clearly identifiable eye movements, quantitative studies of eye movements or other phasic activity have not been reported. During PS , nuchal EMG displays short lasting drops in amplitude which seem to indicate the presence of at least phasic inhibition or relaxation. Carli (1969) reported that mono and polysynaptic reflexes were inhibited during rabbit PS. Unfortunately, he did not indicate quantitatively the depth to which this inhibition occurred, nor whether this finding was generalizable to complete PS episodes rather than selected moments within the episodes. The relevance of these two latter questions is made evident by the finding that the overall activity of the nuchal musculature, during

PS, measured in total voltage, does not reach levels significantly lower than in SWS (Pivik, Sircar & Braun, 1980). Cortical and hippocampal leads show 8-9 Hz activity which is more regular than in the W state. Spontaneous PGO spikes have not been reported in rabbits under non-medicated conditions, but reserpine has been reported to produce PGO spikes in rabbits (Verley, Garma & Scherr, 1969).

Definition and history of tonic immobility

Tonic immobility (TI) is a reversible state of paralysis and reflex depression most easily induced by dorsoflexion and restraint. The term "tonic immobility" has been interchanged with others such as "fascination", "death feint", "animal hypnosis", "immobilization reflex", "playing possum", and "totstellreflex" (Chertok, 1964).

The first experimental induction of TI is usually attributed to the Jesuit father Athanasius Kircher. In 1646, he performed the famous Experimentum Mirabile De Imaginatione Gallinae which consisted simply of tying a hen's feet, placing the hen on a board, restraining it until struggling ceased, and drawing a chalk line from the beak outwards. When the hen's feet were untied it remained transfixed. Kircher believed that the chalk line was interpreted by the animal as a bond which maintained a relation of paralytic submission to the "conquerer".

This explanation of TI was later disproved by Czermak (1872) who demonstrated that it was possible to induce a paralytic state in various animals simply by placing them on their backs with temporary mild restraint. He interpreted the reaction as a form of hypnotically induced central nervous system inhibition, somewhat akin to sleep.

Preyer (1873) and Danilewski (1886) followed with an interpretation of TI which is still largely accepted today. They conceived of TI as an adaptive fear reaction. Danilewski (1890), Biernacki (1891), Verworn (1897) and Mangold (1914, 1934) were later to report that voluntary motor reactions or reflex reactions to drugs and to various forms of stimulation were greatly depressed during TI, and that muscles went through phases of rigidity and flaccidity.

No species has been reported to be completely refractory to TI, but it is more difficult to induce the reaction in higher animals. The following phyla have been verified to be susceptible to TI: arthropods, coelenterates, amphibians, fish, cephalopods, reptiles, rodents, insectivores, fowl, mammals, herbivores, carnivores, elephants, and, under special conditions, even primates. A case of "paroxysmal inhibition" which resembled TI was reported in humans by Hoagland (1928).

Techniques for inducing tonic immobility

The literature is replete with reports of various ways of inducing TI. The most common technique is dorsoflexion followed by restraint. Surprisingly, however, very similar results have been obtained by different approaches such as hooding birds (Patrick, 1924), staring at them (Erhard, 1924), using bright lights (Mowrer, 1920), loud noises (Kunin, 1967), monotonous stimulation (Pavlov, 1934), blinding frogs and guinea pigs (Sidis, 1908), hair clipping in pigs (Marcuse & Moore, 1944), immobilization of monkeys with a dental fixating device (Wendt, 1936) or inversion of their visual fields (Foley, 1938).

Behavioral aspects of tonic immobility

Some of the most consistent characteristics of TI are the following:

- absence of righting reflexes (Klemm, 1966)
- eyes open (Klemm, 1966)
- absence of eye movements (Carli, 1968)
- absence of phasic muscular events (Klemm, 1966)
- limbs at first rigidly extended, later becoming relatively flaccid (Ratner, 1967)

Hoscovek and Svorad (1969) reported that susceptible animals appear to be more "emotional" than non-susceptible ani-

mals. Susceptible animals showed greater urine retention, less locomotor activity, and more non-locomotor activity such as scratching, yawning, biting, and washing. Liberson (1948) and Gley (1895) reported that long term continuous induction of TI occasionally resulted in death. Moore (1962) found that habituation to fear inducing stimuli reduced susceptibility to TI in goats, and Ratner (1967) reported similar results in rats. TI episodes have been reported to persist despite extremely painful stimulation (eg., tracheotomy, laparotomy in chickens, Mangold, 1914). Wendt (1936) reported that he could easily terminate TI in monkeys by blocking their nostrils. When thrown into the air, birds immediately emerged from TI and flew to safety (Patrick, 1924). Auditory, tactile, or olfactory stimuli can arouse an animal from TI provided they are of extremely high intensity (Schwartz, 1956; Carli, 1969).

Electrographic properties of tonic immobility

The sensorimotor and occipital cortex and amygdala of the oppossum show a very low voltage fast frequency EEG profile during TI (Baratt, 1965). In rabbits, the neocortical EEG shows an arousal pattern during TI. If the episode is sufficiently prolonged, synchronized high voltage slow wave EEG with spindle bursts may be observed in the neocortex (Carli, 1969). Hippocampal EEG shows no initial change, but in prolonged TI, reduction of frequency and increase in voltage

appears (Harper, 1971). Klemm (1966) was able to induce TI in rabbits prior to, and during, episodes of epileptiform EEG provoked by amphetamine and pentylenetetrazol. The usual spastic behavior concomitant with epileptiform EEG did not appear during TI. Klemm (1965) reported that TI was prolonged by electrical stimulation of the pontine reticular formation and the midline thalamus. He also reported increased multiple unit activity during TI in the median reticular formation throughout the medulla and the pons (1969). Most evoked potential studies of TI indicate that cortical reactivity to electrical, mechanical, and acoustic stimulation is only slightly reduced during TI (Svorad, 1957; Silva, 1959; Vanreeth, 1963; and Klemm, 1966).

Carli's research on reflexes during TI (1969) will be reviewed here to demonstrate the extent to which reflex inhibition is similar in TI and in PS. Selective electrical stimulation of excised high and low-threshold gastrocnemius afferent nerve fibers during TI showed that monosynaptic extensor and flexor reflexes were abolished and that polysynaptic extensor and flexor reflexes were depressed. Similar results were obtained by repetitive stimulation of the sural nerve while recording polysynaptic responses in the posterior biceps muscle (Carli, 1969). These effects were less marked during hypertonic moments within episodes of TI. Carli (1969) reported that generally, TI was characterized by low muscle tone.

Ultradian rythms and tonic immobility

Ultradian variations have been reported in the mean duration of the TI response in bullfrogs and tarantulas (Ternes, 1977). The cycle peaked markedly twice every day ($p < .0001$). Unfortunately, there are no reports on higher species regarding ultradian variations of the TI response. Research carried out in the present laboratory has indicated however that a similar biphasic cycle exists in rabbit TI.

Baratt (1965) reported that TI was much more easily induced in the oppossum if induction was attempted immediately during EEG alpha activity rather than sleep or active waking.

Effects of lesions on tonic immobility

Carli (1971) has carried out the most extensive series of ablations in relation to TI. Basically, his results confirmed previous, less well controlled studies in showing that the cortex, cerebellum, 8th nerve, labyrinth, ventral thalamus and hippocampus are not necessary to induce TI in rabbits. Complete transections above the superior colliculus did not abolish the TI response in rabbits (Spiegel & Goldbloom, 1925; and Carli, 1971). Transection studies in frogs revealed that cuts just posterior to the anterior border of the cerebellum did not affect the induction of TI,

suggesting that in frogs the neural mechanism necessary for the occurrence of TI is situated either in the spinal cord or the medulla. Variable results were associated with different levels of midbrain and pontine section suggesting at least secondary mechanisms at these levels in this species (McBride & Klemm, 1969). To date the effects of selective lesions of brain stem nuclei upon TI have not been reported.

The neurochemistry of tonic immobility

Though several of the putative neurotransmitters (ACh, 5HT, opiates, NA, dopamine (DA)) have been studied in connection with TI, the field is relatively new and undeveloped. This section is a review of nearly every study of TI which has involved neurotransmitters.

Hatton, Woodruff and Meyer (1975) have shown that rabbit TI duration is shortened by physostigmine, an anticholinesterase, and is prolonged by scopolamine, a blocker of muscarinic receptors. In chickens the same drugs have opposite effects (Thompson, Piroch, Fallen & Hatton, 1974; Woodruff, Hatton & Meyer, 1976).

In chickens, a serotonergic mechanism has been suggested to modulate TI duration (Maser, Gallup & Hicks, 1975; Wallnau & Gallup, 1977). The only study of the effects of manipulation of 5HT levels on TI in mammals consisted of injec-

tions of PCPA, in rabbits. No effects were reported (Carli, 1977).

Duration of TI is potentiated by morphine (Davis, 1963; Peters & Hughes, 1978) and is reduced by the narcotic antagonist naloxone (Carli, 1971; Wallnau & Gallup, 1979). Following morphine injection (Lim & Guzman, 1968), rats become susceptible to TI. On the basis of the lack of effect of prolonged pain on TI, and the prolongation and shortening of TI by morphine and naloxone respectively, Carli (1975) suggested that during TI a morphine-like mechanism is active. Further recent investigation of this phenomenon in chickens using an opiate analog, D-Ala2, F5Ph2-metenkephalin NH2, and a non narcotic opiate analog, (D-Phe) metenkephalin, reported marked potentiation of TI (Olson, Kastin, Lahoste, Olson & Coy, 1979) by these chemicals.

The alpha-noradrenaline receptor antagonist, chlorpromazine, and amphetamine which releases NA and blocks its uptake, respectively increased and decreased duration of rabbit TI. Unfortunately, these drugs affect DA activity at least as much as NA and make the results difficult to interpret. Reserpine, which interferes with vesicular uptake, and DOPA, a CA precursor, both prolonged the rabbit TI response. Again, the effects of these drugs are not restricted to NA activity. Alpha-methyl-metatyrosine, which inhibits hydroxyla-

tion of tyrosine, and thereby reduces NA and DA, had no effect on rabbit TI (see Carli, 1977).

Only one study has selectively manipulated DA independently of NA (Wallnau, Carnrike & Dewey, 1979). It was found that the DA receptor blocker, haloperidol, and the DA receptor agonist, apomorphine, respectively increased and decreased durations of chicken TI.

Paradoxical sleep and tonic immobility: similarities and differences

Although PS and TI share some important behavioral, neurophysiological and neurochemical similarities, they are clearly not substitutable states. The two states are similar in that: 1.) They both occur in nature ; 2.) They are characterized by behavioral immobility; 3.) They show highly activated cortical EEG patterns; 4.) They manifest low muscle tone and depression of monosynaptic and polysynaptic reflexes; 5.) acetylcholine, and to a lesser extent, NA, have been implicated in their mediation; and, 6.) both are dependent upon the integrity of the brain stem for expression.

The dissimilarities between PS and TI consist largely of: 1.) the absence of phasic properties of PS during TI; 2.) pupillary myosis during PS and dilation during TI; 3.)

irregular respiration in PS but not in TI; and, 4.) the spontaneous cyclical occurrence of PS in sleep, but no spontaneous initiation of TI in any patterned manner, (although there are data suggesting that susceptibility to TI is influenced by circadian variables). No tonic differences between the two states have been reported except an obvious tension in forelimb extensor muscles in TI which is not observed during PS.

The above dissimilarities indicate a lack of state identity between PS and TI. The similarities however, notably regarding muscle tonus and tonic reflex depression, are compatible with the hypothesis of a common neural mechanism which would control the tonic muscular phenomena of each state.

Hypotheses

Evidence has been presented indicating that neuronal elements in the region of the LC area are involved in the phenomenon of motor control during PS. The major purpose of the present study is to test the hypothesis that the LC may also mediate tonic muscular activity during TI. Moreover, since the effects of LC lesions have not been reported in rabbits, the present project represents an initial study of the general effects of LC lesions on sleep-waking behavior in rabbits. Furthermore, this study is the first to perform localized lesions in an attempt to determine the contribution of specific brain stem nuclei to the generation or maintenance of TI.

It is hypothesized that following bilateral IC lesions: 1.) PS will be replaced by the phenomenon of "PS without atonia", and 2.) the TI response will no longer occur, or will be characterized by marked increases in muscular tonus.

The next chapters explain the methods and techniques used, present the results and discuss those results. Finally, the references are listed in the bibliography, and additional data are given in the appendices.

Method

Subjects

Subjects were forty one male, New Zealand White, 2-2.5 month old rabbits, weighing 3-3.5 kilograms at the time of surgery. The animals were housed in a ventilated room controlled for humidity (54%) and temperature (70 degrees F), and were maintained on a 12 hour light-dark cycle from 8:00 AM to 8:00 PM. Food and water were provided ad lib at all times. The animals were routinely monitored for the two most common diseases which affect rabbits, namely ear mites and snuffles (common cold). The former was treated with medicated mineral oil (Hexamite) and the latter with an antibiotic (Tetracycline) dissolved in the drinking water. No other diseases affected the colony.

Surgical procedure

Following shipment, the animals were adapted to the holding area for one week. Surgery was then performed following 24 hours of food and water deprivation. Chlorpromazine (Largactil, .4mg/kg) was administered intramuscularly as a preanaesthetic one half hour prior to surgery. Sodium Pentobarbital (Nembutal) diluted in equal parts of distilled

water was then injected into the marginal ear vein in small increments until the crossed extensor pinch reflex was depressed, and was titrated as required to maintain this depth of anaesthesia. A mineral corticosteroid (Decadron, .1ml/kg) was injected intramuscularly at 3 hour intervals to replenish stress-induced depletion of corticosteroids in this species.

Following surgery, Penicillin (Penlong-S-Plus, .1 ml/kg) was administered intramuscularly and an antibiotic powder (Sulfathiazol) was sprinkled over the wound.

Electrodes were implanted for chronic recording of EEG (3 pairs of stainless steel flat ended #0-80 screws over frontal, parietal and occipital cortex), EOG (two pairs of similar screws placed anteriorly and superiorly to the eye orbits), and EMG (four multistrand, teflon coated stainless steel wires placed deeply into the deltoid neck muscles). Coordinates for cortical electrode placement were 27 mm anterior to bregma and 2 mm lateral for frontal cortex, 6 mm anterior and 3 mm lateral for parietal cortex and 6 mm posterior and 5 mm lateral for occipital cortex. A topical anaesthetic (Xylocain Hydrochloride) was injected into tissues prior to penetration or application of pressure. A stainless steel cannula made from 18G caliber injecting needles, 25 mm long and 1.5 mm wide, stereotaxically aimed at

each locus coeruleus, was implanted, and securely anchored in acrylic. Stainless steel rods plugged the cannulae to prevent either entrance of foreign substances into, or loss of cerebrospinal fluid from, the cannulae. The skull was oriented such that lambda was 1.5 mm lower than bregma following the specifications in McBride and Klemm's (1968) and Fifkova and Marsala's (1967) rabbit atlases.

Stereotaxic coordinates are commonly established by referencing all measurements to the junction of skull sutures at bregma. However, the skull size of rabbits in the age range used in this study varied tremendously. The lambda-bregma distance varied from 17.1 mm to 20.1 mm. Though this could conceivably be explained by the observed variation in body weight (2.7-3.8 kg), closer analysis indicated that variation in skull size was not fully dependent upon body weight since the correlation between body weight and lambda-bregma distance was only .38 ($p < .01$). Since rabbits manifest significant variability in skull size at least in the late adolescent phase, research based on stereotaxic implantation during this time, must make allowance for greater stereotaxic error.

Pilot work for this study indicated that the 3 mm variation in lambda-bregma distance reflected a similar variation in underlying brain structures. Consequently, a more pre-

cise stereotaxic method was required. On the basis of histological verification of lesion sites in the first five animals of this study, a correction for anterior-posterior coordinates for LC lesions was established at 70.05% of the lambda-bregma distance, posterior from bregma. This method, which takes into account the relation between two skull sutures instead of one, with the point of implantation situated between, provided clear improvement in the accuracy of the lesion sites. The LC was determined to be 2 mm lateral to midline and 16 mm deep from skull surface in this study.

Recording environment

The animals were recorded for baseline sleep following 1 to 2 weeks of recuperation and adaptation to the recording cage (>24 hours). Adaptation was considered suitable if the rabbits ate and drank as they did in the holding room. Food, water and environmental control of temperature, humidity and lighting were maintained as in the holding area. Light intensity in the recording cage was maintained at 9.3 foot candles (100 lux). Both the recording cages and rooms which enclosed the cages were sound dampened and electrically shielded. A counterweight boom and a swivel commutator system (Airflight Electrocannular Slipring Assembly, Model CAY 675-24) ensured free movement of the animals. Polygraphic recordings were always taken from 11:00 AM to 5:00 PM. This time of day was chosen for recordings because

it has been established (Narebski et al, 1969) that the rabbit manifests the highest levels of each sleep state during this time of day. During the 6-hour recording periods, the animals were observed remotely with a video monitoring system. Occasionally, subjects were recorded simultaneously in separate recording rooms. Figure 1 indicates the equipment used in this study and the connections between the components. The chronological sequence of procedures carried out in the present study from the time of shipment of the animals to the permanent mounting of stained histological brain samples was as follows:

01. ADAPTATION (1 WEEK)
02. IONIC IMMOBILITY RECORDINGS (OPTIONAL)
03. FOOD DEPRIVATION (24 HOURS)
04. SURGERY
05. RECOVERY (1 WEEK)
06. BASELINE RECORDINGS
07. LESIONING
08. RECOVERY (5 DAYS)
09. FIRST POST-LESION RECORDING (DAY 5)
10. SECOND POST-LESION RECORDING (DAY 14)
11. THIRD POST-LESION RECORDING (DAY 30) (N=6)
12. PERFUSION
13. FORMALIN BATH (3 DAYS)
14. BRAIN BLOCKING
15. SUCROSE-FORMALIN BATH (1 WEEK)
16. HISTOLOGICAL SECTIONING AND SKETCHING
17. STAINING AND MOUNTING

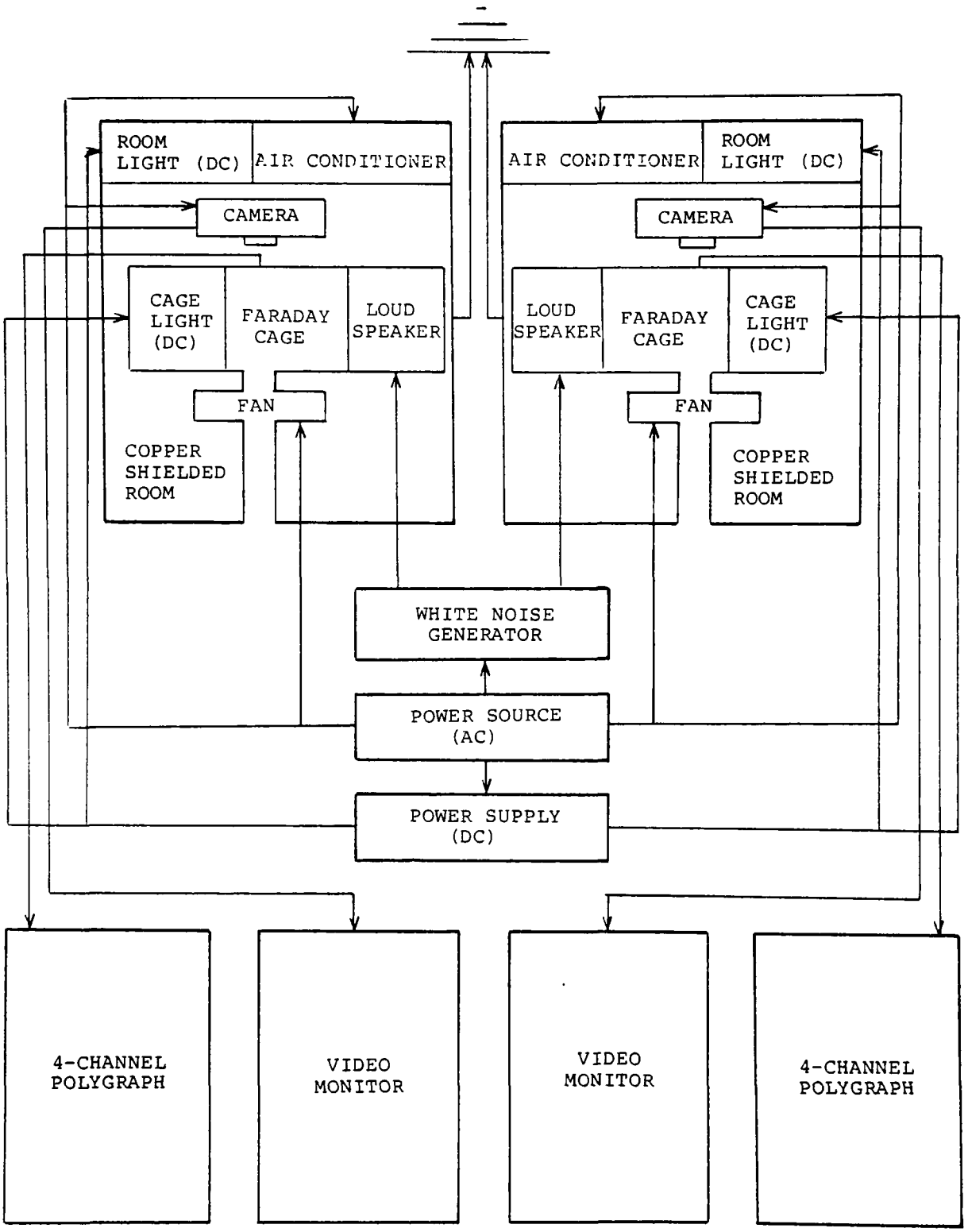

Figure 1: Equipment flowchart.

Scoring criteria for sleep and waking states

EEG records were categorized by visual analysis of 30 second epochs into 4 states: waking (W), D (D), slow wave sleep (SW) or (SWS) and PS. Prevalence of characteristics of a state within an epoch (> 50%) determined the state classification of the epoch. Polygraphic measures (EEG, EOG and EMG) together with behavioral observation were used to identify each state. Waking was defined by the presence of low voltage fast EEG, gross body movements or activated EMG and absence of phasic phenomena in the periorbital and facial areas. Drowsiness was characterized by periods of isolated spindles (less than 3 per epoch), stable EMG, and absence of phasic EOG activity. Slow wave sleep was characterized by a decreased frequency in the background EEG, regular spindling (more than 2 per epoch), with EMG and EOG characteristics similar to those noted above for D. Paradoxical sleep was defined by EEG activation, a theta rhythm more regular than that of W, EOG activation (isolated spikes as well as bursts), and variable EMG with occasional twitching. During the course of the study, a fifth scoring category called "phasic activation" or (P) was utilized. This phenomenon is comparable to the "PS without atonia" described by Morrison et al (1980) in LC-lesioned cats. It is characterized by the same attributes as PS, except for the absence of muscle atonia or relaxation. Instead, head jerks, severe body twitching, abrupt arousals and uncont-

rolled explosive flight behavior were common. The full description of the P phenomenon is given in the results section and was used in its entirety for scoring. An external judge scored the sleep records blindly. The percentage of agreement between the two scorers was 96% for baseline recordings. Post-lesion polygraphic records were analyzed with increased dependence on behavioral observations made by the first scorer. Figures 2 to 6 illustrate the states of sleep and waking in unlesioned rabbits as recorded in the present study.

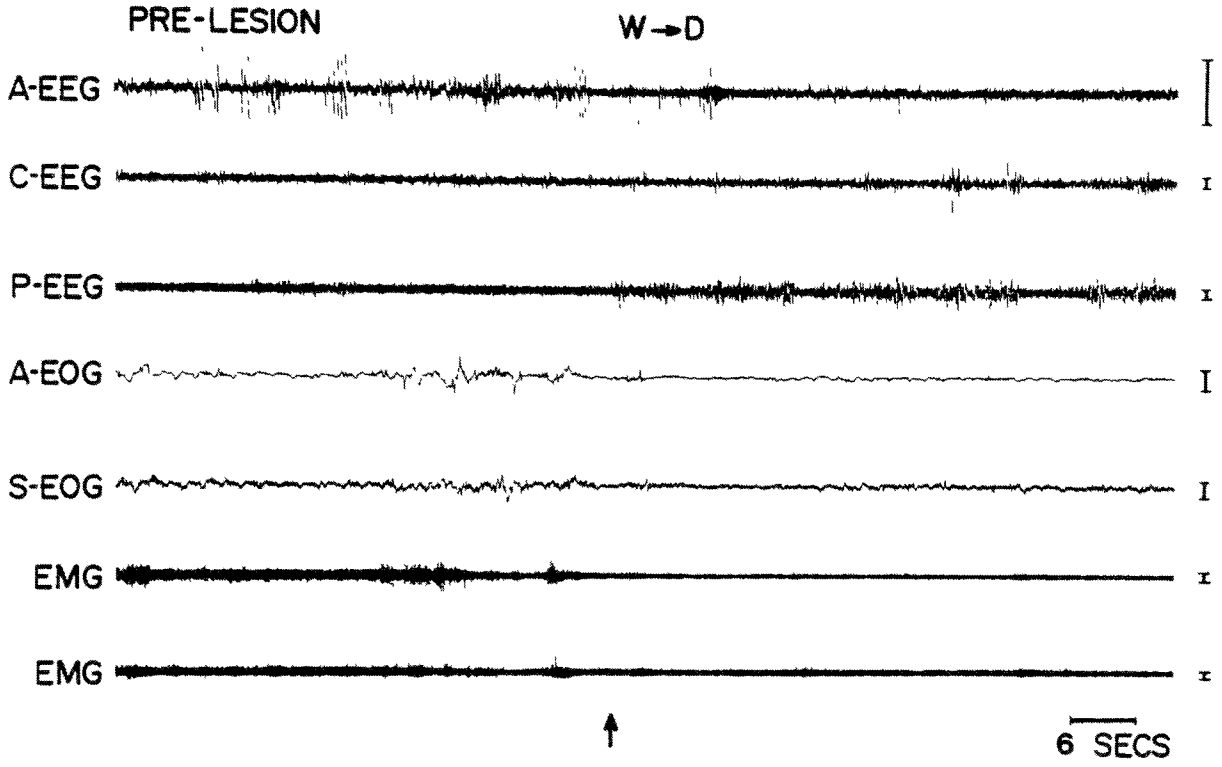

Figure 2: Transition from waking to drowsiness. W = waking, D = drowsiness, A-EEG = anterior EEG, C-EEG = central EEG, P-EEG = posterior EEG, A-EOG = anterior EOG, S-EOG = superior EOG, EMG = electromyogram, the vertical arrow indicates the moment of passage from one state to another, vertical calibration corresponds to 50 Uv.

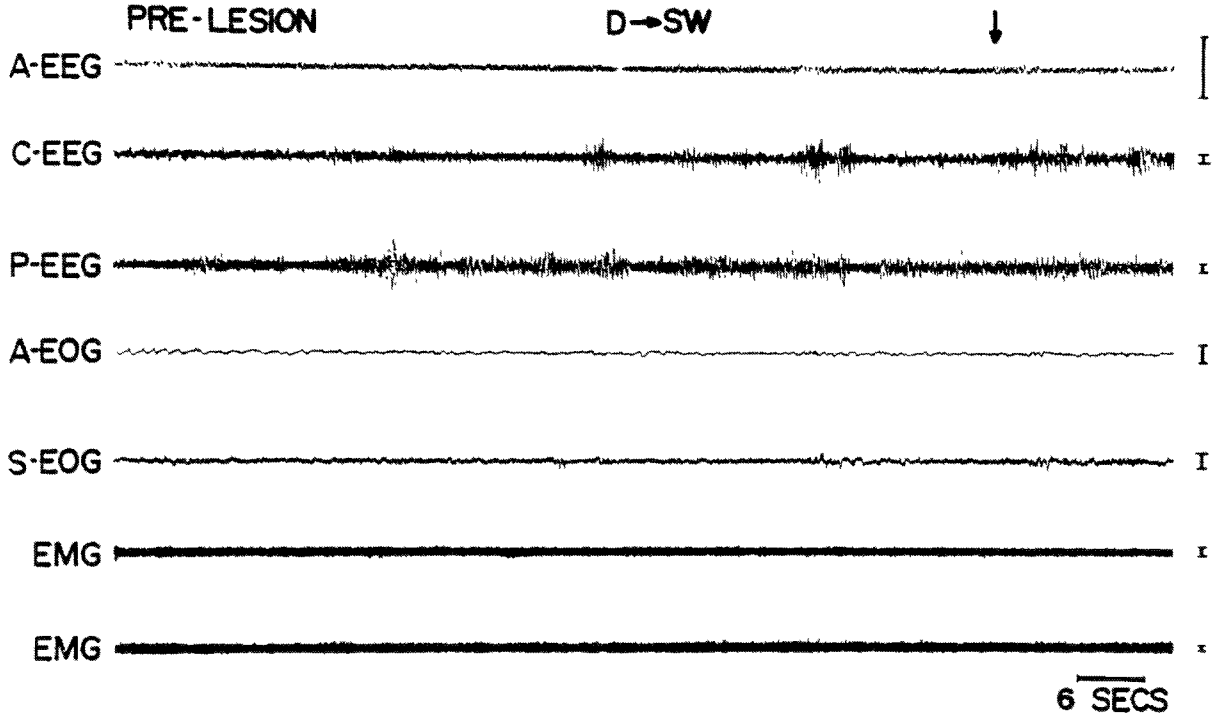

Figure 3: Transition from drowsiness to slow wave sleep. D = drowsy, SW = slow wave, A-EEG = anterior EEG, C-EEG = central EEG, P-EEG = posterior EEG, A-EOG = anterior EOG, S-EOG = superior EOG, EMG = electromyogram, the vertical arrow indicates the moment of passage from one state to another, vertical calibration corresponds to 50 Uv.

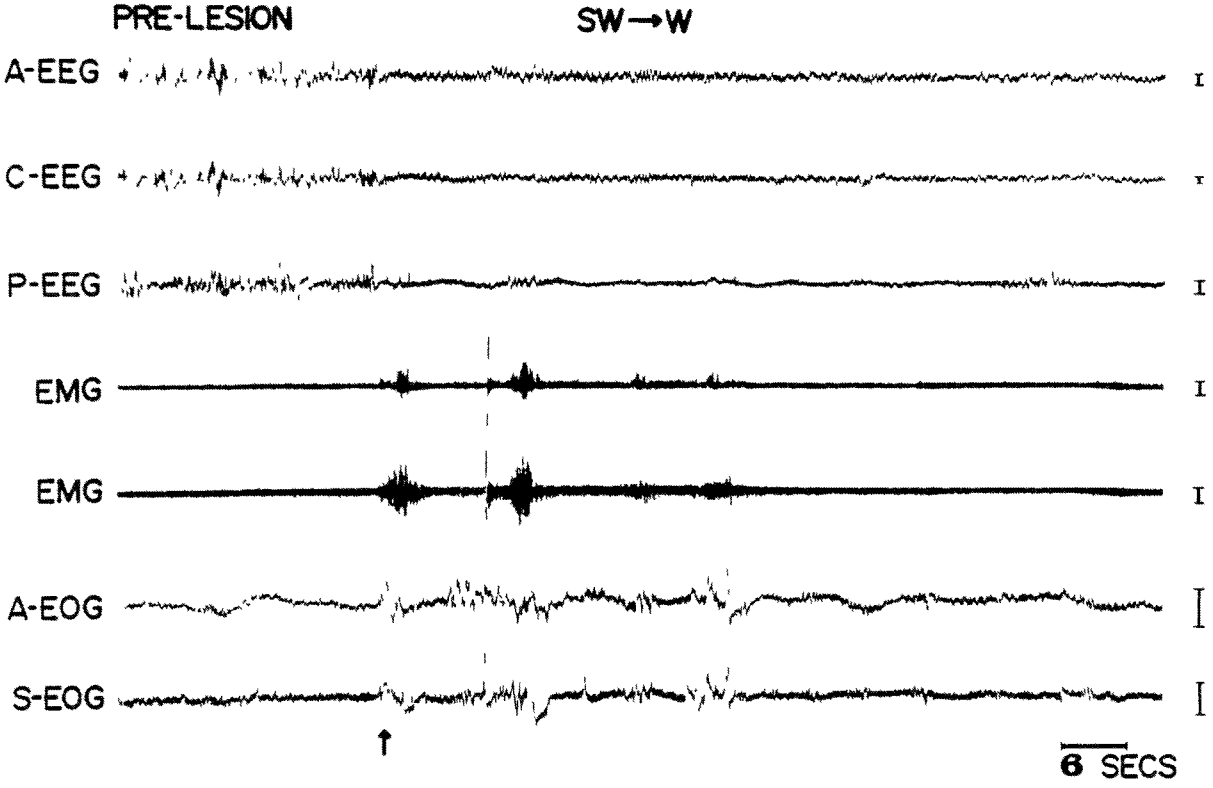

Figure 4: Transition from slow wave sleep to waking. W = waking, SW = slow wave, A-EEG = anterior EEG, C-EEG = central EEG, P-EEG = posterior EEG, A-EOG = anterior EOG, S-EOG = superior EOG, EMG = electromyogram, the vertical arrow indicates the moment of passage from one state to another, vertical calibration corresponds to 50 Uv.

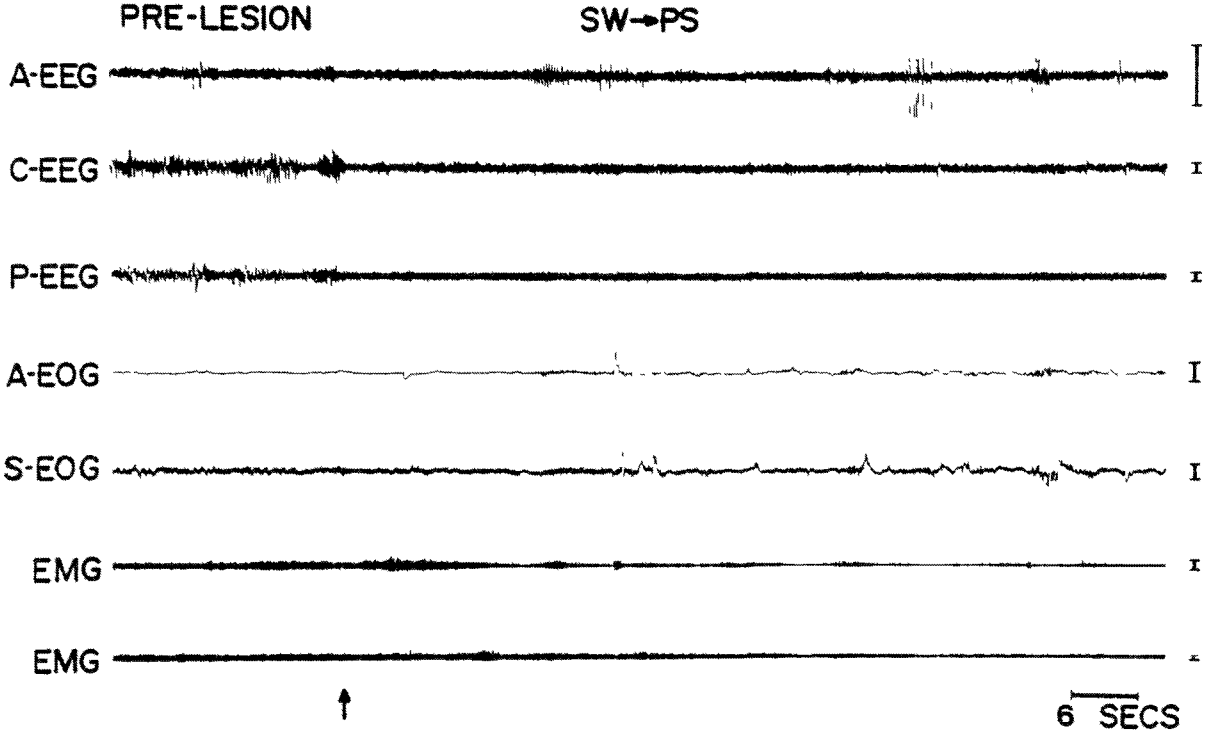

Figure 5: Transition from slow wave sleep to paradoxical sleep. SW = slow wave sleep, PS = paradoxical sleep, A-EEG = anterior EEG, C-EEG = central EEG, P-EEG = posterior EEG, A-EOG = anterior EOG, S-EOG = superior EOG, EMG = electromyogram, the vertical arrow indicates the moment of state change, calibration corresponds to 50 Uv.

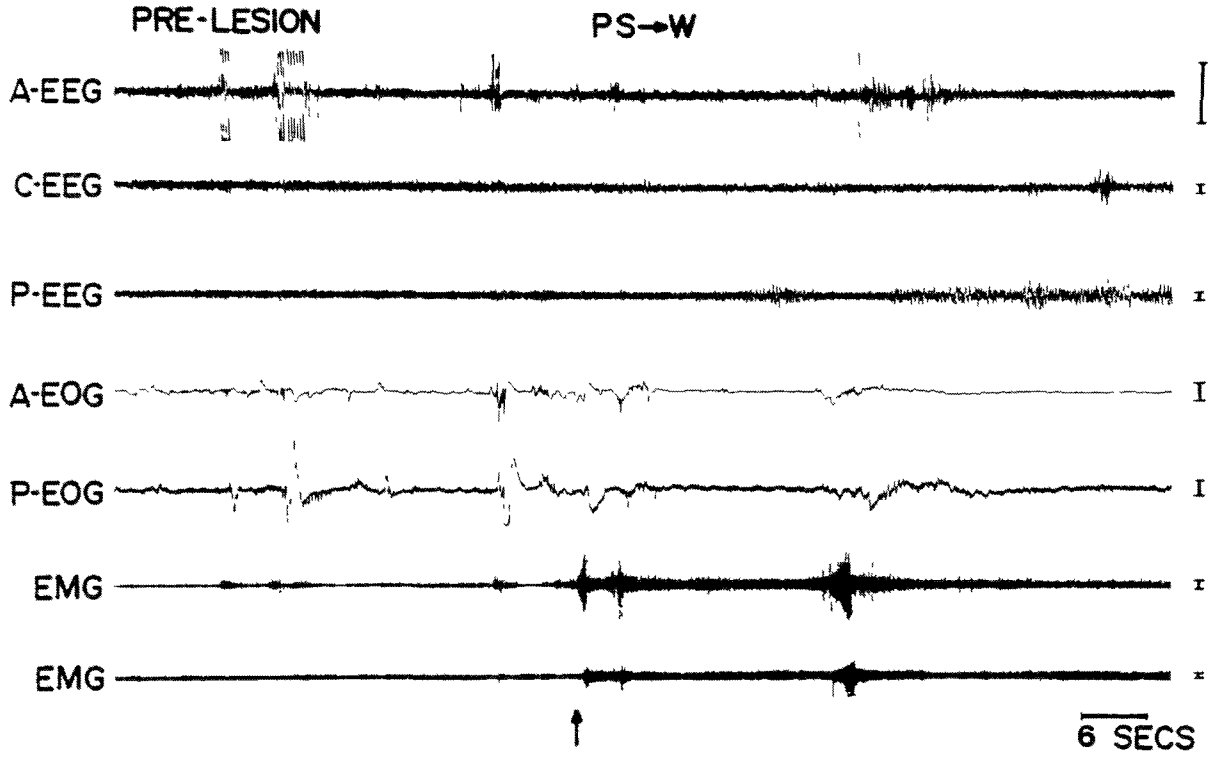

Figure 5: Transition from paradoxical sleep to waking. PS = paradoxical sleep, A-EEG = anterior EEG, C-EEG = central EEG, P-EEG = posterior EEG, A-EOG = anterior EOG, S-EOG = superior EOG, EMG = electromyogram, the vertical arrow indicates the moment of passage from one state to another, vertical calibration corresponds to 50 Uv.

Polygraph settings and electrode derivations

For each recording session four to seven channels of bipolar recordings were obtained on either a Beckman R612 8-channel polygraph or a Nihon-Kohden Multipurpose 8-channel polygraph at a paper speed of 5 mm/second. Filtering characteristics for electrographic data were as follows: EEG-- high frequency filters at 15 Hz and low frequency filters at .1 Hz; EMG-- high frequency filters at 100 Hz and low frequency filters at .3 Hz; EOG-- high frequency filters at 30 Hz and low frequency filters at .3 Hz; Sixty Hertz notch filters were used on all channels to reduce this common source of interference, even though such interference was rarely present in unfiltered tracings.

Lesioning

Shortly after the 6-hour baseline recording session the rabbit was placed in a restraining device designed to minimize body movement and rigidly fix the head without causing pain. The cannulae permitted insertion of the lesioning

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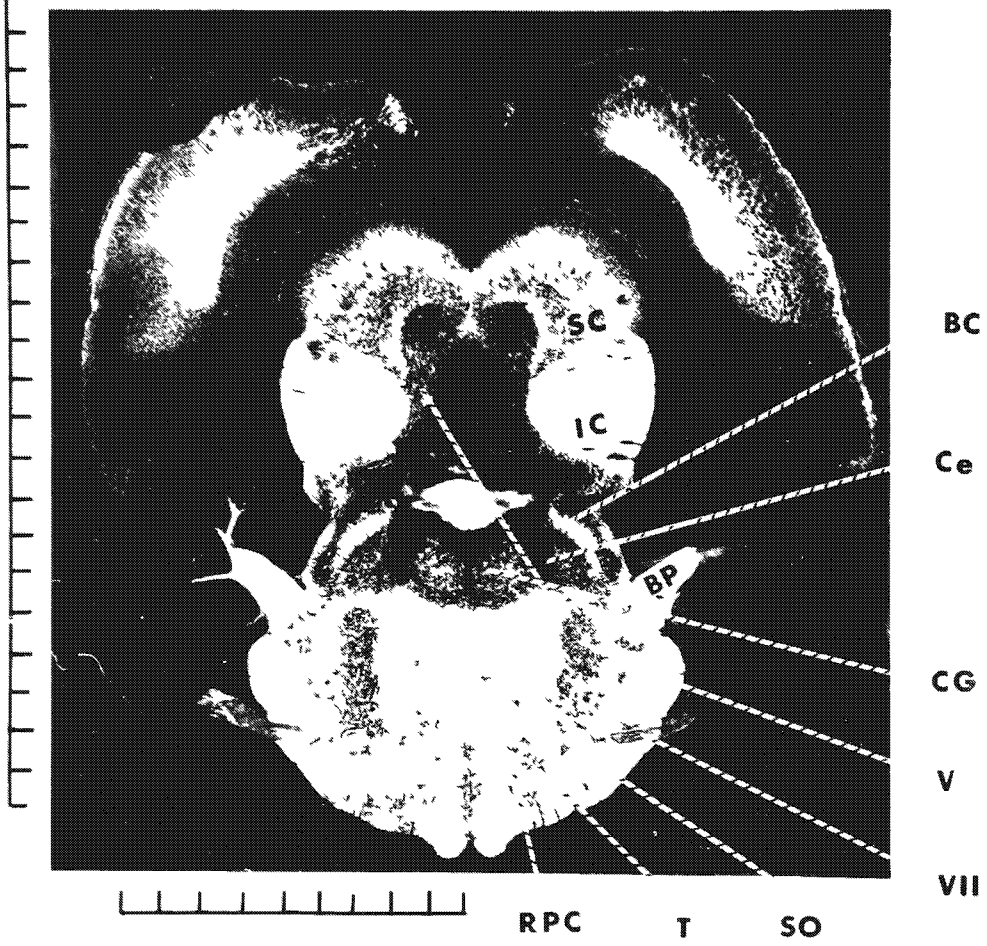
[2]In the adult New Zealand White rabbit the LC-LSC complex is approximately 2mm long rostrocaudally and 1mm wide. Though Meesen & Olzewski have distinguished a more tightly packed principal nucleus coeruleus, and a more diffuse nucleus subcoeruleus, in the rabbit, not all authors make this distinction (Bubenik & Monnier, 1972). Cell morphology and neurotransmitter characteristics do not appear to differ from the LC to the LSC (Jones & Moore, 1974). Future reference to the LC in this investigation will therefore subsume the LSC.

probe directly to the target area,[2] (see figures 7 to 9) and since brain tissue is devoid of pain receptors, lesioning was effected without anesthesia. The stainless steel plugs were removed from the cannulae and a lesioning electrode (.75 mm in diameter) was lowered into each cannula to a distance 2 mm beyond the tip (16 mm from skull surface). A David Kopf Radio Frequency Thermostatic Lesion Maker (Model RFG-4) generating a 500,000 Hz sinusoidal pulse lasting 60 seconds and adjusted to 60 degrees Fahrenheit at the electrode tip was used to create the lesion. The plugs were then sterilized and replaced into the cannulae. The animal was given 5 days to recover, and in some cases was nursed with subcutaneous injections of 5% Dextrose solution and a solution of Ringer's Lactate (50 ccs).

Figure 7: Coronal section of rabbit brain. Prepared with frozen sectioning and with a fiber stain (reproduced with permission from McBride & Klemm, 1969).

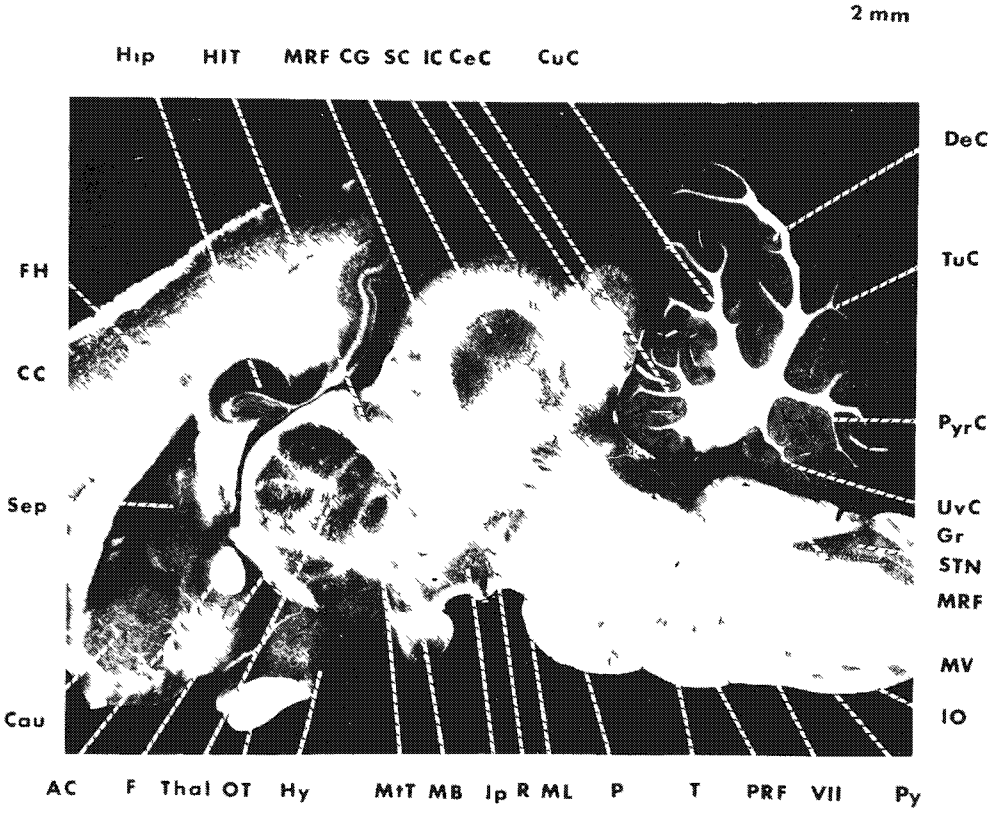
P 15



P 1

BC Brachium conjunctivum
BP brachium pontis
CC cerebellar cortex
CG central gray
IC inferior colliculus
RPC retikulärer pontiner Kaudalteil
SC superior colliculus
SO superior olive
T trapezoid
V motorischer Trigeminuskern
VII Fazialiskern

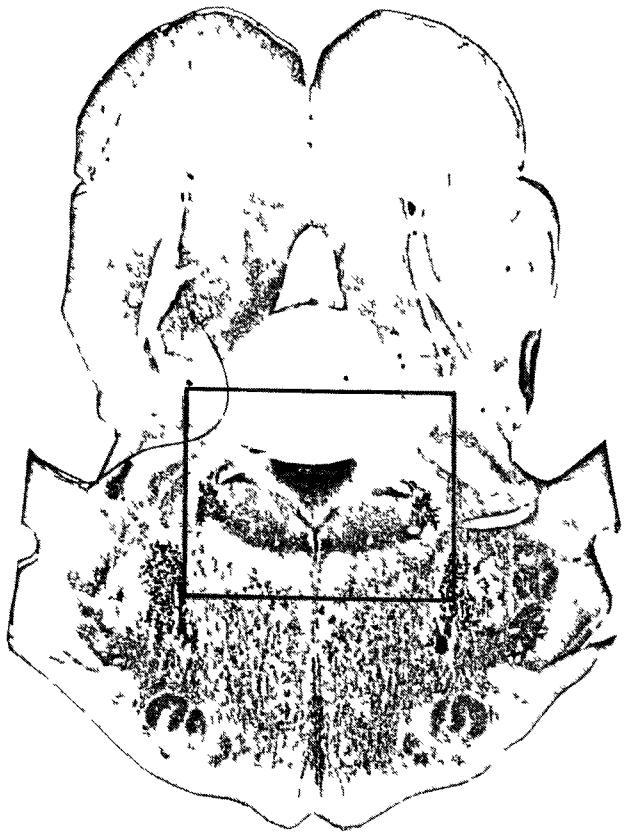
Figure 8: Sagittal section of rabbit brain. Prepared with frozen sectioning and with a fiber stain (reproduced with permission from McBride & Klemm, 1969).

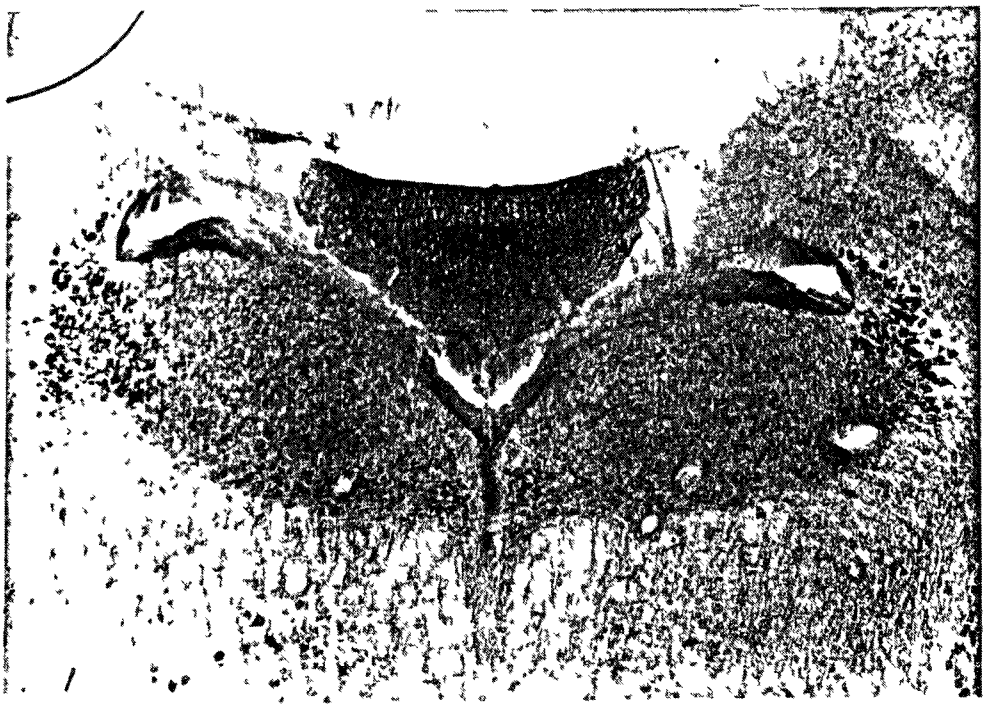


2 mm

AC = anterior commissure
Cau = caudate nucl
CC = corpus callosum
CeC = central lobe cerebellum
CG = central grey
CuC = culmen, cerebellum
DeC = declive, cerebellum
F = fornix
FH = fimbria of hippocampus
Gr = nucl gracilis
Hip = hippocampus
HIT = habenulo-interpeduncular tract
Hy = hypothalamus
IC = inferior colliculus
IO = inferior olive
Ip = interpeduncularis nucl
MB = mammillary body
ML = medial lemniscus
MRF = midbrain and medullary reticular formation
MtT = mammillo thalamic tract
MV = medial vestibular nucl
OT = optic tract
P = pontine nucl
PRF = pontine reticular formation
PyrC = pyramis cerebellum
Py = pyramid
R = red nucl
SC = superior colliculus
Sep = septum
STN = solitary tract nucl
T = trapezoid
Thal = thalamus
TuC = tuber cerebellum
UvC = uvula cerebellum
VII = facial nerve

Figure 9: Cresyl-violet stained coronal frozen section (28U) of unlesioned rabbit locus coeruleus. The section is situated at the midportion of the locus coeruleus and illustrates the dark perikaya characteristic of this nucleus.





Tonic immobility

Measures of tonic immobility were taken at approximately 5:00 PM following each recording. For TI sessions the animals were disconnected from their recording cables and were removed from the recording cages in order to prevent all possible physical constraints. A minimum of three such sessions were obtained from each subject; the first following initial sleep recordings of unlesioned animals, the second, 5 days after lesioning, and the third 14 days after lesioning. In a few instances, when a pattern of P following SWS was evident, a fourth recording was taken 3-4 weeks after the lesion. On a few occasions, polygraphic recordings of TI were taken pre and post lesion in addition to the standard number of TI recordings taken without cable attachments. TI was induced in the manner described by Klemm (1966), namely, the front paws were held in one hand, the hind paws in the other and the animal abruptly turned upside down and immediately deposited into a V-shaped trough. The trough is routinely used to stabilize the dorsoflexed position of the animal during induction of TI. Without the trough, the animal's own weight would tip him over and shorten the duration of TI in an arbitrary fashion. Each TI episode was timed with a stopwatch and the laboratory was kept silent during TI testing. Ten episodes of TI were usually induced per session except when exceptionally long lasting TI episodes occurred. In such instances a minimum of 5 epi-

sodes was induced. The termination of each TI episode was determined when the animal spontaneously righted itself. Since repetitive and prolonged induction of TI causes significant stress (Liberson, 1948), a 5 minute rest period was given each time 5 minutes had elapsed regardless of whether one or several episodes had been induced. No episode was ever interrupted. During the 5 minute rest period the animal was returned to the recording cage.

Duration of the survival period

Studies involving NA bioassays following LC lesions have demonstrated a massive telencephalic reduction of this neurotransmitter reaching 30% after 3 days in rats (Worth, Collins, Kett & Austin, 1976) and stabilizing at 50 to 86% depletion after 7 days in cats and rats (Osumi, Oishi, Fujiwara & Takaori, 1975; Roussel, Pujol & Jouvet, 1976; Jones, Harper & Halaris, 1977; Anlezark, Grow & Greenway, 1973). Macaque cortex registered 87% depletion at 35 days following bilateral LC lesions (Huang, Redmond, Snyder & Maas, 1975).

Studies examining NA depletion following LC lesions have not been made in rabbits, but the three species surveyed (cats, rats and macaques) all showed massive reductions of brain NA after LC lesions. It is logical to assume that similar reductions in brain NA occur in rabbits following LC

lesions. In view of the above evidence, it was decided that since the first post lesion recording should be taken at a time when an intermediate amount of NA depletion was expected, a 5 day interval would be appropriate. The last post lesion recording was scheduled 14 days after lesioning, an interval indicated by the above evidence as adequate for maximal or near maximal NA depletion.

Histological procedures

The animals were sacrificed by an overdose of nembutal followed by intracardiac perfusion with .9% sodium chloride solution and 10% formaldehyde. The head was mounted stereotaxically[3] for blocking the brain at an angle perpendicular to horizontal for coronal sectioning. The blocked sections were placed in 10% Formalin for 3 days, 30% sucrose for two days, and finally, were frozen with pressurized CO2 and sectioned into 28 micron sections using a Lipshaw Model 1900 cryostatic microtome. Sections were stained using the Cresyl Violet cell body stain. Lesions were localized using a projector which magnified the section by a factor of 200. Fifteen to twenty five tracings were made of the sections

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[3]This technique would not have been possible with a conventional rabbit head holder such as the one marketed by the David Kopf Company. The additional anterior and central fixation points provided by a new rabbit adaptor (Pivik & Braun, 1979) made it possible to remove the brain posterior to the diencephalon while maintaining the head in the stereotaxic apparatus.

throughout the excursion of the lesion for each brain. Five samples, as equally spaced as possible, were selected from each brain for permanent histological mounting. Reference was made to the atlases of McBride and Klemm (1968), Meesen and Olzewski (19) and Fifkova and Marsala (1967) for structure localization.

Statistical techniques

The study contained basically six dependent measures, namely, W, D, SW, PS, P, and TI, and one independent variable, namely, the brain stem lesions. Although this was in fact a multivariate repeated measures design, the number of subjects and the distributions of the dependent variables did not warrant the use of multivariate analysis of variance. Few authors have recorded sleep in an unlesioned control group so that any quantitative assessment of the effects of lesions on sleep has been quite difficult to interpret. The use of repeated measures in the present project increased the interpretability of the results and allowed for the use of more powerful statistical techniques than does the use of independent groups. Animals lesioned in areas other than the LC were classified into 3 comparison groups on the basis of lesion location, i.e., either 1) in the reticular formation, 2) outside the reticular formation, or, 3) involving parts of the LC. The statistical design, in univariate terms, consisted therefore of a two way re-

peated measures design with a 4 level "group" factor and a 3 level "time" factor. Table 1 summarizes the design of the study and also indicates the number of valid replicates per cell. The reasons for the lost replicates will be given in the results chapter. Analyses were carried out on the basis of the complete design (therefore including main effects) and were repeated on partial data bases for simple main effects. The results of these analyses were so similar that it was decided to present only results based on simple main effects comparisons.

TABLE 1

Design of the study with number of valid replicates per cell

"GROUP" FACTOR	"TIME" FACTOR: BASELINE MEASUREMENTS	5 DAY POST-LESION MEASUREMENTS	14 DAY POST-LESION MEASUREMENTS
LC	N = 11	11	9
PLC	11	11	11
R	5	5	4
NR	5	5	4

Results

Baseline sleep-waking patterns

Subjects recovered completely from initial surgery in less than a week. Rabbits are known to manifest very little PS when insufficiently adapted to the recording situation (Narebsky et al, 1969) and the values presented in Table 2 and figure 10 are not at odds with previous reports, (Tabuschi & Himwich, 1969) suggesting that the animals in this study were adequately adapted following the initial surgery. Moreover, the sleep patterns observed in the rabbits prior to lesioning were very similar to those recorded from 5 rabbits without implanted cannulae under identical or similar recording conditions in our own laboratory.

TABLE 2

Statistics for sleep-waking states in unlesioned rabbits

State	Frequency	SD	Percentage	SD	Duration	SD
W	41	10.09	33	7.16	3.10	1.28
D	43	08.35	10	2.97	0.82	0.16
SW	43	08.78	49	7.33	4.19	0.82
PS	13	05.50	08	3.51	2.35	0.87

Note. Based on 6 hour recordings. "Frequency" indicates the mean number of occurrences of the state in the 6-hour recordings. "Percentage" refers to the mean percentage of total recording time (TRT) spent in each state. "Duration" indicates the mean duration in minutes of the episodes for each state.
(N=40)

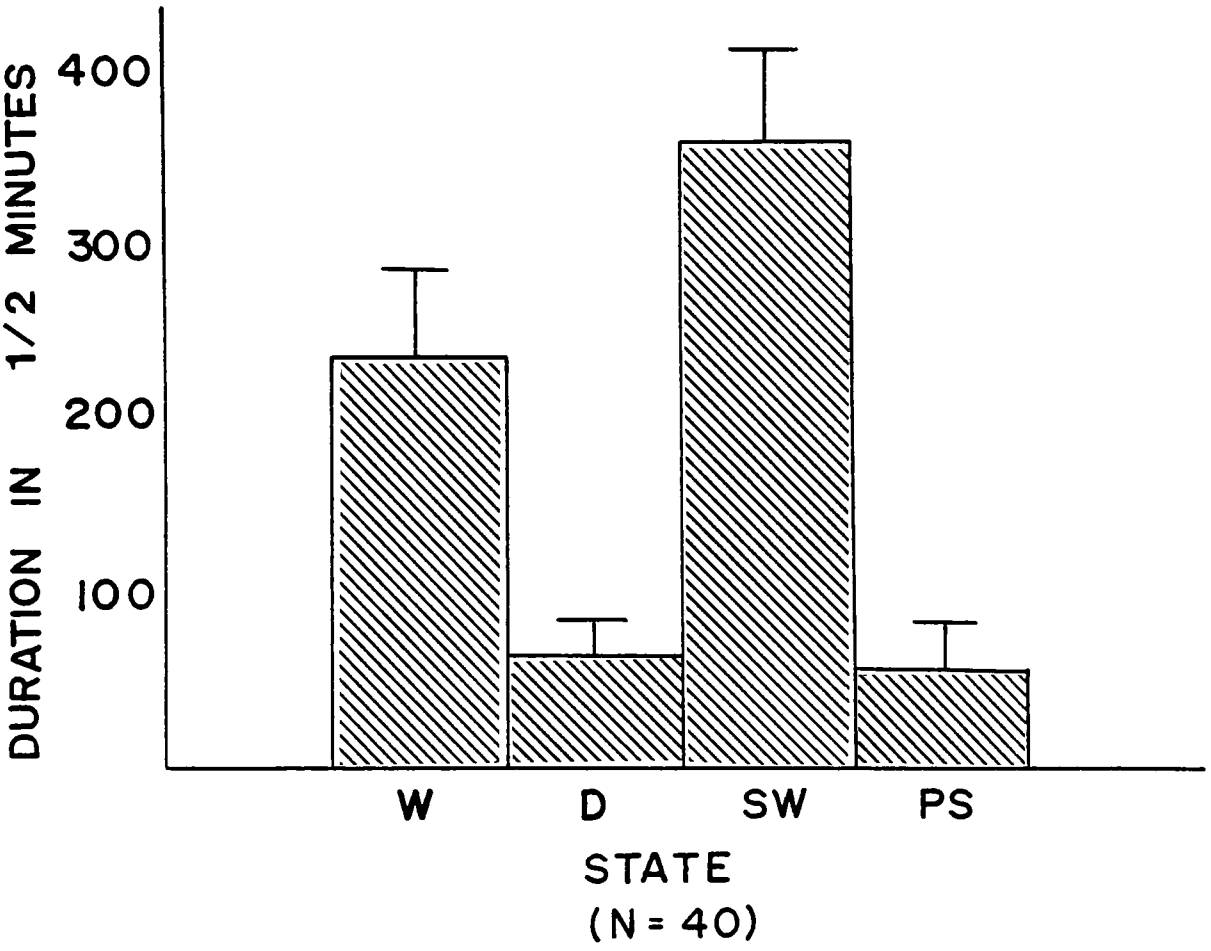


Figure 10: Time spent in sleep-waking states by unlesioned rabbits. Histogram of mean time and standard deviations, in half minutes, spent in each state in unlesioned rabbits during the 6 hours.

The preceding analyses have been the major mode of analysis used in describing sleep states in the rabbit. Previous studies have not considered the temporal relation between states of sleep and waking beyond the presentation of sleep histograms. Narebski et al (1969) included a "hypnogram" in their report which corresponds to the sleep histogram presented here (see figure 11).

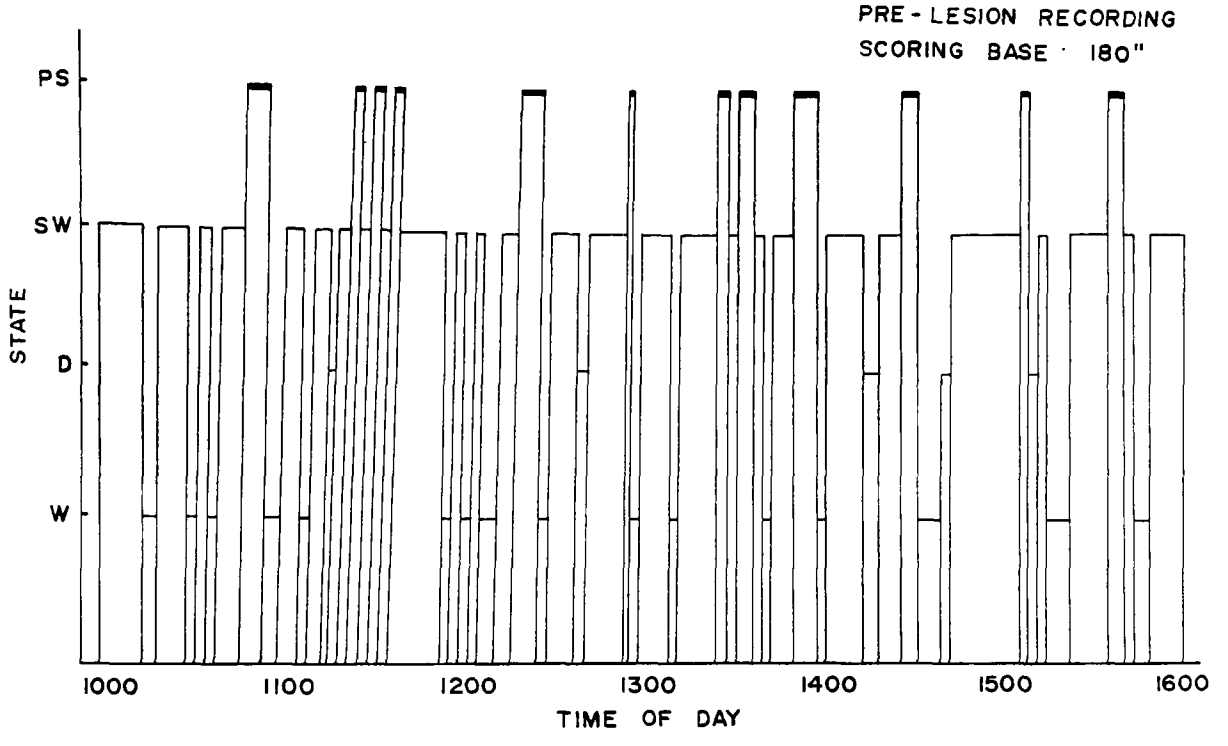


Figure 11: Histogram of daytime sleep and waking in the unlesioned rabbit.

Table 3 illustrates similar aspects of state change relationships, with exact proportions of state changes, based on all pre lesion recordings. This table shows that PS, for example, was followed by W on most occasions and D and SWS less frequently. PS was nearly always preceded by SWS and rarely by D. This sequence matrix of sleep-waking states in the rabbit corresponds to what has been reported in other species. Since the effects of lesions may be reflected in patterns of state alternation as well as state duration, it was decided to analyze the patterns of state alternation in post lesion records as well as in baseline recordings (see table 4).

TABLE 3

Percentile Proportions of State Changes in 40 Unlesioned Rabbits

STATE	W	D	SW	PS	ROW %
W	-	6	17	7	30
D	23	-	5	2	30
SW	6	24	-	1	31
PS	0	1	8	-	9
COLUMN %	29	31	30	10	TOTAL=100

Note. The abcissa represents the first element of the state change, the ordinate, the second. For example, 23% of all state changes consisted of W followed by D. Data are composite sums of state changes from 40 baseline recordings transformed into percentile proportions.

Special attention will be paid to the PS state because this sleep state is one of the more important dependent variables in this study.

During the course of the present study it was noted that contrary to published reports (Carli, 1969) and general acceptation, in unlesioned rabbits, EMG during PS did not undergo sustained reduction, and that frequently high levels of EMG activation were evident.

A feature of rabbit PS not previously reported is clusters of high amplitude 6-8 Hz activity recorded most clearly from the anterior ECG channel. These clusters occurred on the average 2.29 times per minute of PS, reached an amplitude of up to 10 times the background EEG and lasted 1-2 seconds (see figure 12).

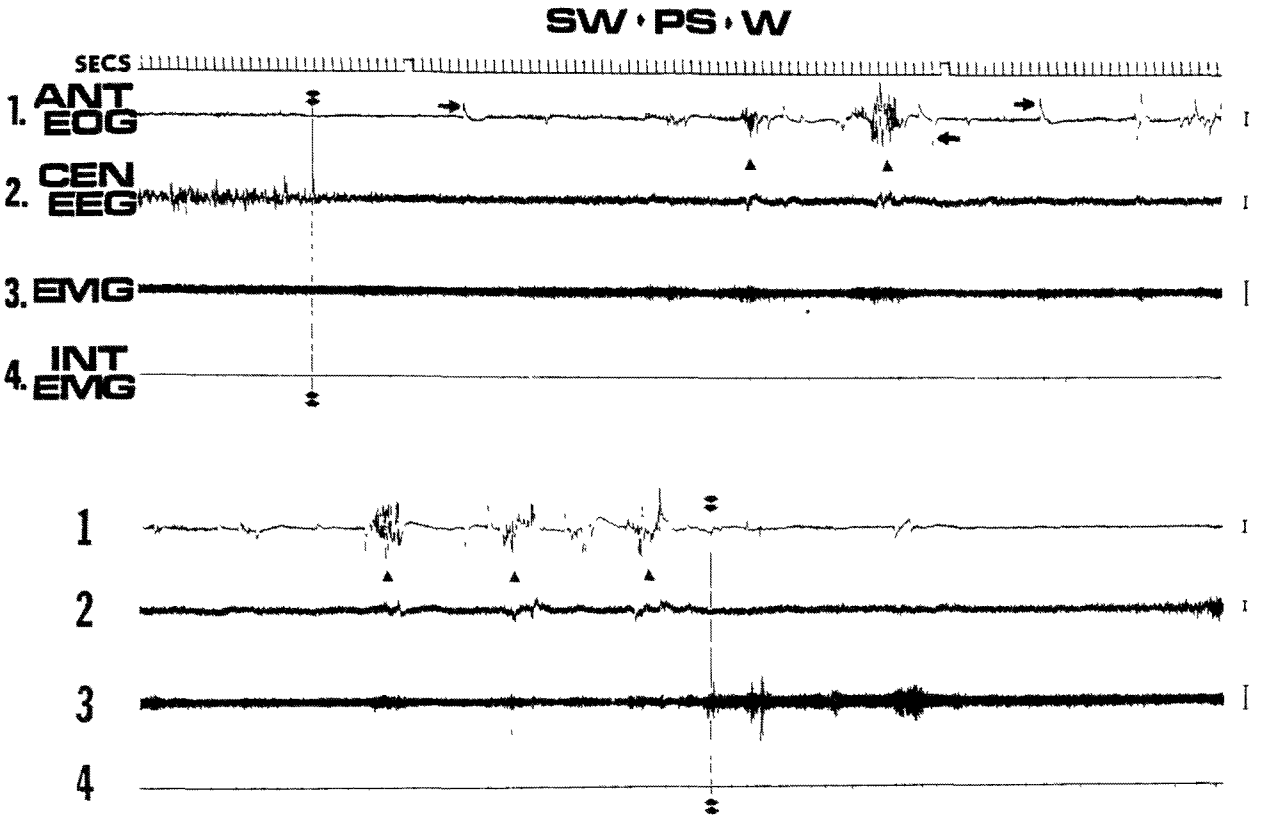


Figure 12: Phasic events during paradoxical sleep. Polygraphic illustration of phasic activity during PS in the unlesioned rabbit. The horizontal arrows indicate isolated eye movements. The vertical arrows indicate bursts of volleys of phasic activity. SW = slow wave sleep, PS = paradoxical sleep, W = waking, secs = seconds, ANT EOG = anterior EOG, CEN EEG = central EEG, EMG = electromyogram, vertical bars indicate the moments of state change, calibration = 50 Uv.

For scoring purposes, a "high amplitude volley" was defined as any burst of at least 4 waves with an interpeak interval of less than 200 milliseconds. Two volleys were considered distinct if they were separated by more than one second.

These bursts of phasic activity have not been detected in occipital transcortical recordings, and have never been observed in quiet waking, D, or SWS. A phenomenon resembling the high amplitude waves of PS did appear on rare occasions in active waking. These volleys were definitely not cortical spindles since the latter never appeared in the anterior EOG channels even during SWS and were always at a frequency of 13-15 Hz. The phasic volleys are not likely to be chewing movements because such movements effected a regular 4 hz artifact in all rabbits. Behavioral observations during PS in this study indicated that these high amplitude volleys occurred simultaneously with eyelid tremors. It was not possible to determine whether these tremors were accompanied by eye movements. The volleys, contrary to isolated eye movements described by Narebsky et al (1969), never preceded PS, and were less frequent than isolated eye movements which occurred on the average 8.77 times in each minute of PS. The density of these high amplitude volleys did not correlate with the density of isolated eye movements ($r = .09$).

Location of the lesions

Despite improvement of stereotaxic accuracy (modification of stereotaxic rabbit adaptor and of method of calculating coordinates), only 28% of the lesions were selectively centered on the locus coeruleus (i.e., at least 80% destruction of the target nucleus with minimal destruction of surrounding areas). In 8 cases, lesions were markedly asymmetric. These cases were omitted from all post lesion analyses. For a detailed presentation of the 32 remaining bilateral lesions see Appendices C and D. Inconsistencies and inaccuracies across stereotaxic atlases (coordinates in the present investigation differed by up to 3 mm from other atlases for LC) and skull variability in rabbits, contributed to this inaccuracy in the placement of lesions. Lesions were localized in the following areas:

1. Eleven lesions selectively involved the LC (> 80% destruction of LC). This group was called the "LC" group.

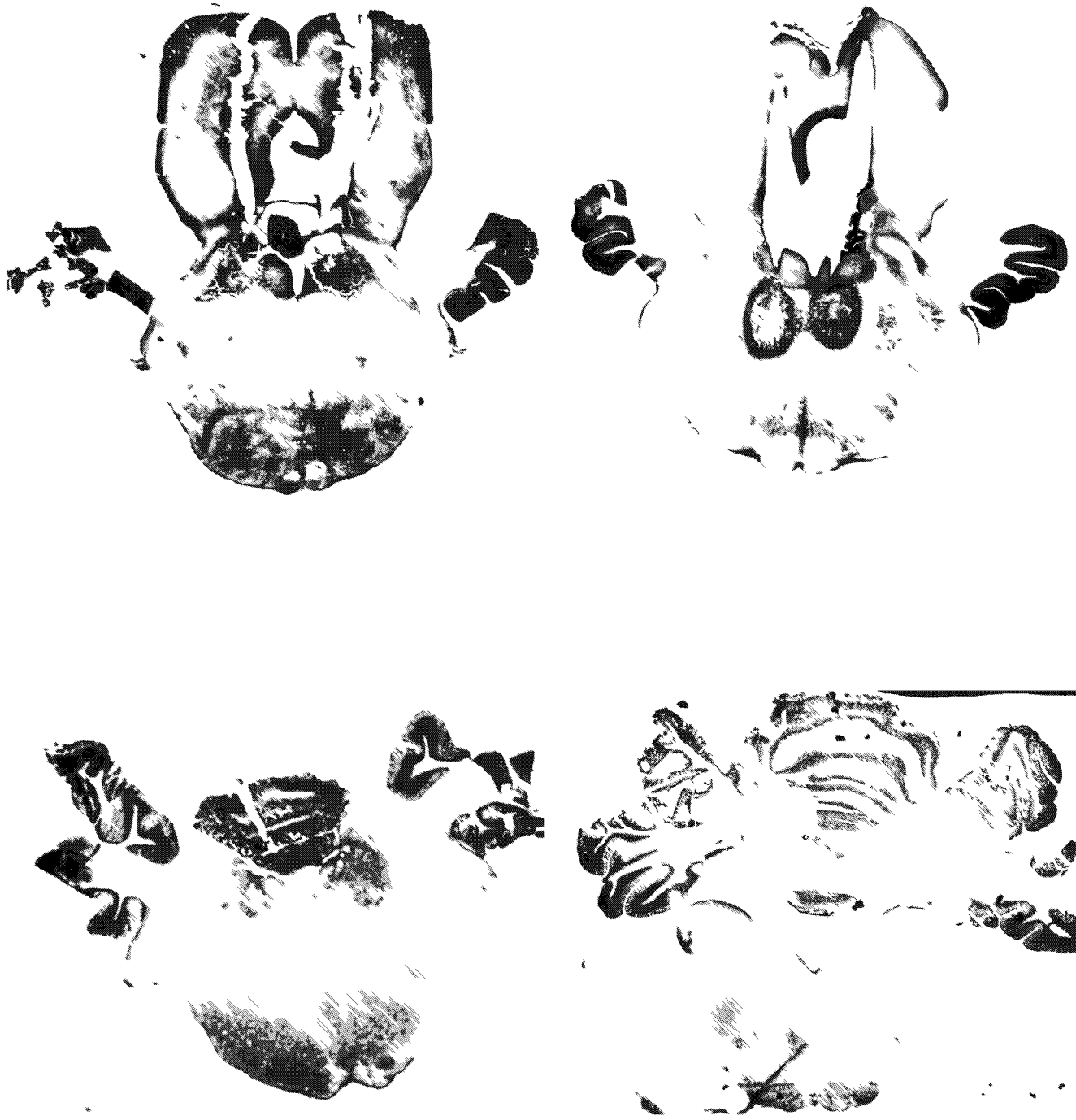
2. Eleven lesions involved only a portion of the LC (30-50 %). They involved the caudal LC (N=5), the lateral LC (N=2), the lateral- caudal LC (N=2), the dorsal LC (N=1) and dorsal-caudal (N=1) LC. For purposes of statistical analysis this constellation of lesions was grouped into a "partial-LC" or "PLC" group.

3. In five animals, bilateral lesions were localized to the NRPC (N=3) or the NRPO (N=1) or both (N=1). All five lesions completely spared the LC. This group was called the "reticular" or "R" group.

4. In another group of five animals lesions were centered on non-reticular structures, namely the Vestibular nuclei (N=2), the Central Grey matter (N=1), the Colliculi (N=1) and the Cerebellum (N=1). None of these lesions involved the LC. These lesions were grouped into a "non-reticular" or "NR" group.

Figure 13 illustrates examples of lesions from these 4 groupings. Of the 32 symmetrically lesioned animals, there were three experimental mortalities. Two LC cases were lost after the first post lesion recording. One animal (LC-37) whose behavior progressively deteriorated following lesioning, died before a second post lesion recording could be taken. The other, (LC-34), because of an error in laboratory procedure, was sacrificed prematurely. One member of the "reticular" group died after 7 days of deterioration following lesioning (NRPC and Nucleus Gigantocellularis Reticularis). To summarize, the final composition of the groups was LC (N=11), PLC (N=11), NR (N=5) and R (N=5). (Detailed drawings of each lesion are presented in Appendix C)

Figure 13: Illustrations of the lesions. Photographs of 28U brain sections stained for cell bodies with the cresyl violet method. In the top left corner is an example of an LC lesion. At the top right hand side is an example of an "R" lesion involving the NRPO. The lower left hand corner illustrates an "NR" lesion involving the medial vestibular nucleus. The lower right hand corner represents another "NR" lesion located in the cerebellum.



Immediate behavioral effects of the lesions

The placement of cannulae positioned over the LC during surgery made it possible to effect lesions without discomfort in the restrained conscious animal. The lesioning procedure lasted approximately two minutes, and during this procedure, the animals manifested agitation indistinguishable from that observed under the same conditions of restraint without lesioning. Respiratory rates increased but only for the short duration of the lesioning procedure. The only changes which occurred frequently in the rabbits at the time of lesioning were nystagmus, marked generalized loss of skeletomotor tonus, and increased respiratory rates.

Though no precise measurements of respiration rates or motility were made at the time of the lesions, observations were made in a relatively systematic manner. In terms of obvious motor abnormalities such as dystonias, dystaxias, and inactivity, the "NR" group suffered the severest symptoms. These symptoms were observed in decreasing order of severity in the "R" group. In the "LC" group only inactivity was observed immediately following the lesions, and this lasted no more than a few days.

Long term vegetative and motor effects of lesions on the waking state

Unlike cats and rats, rabbits did not manifest absence of micturition following LC or other lesions. It was never necessary to void the bladders by massage. A temporary syndrome of adipsia, aphagia and diahrrea was observed in all lesioned animals except two NR cases in which the latter symptom persisted until the time of sacrifice. The LC-lesioned animals lost approximately one kilogram of weight during the first post lesion week, and remained at that level for the survival period. Animals with non-reticular lesions had regained normal weight levels by the 14th post lesion day.

Respiration was normal 3-4 days following the lesions in all cases except NR-1 and PLC-11 whose respirations were laborious until sacrifice, 14 days after the lesions. These observations are based on visual monitoring of respiratory rate.

No dystonias, ataxias, or other abnormal movements were observed during waking in LC-lesioned animals. Not unexpectedly, these behaviors were present in animals with lesions involving the vestibular system or the cerebellum. All 3 lesions centered on the NRPC produced persistent motor abnormalities. In 2 cases, spastic neck dystonia was ob-

served, and dystaxia was observed in the 3rd. Lesions involving the NRPO and the rostral part of the NRPC did not produce any obvious motor abnormalities.

Contrary to effects observed in LC-lesioned rats, no uro-genital disorders such as permanent erections, bleeding of the penis or in the bladder, or bladder or penile infections were observed.

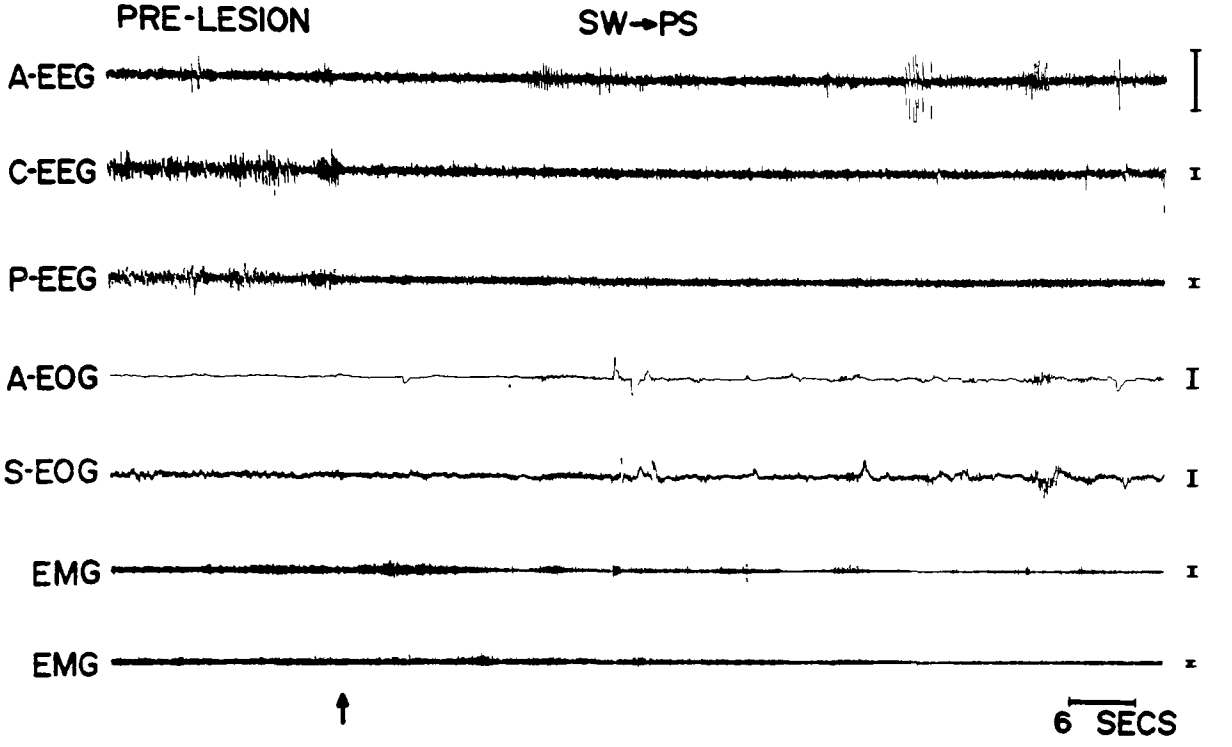
Though LC-lesioned rabbits still appeared slightly scruffy and dirty, grooming was observed in most cases by the 14th day post lesion. Only one case, with a generally debilitating syndrome, did not recover grooming (NR-1). In this animal the lesion encompassed a massive area involving all 4 colliculi.

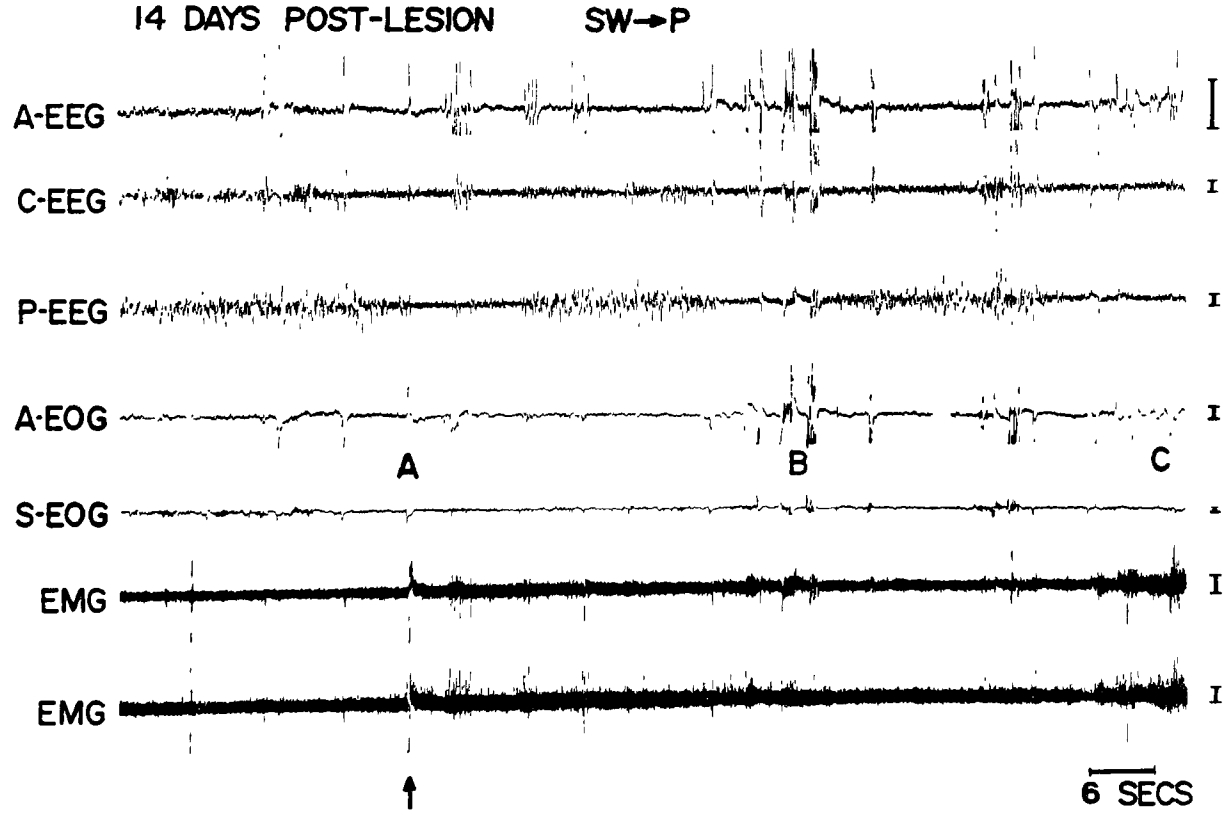
Inactivity was observed in all groups immediately following the lesions, but this was usually a temporary phenomenon. There is evidence from a previous report (Morrison et al, 1980) that the motility level increases in cats within the week following LC lesions. In the present study, by the end of the second week post lesion, overall waking time during recordings had increased by 17.5% in the LC group. It was found, however, that this increase was comprised primarily of waking without EMG activation (11.5%) rather than active waking (7% increase).

Phasic activation

Figure 14 shows that selective effects of LC lesions are distributed throughout sleep; i.e., sleep is severely fragmented not only by the extended periods of phasic activation, but also by frequent abrupt and brief episodes of twitching which interrupt SWS. A similar phenomenon was described for the first time by Jouvet and Delorme in 1965. They stated that the phasic events of PS progressively invaded SWS.

Figure 14: Paradoxical sleep and 'phasic activation'. Illustration of transitions from SWS to PS and from SWS to P in the same rabbit before and after a LC lesion. A= Abrupt ear movement. B= Hallucinatory-like movements C= Sequence of agitation culminating in violent collision with the cage wall. SW = slow wave sleep, PS = paradoxical sleep, P = phasic activation, A-EEG = anterior EEG, C-EEG = central EEG, P-EEG = posterior EEG, A-EOG = anterior EOG, S-EOG = superior EOG, EMG = electromyogram, vertical arrows indicate the moment of state change, calibration = 50 Uv.


PRE-LESION
SW→PS
A-EEG
C-EEG
P-EEG
A-EOG
S-EOG
EMG
EMG
6 SECS


14 DAYS POST-LESION
SW→P
A-EEG
C-EEG
P-EEG
A-EOG
A
B
C
S-EOG
EMG
EMG
6 SECS

Previous studies have not attempted to quantify the occurrence of P produced by LC lesions because of the difficulty in differentiating P from W on the basis of the usual method of scoring polygraphic records by visual inspection. In the present experiment, behavioral observations from video monitoring coupled with electrographic recordings made it possible to differentiate P from W, therefore to quantify P.

Temporally extended episodes of predominantly phasic activation (P) were identified according to the joint presence of the following characteristics:

1. Extreme twitching, sudden head jerking, or any other unusual and abrupt movement lasting at least half an epoch (i.e., 15 seconds)

2. Clearly identifiable cortical desynchronisation.

In the present study, evidence for an LC lesion effect of "PS without atonia" was confirmed and expanded. It was found that D and SWS tended to precede P and W tended to follow P in proportions similar, but not identical, to those observed for PS in the pre lesion condition. Although systematic data were not collected in the present study with regard to pupil size, it was possible on a few occasions to observe pupillary myosis during "phasic activation". Myosis

is also known to occur during PS. There were also high amplitude waves recorded from anterior EOG emplacements, described previously in normal rabbit PS, which occurred during phasic activation (see figure 14). The maximum durations of P and PS were very similar in this study (10 minutes and 10 minutes, 20 seconds, respectively), although the mean duration of PS episodes (140 seconds in the LC group prior to lesioning) was considerably longer than the mean duration of P episodes (61 seconds in the same group 14 days after lesioning). However, the total time spent in P corresponded quite closely to the time spent in PS by unlesioned animals. Phasic activation correlated negatively with PS ($r = -.44$, $p < .1$), indicating somewhat of a reciprocal relationship between the two states. Finally, the progression of EEG desynchronization occurring in occipital EEG recordings tended to precede the desynchronization occurring at more anterior derivations at the beginning of both PS and P episodes.

Effects of the lesions on the time spent in each state

A univariate repeated measures analysis of variance with two factors was performed to assess the effect of the lesions on sleep and waking states. One factor ("groups") consisted of four groups of subjects based on lesion sites, namely LC, PLC, NR and R. The other factor ("time") consisted of the three recording sessions, namely baseline, 5 days post lesion and 14 days post lesion. The dependent

measures were the amounts of time spent in W, D, SWS, PS and P per recording. Tests were computed to assess the validity of these data for meeting the assumptions of normality, homogeneity of variance, and symmetry of variance-covariance matrices. All variables satisfied the assumptions (see Appendix E). Post-hoc analyses were based on the Scheffe method.

The amount of PS was significantly reduced at both 5 and 14 day intervals for the LC group only ($p < .001$). Correspondingly, the amount of P was significantly increased 5 days and 14 days after the lesions only in this group ($p < .001$). No other simple main effects reached the .05 level of probability either on the "time" or the "group" factors. No interaction effects were observed (For illustration see figure 15, for means and standard deviations see Appendix F).

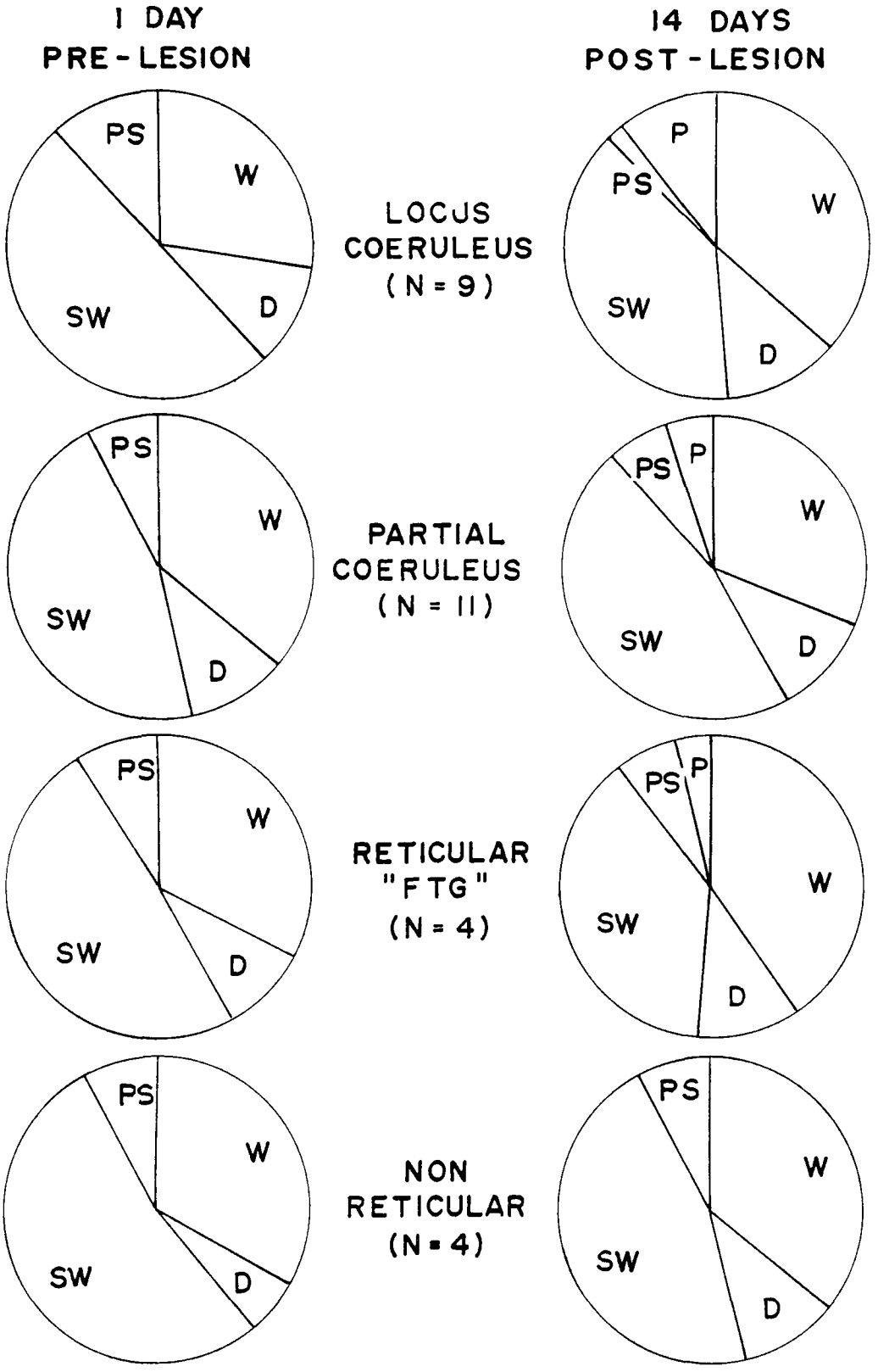


Figure 15: Amounts of time spent in each state pre and post lesion

Effects of the lesions on the incidence of sleep-waking states

Figure 16 shows that there is a slight increase in incidence of each state, except PS, in each group (except the NR group) 5 and 14 days after the lesions. The NR group, which demonstrated no overall change in state incidence post lesion, showed a marked increase 5 days post lesion followed by a return to baseline level by 14 days post lesion. Figure 17 illustrates the same effect over time with all the states combined. Overall, there was a slight increase in the incidence of state changes in all groups except the NR which returned to an average level characteristic of unlesioned rabbits.

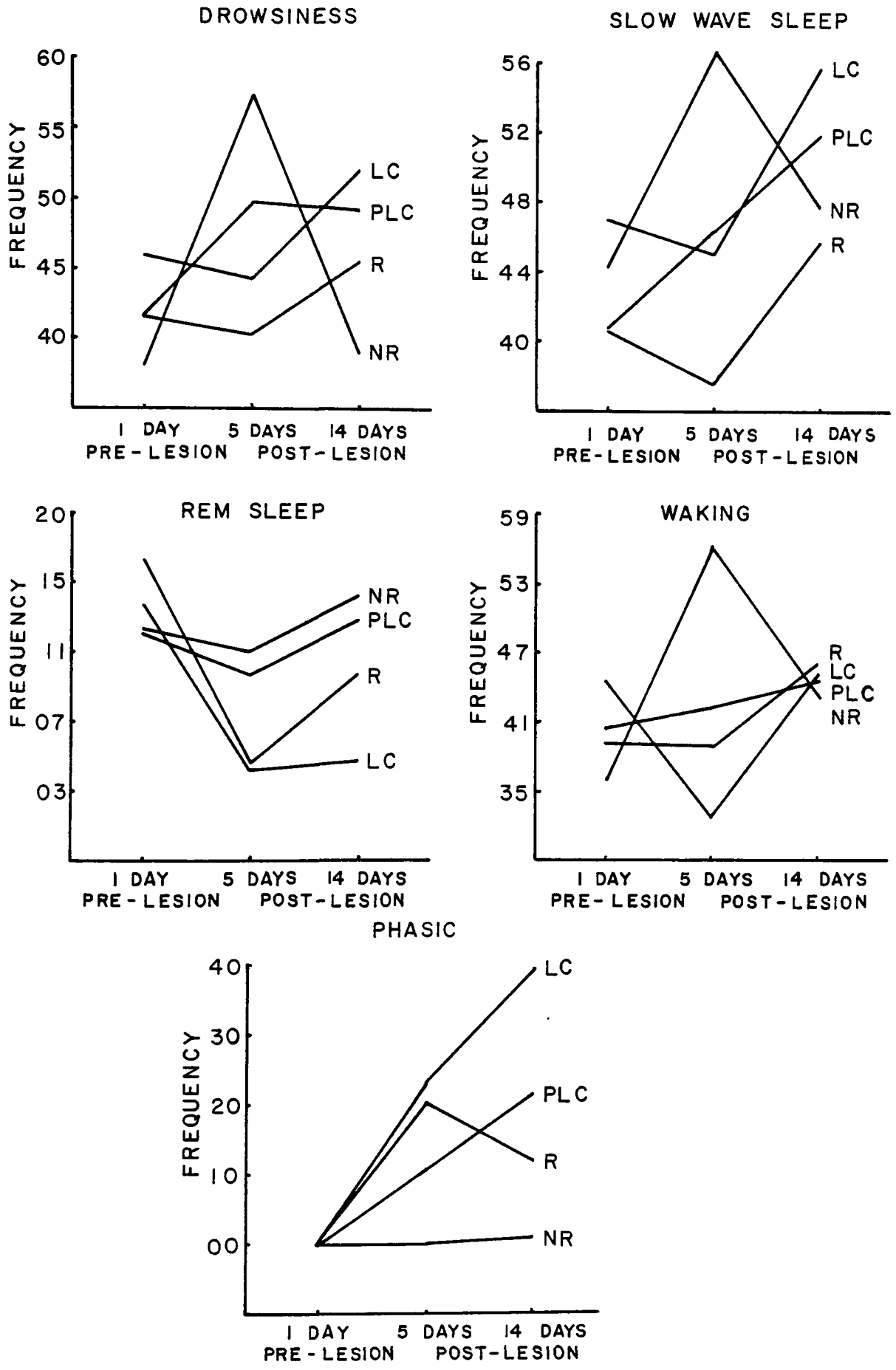


Figure 16: Frequency of occurrence of each state.

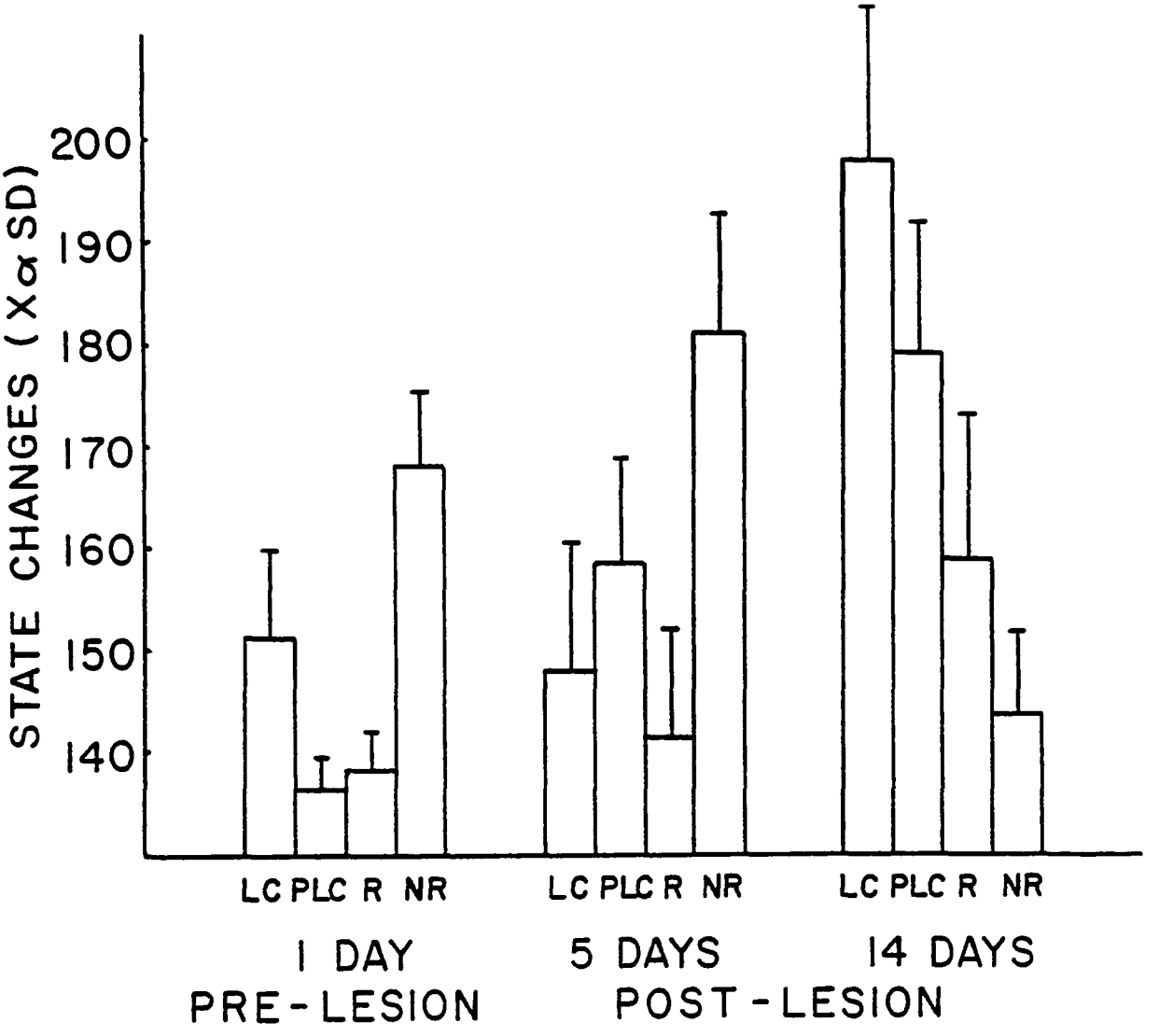


Figure 17: Frequency of state changes per recording per group.

Effects of lesions on the patterns of state alternation

At 14 days post lesion, the pattern of of sleep-waking state alternation was massively disrupted in the LC group, disrupted to a lesser extent in the PLC and R groups, and was unaffected in the NR group (see table 4 and figure 18). The common pattern of state alternation in the unlesioned rabbit from W to D to SWS to PS to W becomes somewhat obscure in the recordings of LC, PLC and R-lesioned animals, because of the interspersion of P throughout the records. It can be seen, however, that the pattern of relation between W, D, and SWS remains basically unaffected in all groups at the 14 day interval. The relation of PS to the other states changes most markedly because of the drastic reduction in incidence of this state, especially in the LC group. Also, each state comes into relation with the P activation according to the pattern previously exhibited with respect to PS, i.e., P is usually preceded by SWS or D (the latter less frequently) and P is generally followed by W.

TABLE 4

Pre and post lesion patterns of sleep-waking state alternation

		1 DAY PRE-LESION					14 DAYS POST-LESION				
		W	D	SW	PS	P	W	D	SW	PS	P
LC	W	0	9	26	9	0	0	10	19	1	16
	D	34	0	9	3	0	32	0	8	1	11
	SW	9	32	0	1	0	11	34	0	0	11
	PS	0	2	12	0	0	0	1	3	0	1
	P	0	0	0	0	0	2	8	26	4	0
PLC	W	0	9	22	9	0	0	10	18	8	9
	D	33	0	7	2	0	32	0	7	3	7
	SW	7	32	0	1	0	12	32	0	1	6
	PS	0	1	10	0	0	0	2	11	0	0
	P	0	0	0	0	0	1	5	15	1	0
R	W	0	8	20	11	0	0	9	25	4	8
	D	30	0	7	5	0	35	0	4	5	2
	SW	9	32	0	0	0	10	33	0	1	2
	PS	0	3	14	0	0	0	2	8	0	0
	P	0	0	0	0	0	1	3	8	0	0
NR	W	0	4	25	8	0	0	7	27	8	1
	D	25	0	9	4	0	29	0	6	3	0
	SW	10	33	0	1	0	14	29	0	4	1
	PS	0	2	11	0	0	0	2	12	0	0
	P	0	0	0	0	0	1	0	1	0	0

Note. Mean number of state changes in each group for baseline and 14th day post lesion recordings. The abcissa represents the first element of the state change, the ordinate, the second. For example, in the LC group pre lesion, D was followed by W exactly 9 times. The entries in this table are absolute numbers.

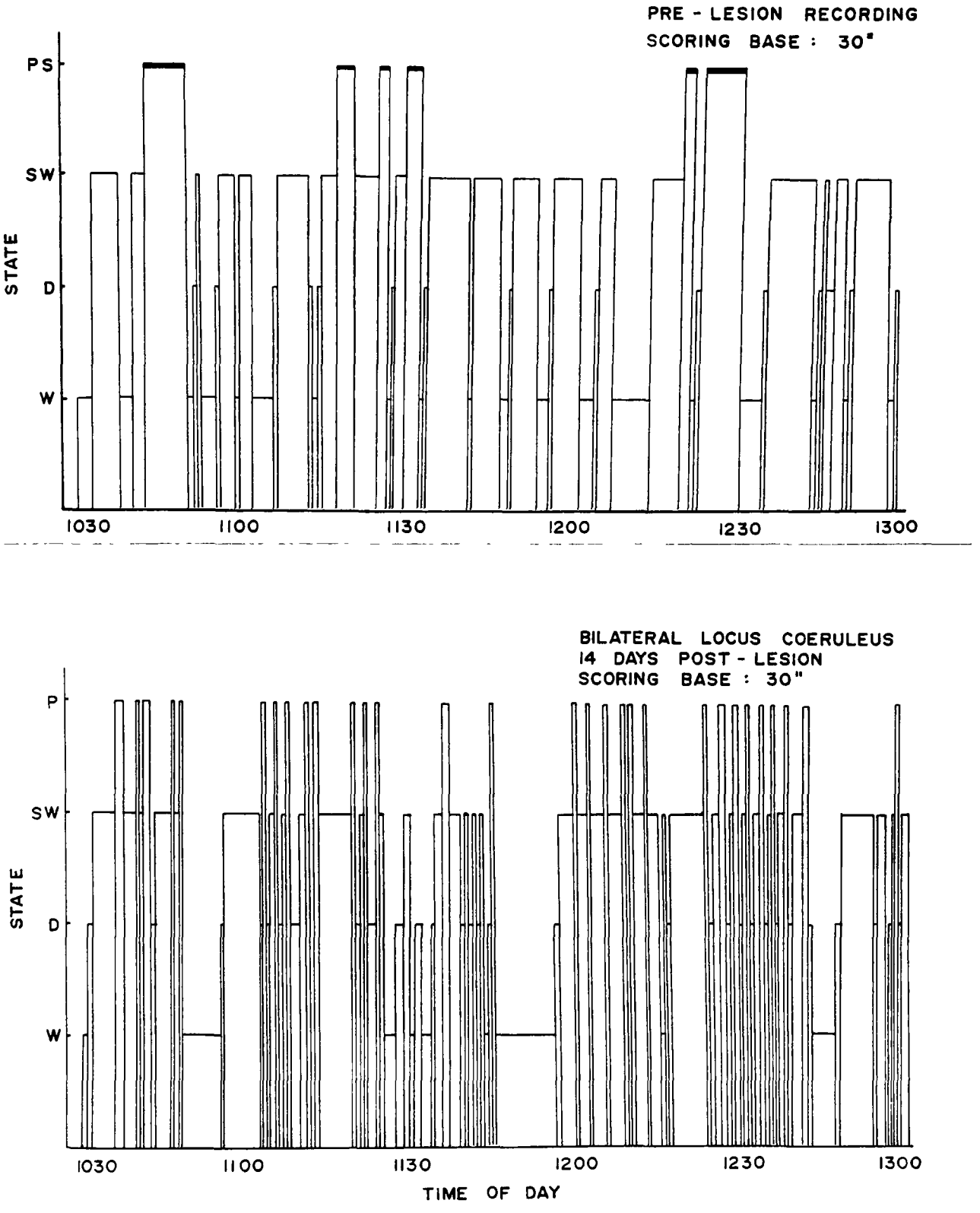


Figure 18: Histograms of pre and post-lesion sleep.

The findings reported in this section and the two preceding sections indicate that lesions which encompass the LC area have a strong and selective effect on sleep, and that in general, lesions of comparable size in other parts of the brain stem do not produce significant alterations in the sleep pattern. However, the next section will serve to qualify and refine this general statement.

Effects of lesions on sleep and waking: subdivisions of the lesion groups

The issue regarding which part of the LC, if any, is sufficient to produce P cannot be fully answered by data gathered in the present study. However, some relationships pertinent to this issue may be indicated. For example, the amount of LC destroyed regardless of location correlated more with the reduction in PS ($r = -.45$, $p < .05$) than with the increase in P ($r = .37$, $p < .25$). Of 5 cases exhibiting the highest amounts of P and total abolition of PS, 4 were centered on the LC and 1 involved the lateral LC. Without exception, lesions involving only the caudal part of the LC (N=5) produced P, (mean time = 12 minutes, 50 seconds at 14 days post lesion), and PS reduction (mean time = 27 minutes, 50 seconds at 14 days post lesion), but not to the same extent as more complete LC lesions. Four lesions involving only the lateral or lateral-caudal LC produced similar but slightly more marked effects (mean time in P = 32

minutes, 25 seconds, mean time in PS = 17 minutes, 9 seconds; both measurements were taken at 14 days post lesion). None of these effects was as marked as those observed following complete LC lesions (mean time in PS = 6 minutes, 35 seconds, in P = 38 minutes, 32 seconds). One lesion involving the dorsal LC (PLC-9) did not produce P or PS reduction. Only complete LC lesions produced dramatic "hallucinatory-like" movements, including abrupt, violent motor activation, culminating in collision with cage walls. Such behavior was never observed in waking.

LC lesions were not, however, the only lesions followed by abolition of PS. Brief episodes of P (totalling between 8 and 32 minutes per record) were observed in 3 animals with lesions involving the entire NRPC and in one animal (R-18) with a lesion involving the NRPO and a small part of the NRPC. One NRPC lesion and one lesion involving both the NRPO and NRPC were followed by total abolition of PS. The one animal with a lesion involving only the NRPO did not manifest abnormal sleep. None of the non-reticular lesions was associated with signs of abnormal sleep.

In a subsample of 6 LC-lesioned rabbits, the survival period was extended to 30 days after the lesion, at which time a 4th recording was taken. The effects of LC lesions on PS and P described in previous sections at 5 and 14 days

post lesion were maintained and even slightly enhanced 30 days after lesioning. (see table 5). The violent "hallucinatory-like" behavior observed in LC-6 and LC-8 following the lesions persisted throughout the 30 day post lesion period. The effects of LC lesions described above are therefore very long lasting, and may, as Henley and Morrison contend (1974), be permanent.

TABLE 5

Mean episode frequencies and durations and %s of total recording time

State	Frequency	%	Duration
	5 Days Post-Lesion		
W	27.66	35	4.58
D	43.16	09	0.88
SW	48.00	46	3.47
PS	16.00	03	0.81
P	30.16	07	0.88
	14 Days Post-Lesion		
W	44.83	34	2.69
D	51.83	11	0.79
SW	58.83	42	2.57
PS	04.00	01	0.92
P	45.33	12	0.96
	30 Days Post-Lesion		
W	42.83	35	2.82
D	48.00	09	0.73
SW	60.50	43	2.53
PS	01.33	00	0.69
P	50.00	13	1.00

Note. The subjects are LC-6, LC-8, LC-25, LC-35, LC-36, LC-39.
Duration is in minutes.

Tonic immobility in unlesioned rabbits

Three aspects of TI were measured in the present study. Duration of TI episodes was calculated from the moment the animal was released to spontaneous self righting. Ease of induction, or failure rate, was also calculated. A failure of induction of TI was defined as any attempt to induce TI which produced no response or a response lasting less than 8 seconds. Finally, polygraphic recordings were obtained in a subsample of rabbits.

Mean duration of TI in baseline recordings of 40 unlesioned rabbits was 1 minute, 40 seconds (SD = 1 minute, 36 seconds). Mean percentage of failures of induction out of the total number of attempted inductions at baseline was 17.25% (SD = 15.32%).

In 15 animals, samples of TI were recorded polygraphically[4] and contrary to expectations, TI was not prolonged but was slightly shortened ($p < .25$, Friedman

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[4]In 5 animals, several episodes of TI were induced prior to surgery using an induction method identical to that used throughout the study. The mean durations of TI were 21.44 seconds prior to surgery and 30.04 seconds 8-9 days after the surgery (1 day prior to lesioning). These values, given the tremendous variation in the TI response in this species (see Appendix F), did not approach a statistically significant difference. The correlation between the two distributions of TI inductions reached .55 but was not statistically significant.

test) when the 15 subjects were dorsoflexed with the recording cable attached prior to lesioning. It had been thought that the additional restraint produced by the cable attachment would potentiate TI, as do "fear-inducing" stimuli such as loud noises, glass eyes, etc. (Ratner, 1967). The mean of the TI durations with and without cable attachment was 160.36 seconds and 187.38 seconds, respectively. The correlation between TI with cable attachment and without attachment was -.02.

In polygraphic measurements and behavioral aspects of TI in unlesioned rabbits in this investigation, it was consistently observed that muscle tonus during TI was in fact higher than that of SWS. Figure 19 is an example of a polygraphic recording of TI. Figure 20 illustrates a V-shaped trough being used during rabbit TI.

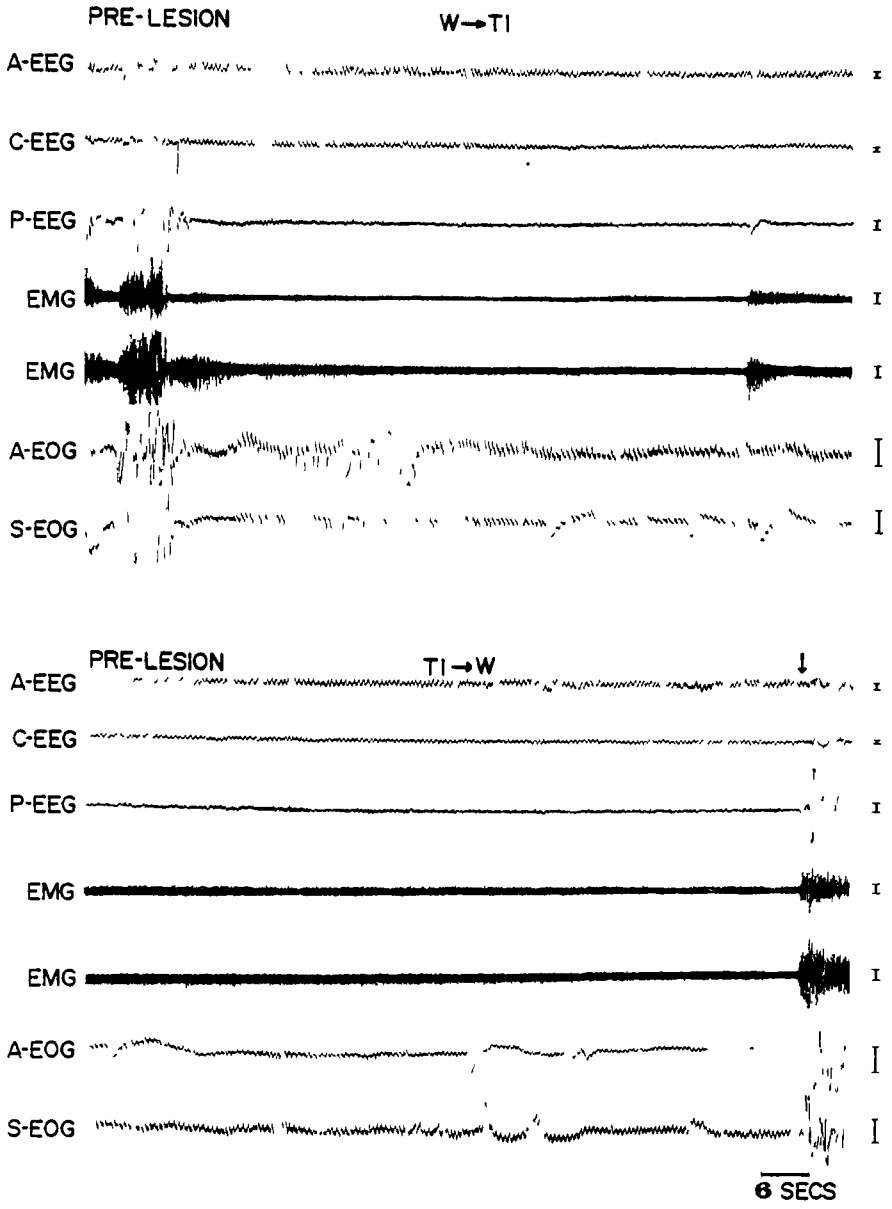

Figure 19: Tonic immobility in an unlesioned rabbit. Continuous polygraphic recording of an episode of tonic immobility. W = waking, TI = tonic immobility, A-EEG = anterior EEG, C-EEG = central EEG, P-EEG = posterior EEG, A-EOG = anterior EOG, S-EOG = superior EOG, EMG = electromyograph, the vertical arrow indicates spontaneous righting of the animal, calibration = 50 Uv.

Figure 20: Photograph of an episode of tonic immobility.

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93a
93a

Effects of the lesions on tonic immobility

Univariate repeated measures analyses were not performed on the mean and median durations of TI or the rates of failure of induction because although tests of the assumptions of normality were adequately met, tests of the assumptions of homogeneity of variance and of symmetry of the variance-covariance matrices were not. The decision was made to proceed with a non-parametric analog of repeated measures ANOVA (Friedman test) for the "time" factor simple main effects and of non repeated ANOVA (Chi square) for the "groups" factor simple main effects. Interaction effects were not considered.

The same 4 subgroups (LC, PLC, R, NP) were used in the statistical analyses of TI. Though figure 21 seemed to indicate a massive increase in duration of TI five days after lesioning in the "reticular" group, and to a lesser extent in the "partial LC" group, these effects did not reach statistical significance at the .05 level of probability. This failure to attain significance may have been due to the tremendous variability observed in the TI response generally. There were in fact no statistically significant differences between mean TI durations either within the "group" factor or the "time" factor. These analyses performed on the median durations of TI produced the same results.

The increase in mean TI duration in the "reticular" group after 5 days was entirely due to the results in one case, R-31. This lesion involved the caudal part of the NRPO and the rostral part of the NRPC. Since the TI response is so variable, and because this effect was due to a single case, no attempt at interpretation will be made. The effect however, was transient, since TI duration in R-31 dropped to below baseline level 14 days following the lesion.

The transient increase in TI duration observed in the "partial-LC" group at the 5th day post lesion resulted primarily from results of animals PLC-9, PLC-38 and PLC-41 where the LC was affected laterally, caudally and caudally, respectively. Since all 4 caudally lesioned PLC cases and both laterally lesioned PLC cases did not demonstrate the effect, it is difficult to attribute the results to selective LC lesions.

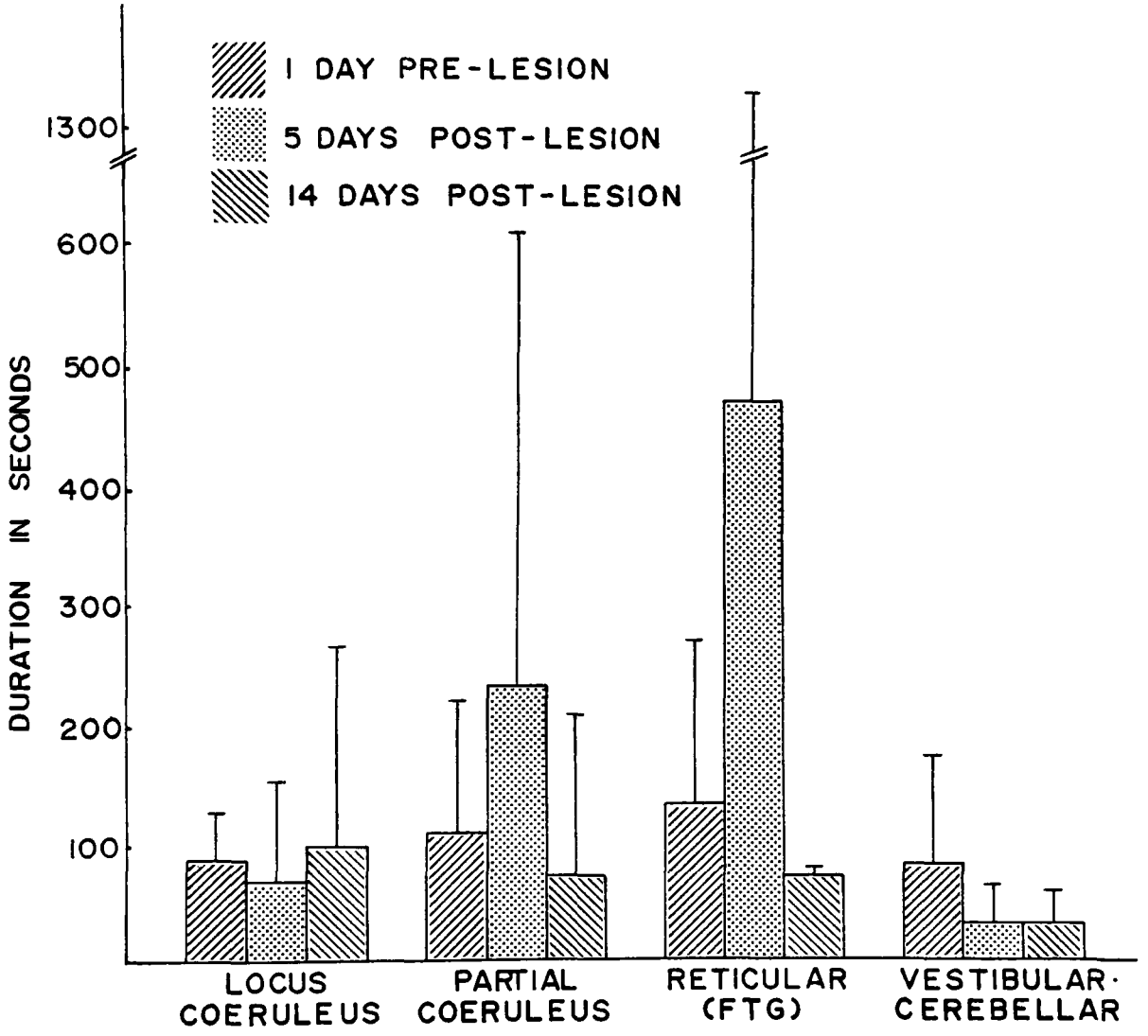

Figure 21: Mean durations of tonic immobility. Mean durations of tonic immobility in seconds and standard deviations for each recording and each group.

Figure 22 presents the results of the analysis of TI induction data for rate of failure. The percentages of TI-induction failure rates clustered between 10 and 20% at baseline for each group, increased 5 days post lesion in all groups except the R group, and became quite variable by 14 days post lesion (failure rates ranging from 10% to 60%). None of these effects was statistically significant.

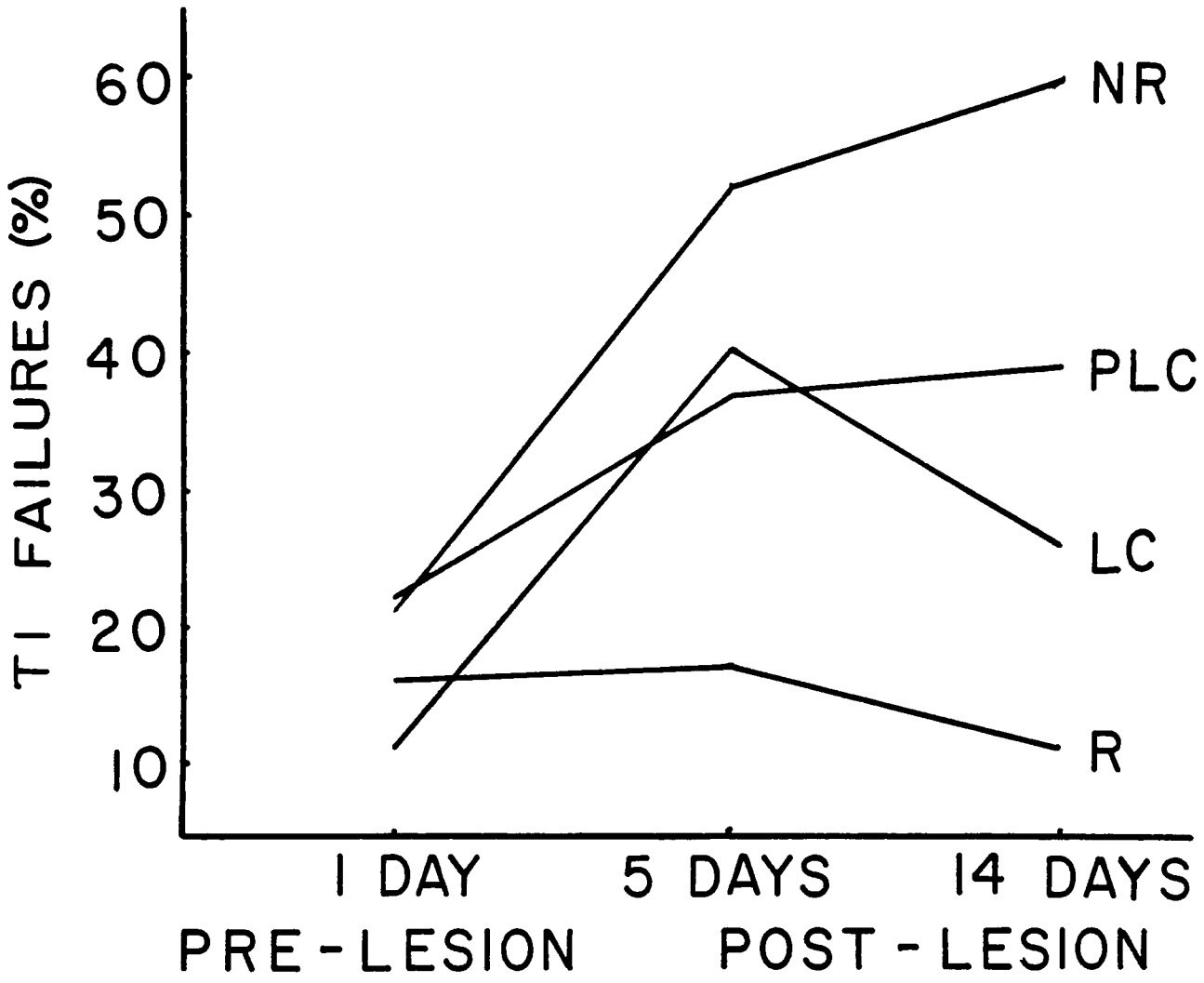

Figure 22: TI-induction failure rates over time. Percentages of failure rates pre and post-lesion in each group.

As was explained in the review of the literature, the TI state in unlesioned rabbits is always characterized by absence of phasic activity (such as eye movements, muscle twitches, cortical spikes, etc.) and by cortical desynchronization except in unusually long episodes (> 10 minutes) where high voltage slow waves appear. These same phenomena persisted following LC lesions. However, in two cases (LC-6, LC-8) a few exceptionally long episodes of TI (> 20 minutes) were recorded after lesioning. Phasic muscular activation (twitches but not eye movements) were observed in these episodes. These phasic intrusions were accompanied by mixed cortical activity and followed periods of high voltage slow EEG activity. These brief phasic episodes did not result in termination of the TI state. Instead, on these occasions slow waves and exclusively tonic motor activity followed the phasic episodes, and were again followed by phasic activity.[5]

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[5]Unfortunately, the polygraphic records illustrating these phenomena were lost during a move to a second laboratory during the course of the study.

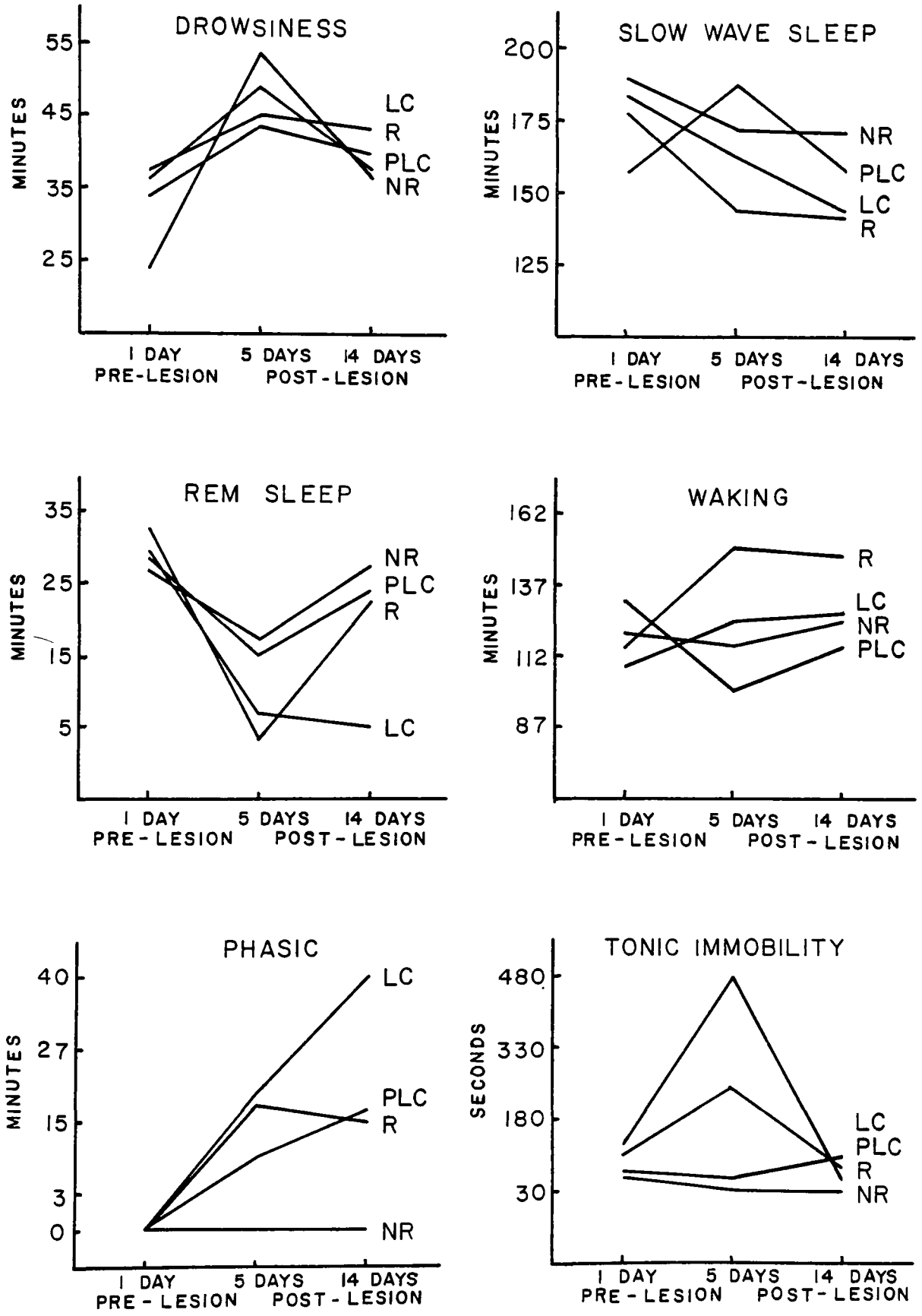


Figure 23: Lesion effects on all variables. Illustration of the mean amount of time spent in each state for each group.

Correlations between the dependent variables

An analysis of the relationships among the six dependent variables was conducted on baseline data. Table 6 indicates that the times spent in each state during baseline recordings were in some cases significantly correlated. Besides the obvious negative relations between mutually exclusive states such as W and SW, some interesting relations appeared in the matrix. TI, for example, was significantly prolonged in animals which spent less time awake in undisturbed recordings. As would be expected, TI related in the opposite direction to SW. The latter coefficient approached statistical significance. Because of the limited sample size of post lesion groups, only baseline data were analyzed using the correlational method.

TABLE 6

Correlations Between the Summed Times Spent in Each State Per Recording

1 Day Pre-Lesion (N=40)

State	D	SW	PS	P	TI
W	-.16 (p=.156)	-.87 (p=.001)	-.07 (p=.339)	-	-.29 (p=.034)
D		-.06 (p=.357)	-.38 (p=.007)	-	-.08 (p=.315)
SW			-.26 (p=.051)	-	.25 (p=.061)
PS				-	.14 (p=.186)

Discussion

Lesion effects: general considerations

The results of this study are based on the technique of selective destruction of a brain stem area by radiofrequency lesioning. Although the parameters of this particular lesioning procedure are well controlled, there are still very significant weaknesses inherent in the technique, for example: the interruption of blood flow and/or fiber tracts to and from other nuclei coursing through the lesioned area is unavoidable; generally, there is either incomplete damage of the target nucleus or excessive destruction extending to other structures in surrounding areas; the degree of destruction which appears in histological verification may not reflect subtle neurochemical and physiological disruption in areas adjacent to the necrotized tissue; bilateral lesions are rarely perfectly symmetrical; anaesthesia combined with the lesioning procedure may interact to produce unforeseen disruption of behavior; and, the lesioning electrode as it descends toward its target effects damage which can sometimes have some significant bearing, independent of the targeted lesion, on the dependent variables.

Several attempts to overcome these difficulties were made in the present study. Lesions were as discrete and complete as possible. Cases with asymmetric lesions were excluded from the results. Temperature controlled radiofrequency current was used to effect the lesions because this approach produces more uniform lesions and less damage in outlying areas than techniques based on the use of direct current. The lesion was effected after recovery from initial implantation of electrodes and guide tubes permitting both an independent assessment of the effects of the implantation and guide tubes, as well as allowing the lesions to be made without anaesthesia, thereby minimizing the risk of compounded effects of implantation and lesioning in one surgical procedure. In an attempt to improve the determination of the localization and extent of each lesion, tracings were drawn of wet histological samples at the time of sectioning to avoid loss of resolution sometimes encurred after further manipulation of the samples (staining, mounting, storing, etc.).

Vegetative and motor effects of locus coeruleus lesions in rabbits

Since a classical vegetative LC lesion effect such as loss of spontaneous micturition was not observed in the present study, some discussion will now be devoted to providing an interpretation of this unforeseen effect, as well as

other vegetative phenomena observed in this study. A discussion of the results having direct bearing on the rationale of this study is given in the next section.

Unlike previous studies involving LC lesions in other species, no significant alterations were observed in a) uro-genital function, b) respiration, or, c) tonic motor function in waking. Other lesion studies which have reported such effects in cats, and in rats, have been based on the method of lesioning during general anaesthesia. The trauma and CNS damage occurring during the combined procedure may produce effects nonspecific to LC lesions. However, stimulation studies of micturition and respiration have indicated that the LC, or at least the dorsolateral pontine tegmentum, does play a role in these behaviors.

The absence of these effects in rabbits could be due to a different organisation of the CNS in this species (except for the tonic motor effects as will be seen later). This is not such implausible hypothesis if the complexity of the neural basis of these systems is considered. Micturition, for example, is controlled by at least three brain centers situated in the posterior hypothalamus and the midbrain (Tang & Ruch, 1956; Pitts, 1974; and Wang & Ranson, 1939), as well as the LC center described initially by Barrington and confirmed by several investigators (Wang & Ranson, 1939;

Tang & Ruch, 1956; Wang and Ngai, 1964). The hypothalamus and LC have confirmed facilitatory effects on micturition in cats, whereas the midbrain has an inhibitory effect. The midbrain inhibitory center is situated immediately rostral to the LC in cats (inferior colliculus, isthmus, suprabrachial area). It is possible that in rabbits the inhibitory center is closer to the LC and that LC lesions would involve both the inhibitory center and the major facilitory center. If this were the case, it would be possible that the bladder could maintain relatively normal function considering that the hypothalamic facilitatory system might suffice to maintain normal micturition.

There are three confirmed respiratory centers in the brain stem. Transection studies have demonstrated that the neuraxis above the pons is not necessary for normal respiration. An inspiratory center situated in the caudal medulla has been identified as the inferior reticular nucleus, an expiratory center has been localized to the caudal medulla just dorsal to the inspiratory center, and finally, a pneumotaxic center has been discovered in the area of the dorsolateral pontine tegmentum (Liljestrand, 1956; Pitts, 1946; Johnson & Russel, 1952; Ngai & Wang, 1957). The former two centers have been confirmed in cats, monkeys and dogs, but the latter has been reported only in cats. Bilateral lesions of the LC have consistently produced apnea or

"breath-holding" in cats. However, these lesions, at least in the studies where the lesions were properly described, have involved the parabrachial nuclei as well as the LC. It is believed that the dorsolateral pontine tegmentum does not have a direct effect on respiration, but simply serves to facilitate the medullary inspiratory center. It is also not believed that the dorsolateral pontine tegmentum operates as a respiratory pacemaker (Lambertsen, 1968). This area has been believed to play only a secondary, or modulatory role in respiration. In the present study, the lack of effect of discretely circumscribed LC lesions on respiration indirectly supports more recent findings implicating the medial parabrachial nucleus rather than the LC in the modulation of respiration (Bertrand & Hugelin, 1971).

The forelimb rigidity and dystaxia reported in studies involving bilateral LC lesions in cats (see review of literature), have not been reported in most lesioning or stimulation studies. Furthermore, the studies which did report the effect involved lesions which encompassed larger areas in addition to the LC (brachium conjunctivum, part of the central gray matter and the entire para and suprabrachial area). It seems probable therefore, that the effects must have been due to damage of structures other than the LC.

Sleep and waking in lesioned rabbits

The primary hypothesis regarding sleep-waking states in this study was that LC lesions would result in a state characterized by increased EMG activity occurring at times when PS periods might be expected. This research hypothesis received clear support. In all animals with bilateral LC lesions, episodes of phasic activation occurred after SWS episodes. PS, as it occurred during baseline conditions, was only rarely observed.

Locus coeruleus lesions. Data obtained in the present study suggest that LC lesion effects on muscle tone and behavior are not due merely to the destruction of the normal mechanism of muscle atonia during PS but result from the release of an excitatory mechanism of motoric activation usually inhibited during PS. The absence of sustained atonia suggests that the mechanism for this phenomenon in rabbits, is either inconsistently present or generally not well developed. It is important to note that in the absence of such inhibition the animals did not exhibit startle-like behaviors as were observed subsequent to LC lesions. Consequently, the increased muscle tone and motor behavior observed in the present project subsequent to LC lesions cannot be attributed merely to elimination of tonic motor inhibition in PS. An interpretation more consistent with these pre-post lesion phenomena is that the LC lesions ef-

fect release of motor activation not normally present during PS, but which is released at times when PS would occur.

However, phasic activity observed post lesion may have been exagerated forms of more discrete activity present in baseline recordings. The volleys of anterior EOG activity, also observed in P after lesioning, may reflect activation similar to that ascribed by Morrison and Bowker (1975) to PGO spikes. These authors have interpreted PGO spikes in cats as essentially epiphenomenonal electrical signs of the activation of a "startle network" by the neural turmoil of PS.

The bizarre motor activation patterns of LC- lesioned rabbits did resemble startle-reactions, e.g., overt flight, upward jerking of the head, sudden explosive movement etc., an observation consistent with Henley & Morrison's claim that the motor activation during PS consists of a startle response. Since the animals were recorded in a quiet, stable environment, it is unlikely that this behavior was caused by unusual external stimuli. Rather, Morrison et als' contention that a "response center to novel stimuli" becomes activated, seems more plausible.

Although the post lesion behaviors observed in the rabbit in the present study resemble in a general way those previ-

ously reported for cat and rat after similar lesions, important interspecies differences do exist. For example, rats have not been reported to manifest stalking or attack behavior following LC lesions, and various behaviors produced by LC lesions in cats are indeed not observed to be within the normal behavioral repertoire of rabbits. Similarly, thumping of the hind limbs concomitant with abrupt flight (observed during P in LC lesioned rabbits in the present study) is an adaptive signalling-escape mechanism observed in rabbits and not cats. It appears, therefore, that the motor sequences which are released, disinhibited, or activated by LC lesions are species-specific.

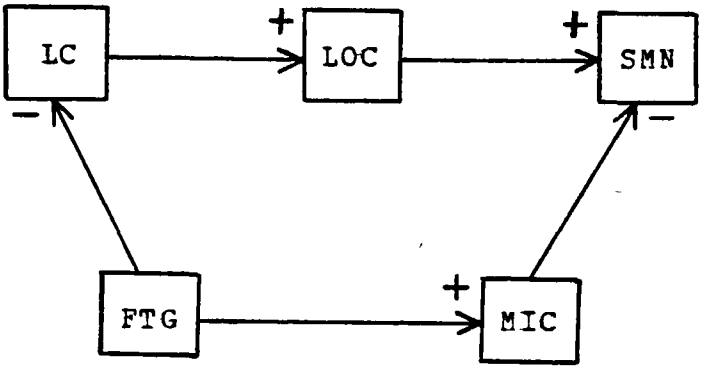
The documentation of subtle changes in the waking behavior of LC-lesioned animals in the present study was cursory, but Morrison et als' (1980) finding of significantly increased motility in LC-lesioned cats was supported, although the effect appeared to be less marked (7% increase in active waking). Morrison's (1979) deduction that LC-lesioned cats should exhibit "excessive exploration", and his impression that LC-lesioned cats lacked the "caution" characteristic of the species, were also supported in the present study. The impression given by the waking behavior of LC-lesioned rabbits was that these animals had become less timid and more prone to bump into obstacles.

A final consideration regarding LC lesion effects is the question of their permanence. With the exception of the Henley and Morrison study (1974) in which six cats were maintained for periods of at least two months post lesion, and in one case up to six months, studies involving lesions of the LC have not exceeded post lesion survival periods beyond 21 days. In the Henley & Morrison study however, the lesions were not all centered on the LC, and some barely involved this nucleus. Furthermore, sleep states and P were not quantified so that it is difficult to judge whether the "PS without atonia" phenomenon described by these authors was a sustained LC effect. Results from the present study, based on quantified analysis of recordings obtained in six LC-lesioned rabbits 30 days post lesion, indicate an uninterrupted increase in the amount of time spent in P, and a correspondingly maintained decrease in the amount of time spent in PS. These observations indicate that the effects of LC lesions on sleep show no signs of abatement after 1 month.

The previous discussion has compared general aspects of LC lesion data from the present investigation with those previously observed in other species. The discussion will now focus on consideration of lesion data as it may relate to Morrison's model of motor control during PS.

Morrison's model of muscular control during PS. This model, representing Morrison's interpretation of the phasic activation which he and others have observed during PS following LC and other brain stem lesions, is illustrated in figure 24 as it was formulated in 1979. In this model, the LC is hypothesized to have direct excitatory connections to a locomotor center which in turn generates the explosive behavior reported to follow pontine lesions. Morrison claims that the LC must be relatively intact and connections between the LC and FTG destroyed for P to occur. His model places more emphasis on control of muscle tonus during PS by the FTG than by the LC. The FTG, not the LC, is postulated to control tonic muscular inhibition during PS by excitatory connections to a medullary inhibitory center (i.e., Magoun's inhibitory center) which in turn effects inhibition at the level of the spinal motoneuron. Furthermore, the FTG is postulated to inhibit the LC.

(BASED ON MORRISON, 1979)



LEGEND:
FTG = Gigantocellular tegmental field
LC = Locus coeruleus
LOC = Locomotor center
MIC = Medullary inhibitory center
SMN = Spinal motor neuron
\+ = Excitatory connection
\- = Inhibitory connection

Figure 24: Morrison's model of paradoxical sleep

At the outset, this model cannot account for the baseline phenomena observed in the present study. According to the model, absence of sustained atonia observed in unlesioned rabbits during the present study should be accompanied by startle-like locomotor activation since the FTG is postulated to control both tonic inhibition and "locomotor" activation. However, startle-like behavior during PS was not observed.

LC lesion data also failed to support Morrison's model. Contrary to the prediction by the model, complete LC lesions produced more P than partial LC lesions, and partial LC lesions produced more P than FTG lesions.

The effects of FTG lesions in the present investigation resulted in: 1.) the complete elimination of PS of the type observed in unlesioned animals, and, 2.) small to medium amounts of P with non-explosive activation. Other studies involving lesions of FTG cells or of the entire NRPC area (Sastre, Sakai & Jouvet, 1979; Jones, 1979) have not reported P as a lesion effect although these authors may have avoided attempting to discriminate P from waking. Though the FTG lesion effects in the present study are in the direction of Morrison's model, they are not as strong as predicted by the model. The other FTG lesion studies contradict Morrison's model.

In conclusion, it appears that although Morrison's idea of a locomotor center was supported by the results of the present study, the internuclear relations postulated to underly the phasic activation during PS without atonia did not receive consistent support.

On the basis of the above criticism, it would seem more fitting to postulate an inhibitory link from the LC to the locomotor center thereby explaining the fact that extent of LC destruction correlates positively with P, and negatively with PS. Futhermore, it seems that excitation of the medullary inhibitory center should be postulated to be provided by efferents coursing through the FTG area. This would account for the absence of effects on muscle tone of kainic acid destruction of FTG cells (Sastre, Sakai & Jouvet, 1979) and for the elimination of the tonic muscular inhibition of PS by non chemical lesions of the FTG.

FTG and vestibular lesions. The effects of FTG lesions in this study partially support Jones' (1979) findings that lesions of the NRPC in cats eliminated the PS state. Since the FTG giant cell bodies were recently demonstrated to be unnecessary for any tonic or phasic aspect of PS (Sastre, Sakai & Jouvet, 1979), it seems that abolition of PS by lesions of this area, in the present study as in others (Jones, 1979; Sastre, Sakai & Jouvet, 1978), may be due to

interruption of fiber tracts originating in other nuclei. Evidence supporting the hypothesis that these fibers originate exclusively in the LC (Sastre, Sakai, & Jouvet, 1979) is sparse. Recent studies of the effects of LC lesions on sleep indicate that an intact LC is not required for the manifestation of several aspects of PS, including PGO spikes, eye movements, muscular twitching, etc. It seems unlikely, therefore, that all fibers coursing through the FTG which, when interrupted, abolish all aspects of PS, should originate in the LC. However, the anatomical basis for the suggestion of LC involvement in FTG lesion effects has been well established in cats and rats since descending LC efferents course through the FTG area, including the NRPO and the NRPC (Fuxe, 1965; Sladek, 1971). It is nevertheless possible that the FTG lesion effects may be partly or entirely due to fibers originating from other nuclei besides the LC.

The polygraphic evidence from the two discrete lesions of the medial vestibular nucleus (NR-5 and NR-13) in this study do not contradict the claim made by Pompeiano (1967) that in order to abolish volleys of eye movements and muscle twitches it is necessary to destroy the entire medial and descending vestibular nuclei. Lesions involving only the medial vestibular nuclei were not sufficient in the present study to abolish or even reduce the frequency of these phenomena.

Neurochemical implications. The roles of known transmitters in the phenomena reported in the present project are without doubt crucial, and, although the methodology did not include specific measures to assess neurochemicals, the possibilities of involvement of certain neurotransmitters in the present study will be considered.

Though the LC supplies most NA terminals to the brain, at least rostrally to the telencephalon, the presence of several other scattered clusters of such neurons in the lateral tegmental field makes it inadmissible, for example, to attribute the observed effects of LC lesions on PS to destruction of the NA system since other NA neurons besides LC neurons (i.e., areas A1 to A5) might suffice to produce the response. Furthermore, since there is evidence of the presence of at least one other neurotransmitter, ACh, and of morphine (Pert, Kuhar & Snyder, 1975; Bird & Kuhar, 1977), in the LC area, the disruption of PS observed to follow LC lesions cannot be attributed to the destruction of even a partial NA system since it may have been the destruction of cholinergic function, or of some other neurochemical function, in the LC which might have caused the observed effects.

Although the evidence for a cholinergic LC mechanism of muscular control during PS is much stronger than the evi-

dence for a similar noradrenergic LC mechanism, Morrison (1979) points out that there is evidence of NA mediation of the brain stem locomotor center during waking.

Brain stem lesions and tonic immobility

The primary hypothesis concerning TI in the present study was that the TI response would be eliminated by LC lesions. The results did not support the hypothesis.

The relative absence of effect of the lesions on TI duration or mean rates of failure of induction, suggests that it is unlikely that the LC or pontine PTG play an important role in the mechanism of induction or maintenance of TI. However, the finding that brief episodes of P were observed during TI, which did not cause the termination of the TI state, indicates a possible link between PS and TI -though not at the level of their respective executive mechanisms.

Baratt's (1965) finding, that TI was more easily elicited in the oppossum immediately following episodes of alpha EEG activity, suggests a relation between TI and brain EEG rhythms. The correlations observed in the present investigation between amounts of time spent in SWS, PS and TI further suggest that TI is related to differences between animals in state preponderance.

No effect on TI was expected for the NR group since previous reports have clearly established that the cerebellar and vestibular systems are not necessary for TI to occur. This expectation was corroborated by the data on TI duration, but the data on failure rates of TI induction gave an indication that both the cerebellum and the medial vestibular nucleus play a modulatory role TI.

Klemm (1965, 1969) reported that brain stem stimulation and multiple unit recordings pointed to the ponto-medullary midline reticular formation as the likeliest candidate for a "TI-center". Our results narrow down this prediction since the midline pontine reticular formation (FTG) was not necessary for TI to be present. However, the particular flexor-extensor relation of TI points not to the medial reticular formation (Magoun's bulbo-inhibitory center) which when stimulated produces flexor tonus and extensor flaccidity, but to the lateral and more rostral reticular formation which produces extensor tonus and flexor flaccidity when stimulated (Eyzaguirre & Fidone, 1975, and Monnier, 1970). Klemm did not thoroughly investigate this brain area with his stimulation and depth recording studies of rabbit TI. Also, it may be unnecessary to posit the existence of a mechanism for the abolition of righting reflexes during TI since such lower reflexes are known to be specifically localized in the mesencephalic reticulum (body righting reflexes) and in the

rostral pontine reticulum (cervical righting reflexes; Magnus, 1924; Monnier, 1970). There is no need to hypothesize the existence of other centers specialized in performing these same functions. It would be more parsimonious to hypothesize an inhibitory link from the "TI-center", probably situated in the lateral ponto-medullary reticular formation, to the pontine and mesencephalic righting reflex centers.

Direct evidence of opiate, ACh, and DA involvement, and indirect evidence of NA involvement in TI have been presented in the literature. It does not appear that an essential or executive function can be unambiguously attributed to any one transmitter at this time. Of the four putative transmitters mentioned above, only the opiates have been shown to be unidirectionally involved in TI in avians and mammals.

It may be appropriate, in light of the lack of common effects on PS and TI of LC lesions to reconsider the difference in muscle response between the two states. TI can be more properly characterized as a cataleptic or catatonic response than as a cataplectic-type response such as PS. Even in rabbits brief cataplectic or atonic periods are observed during PS but not during TI. The recent finding of marked and prolonged "catatonic" muscular rigidity in rats following intracerebrospinal injections of endogenous mor-

phinomimetic brain peptides in rats (Bloom, Segal, Ling & Guillemin, 1980), gives an impressive indication of the special role of opiate action in this particular aspect of muscle function. However opiate-induced rigidity has not been shown to be analagous to TI.

Muscular control of PS and TI: a common mechanism?

The present study has provided additional evidence that LC effects on muscle activity are time-locked into a phase of the sleep cycle. It is possible that the muscular disruption produced by LC lesions requires a "priming" influence of an oscillator intrinsic to the sleep-waking circuitry such that a reflex response like TI would not be susceptible to its influence.

Morrison (1979) and Pompeiano (1967) have suggested that tonic and phasic aspects of muscular function during PS are controlled by different nuclei. It is possible that the LC effects on muscle activity during PS are of a primarily phasic nature. This would explain why such lesions did not affect TI which is clearly a tonic response.

The hypothesized common mechanism of tonic motor control underlying PS and TI was not supported by the present data, but neither was this hypothesis clearly refuted. It is possible that PS and TI share a common mechanism of tonic mus-

cular control, and that only PS is susceptible to phasic activation which masks the presence of tonic muscular control. Clearly, however, aspects of motor control during PS and TI can be differently affected.

INDEX

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Appendix A

Description and location of the locus coeruleus

Description of the nuclear and fiber systems within and surrounding the locus coeruleus.

The locus coeruleus is a pontine reticular nucleus situated ventrolaterally to each horn of the fourth ventricle. Upon macroscopic inspection of human tissue the LC appears as a blue colored mass, hence the name coeruleus, the latin word for blue. The LC, which is immersed in a capillary bed whose density is about 1250 mm of capillary length per cubic millimeter of tissue (Finley & Cobb, 1940), is in fact one of the most highly vascularized structures in the primate nervous system, and probably in lower orders as well. In the adult New Zealand White rabbit, the LC is approximately 2mm long rostrocaudally and 1mm wide.

Some researchers distinguish a tightly packed principal locus coeruleus and a more diffuse postero-ventro-lateral group, called LSC (Meesen & Olzewski, 1949; Dahlstrom & Fuxe, 1965). More recent studies confirm this division on

the basis of differing efferent projections of the two aspects, with the more anterior- dorsal aspect projecting to the telencephalon and the posterior- ventral aspect projecting to the diencephalon (Maeda, Pin, Salvert, Ligier & Jouvet, 1973). This distinction between LC and LSC cannot be made in cats, however, on the basis of cell architectonics according to a report by Jones and Moore (1974).

In the rabbit, the shape of the nucleus is represented differently by Meesen and Olzewski who draw the main and sub-nuclei in the shape of an hour glass, and Bubenik and Monnier (1972) who represent an ovoid shape which conforms closely to the grouping of the pigmented cells of the LC visible macroscopically. Most authors subscribe to Bubenik and Monnier's representation. The histological results obtained in the present study support the latter position.

In the rabbit, according to Meesen and Olzewski, the ventro-caudal part (LSC) is definitely organized more diffusely than the main LC nucleus. Both have large and small cells, with the former cell type manifesting a clearly visible dendrite.

Cell morphology and neurotransmitter chacteristics do not differ from the LC to the LSC, at least in the cat (Jones & Moore, 1974),

Dorsally, one can distinguish the horn of the fourth ventricle. In the more anterior part of the LC, the radix mesencephalica of the trigeminal nerve is situated dorsally as is the nucleus cruciformis. Latero-dorsally, are the subnucleus parabrachialis parvocellularis and further, the brachium conjunctivum. Immediately lateral to the LC is the small nucleus V mesencephalic, followed more laterally by the continuation of the subnucleus parabrachialis parvocellularis. More anteriorly, this is replaced by the nucleus of bechterev. more laterally still is the subnucleus parabrachialis magnocellularis. Latero-ventrally, to the LC is the nucleus trigemini motoris. Immediately postero-ventrally to LC is the nucleus subcoeruleus, but we also see the nucleus reticularis pontis caudalis. More antero-ventrally are region H and the nucleus reticularis pontis oralis. Ventro-medially, is the continuation of both nuclei reticularis pontis caudalis and oralis, and further medially the fasciculus longitudinalis posterior. Medially to the LC is the central grey matter or the stratum griseum centrale with nucleus Q embedded in the mid portion of the central grey at this level. Antero-medially, the stratum marginale appears followed by the nucleus dorsalis tegmenti of gudden. the medio-dorsal flanc of the LC is occupied by the fourth ventricle.

Appendix B

Connections of the locus coeruleus

Detailed description of the afferent and efferent connections of the locus coeruleus.

Afferent connections to LC include fibers from the trigeminal nerve, the ventro-lateral subventricular grey matter, the dorsal longitudinal fasciculus and the lateral lemniscus (Russel,1955). Other researchers report afferents from the gigantocellular tegmental field (FTG) (Nauta & Kuypers, 1958). Ramon-Moliner (1974) suggested that FTG cells may project into cholinoceptive LC cells. the nuclei raphe pontis, raphe magnus, the hyothalamus, the arcuate nucleus, the perifornical area, the mesencephalic central grey, the fastigial nucleus, the contralateral LC, the substantia nigra and the parabrachial nuclei project to the LC (Sakai, Touret, Salvert & Jouvet, 1977

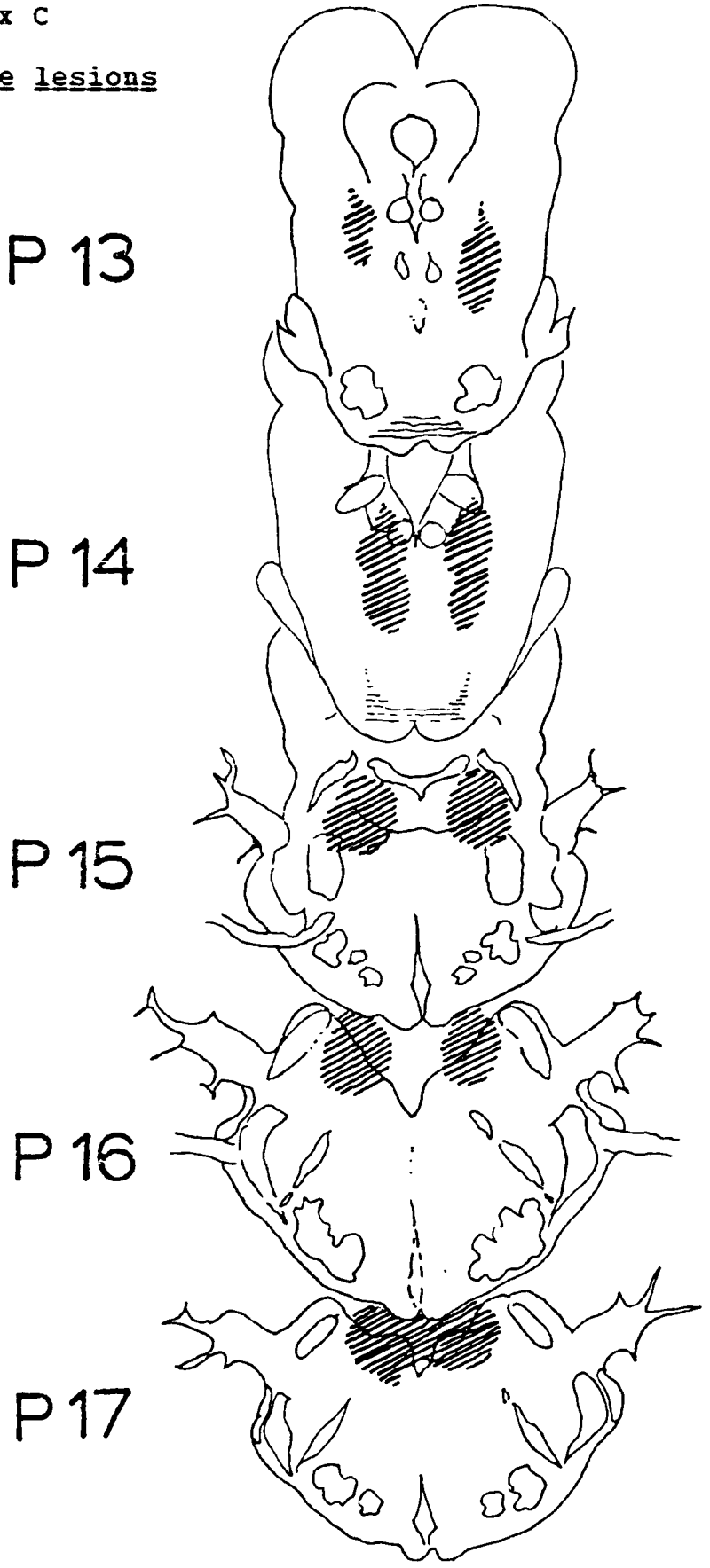
Efferent connections from the LC include the descending lateral tegmento-reticular pathway (Johnson & Russel, 1952), the nucleus prepositus hypoglossi and intercalatus (Briggs &

Kaelber, 1971), most raphe nuclei (Chu & Bloom, 1974, and Pasquier, Kemper, Forbes & Morgane, 1977), medial forebrain bundle, hypothalamus and thalamus (Garver & Sladek, 1976), the cerebellum through the superior peduncle, the dorso-medial lateral tegmental field through the mesencephalic v (maeda et al, 1973, and Pickel, Segal & Bloom, 1974), the amygdala via the stria terminalis, the cingulate cortex, hippocampus, subiculum, pyriform cortex, frontal cortex, the claustrum via the anterior commissure (Pickel et al, 1974), the homolateral ventral inferior olive (Russel, 1955).

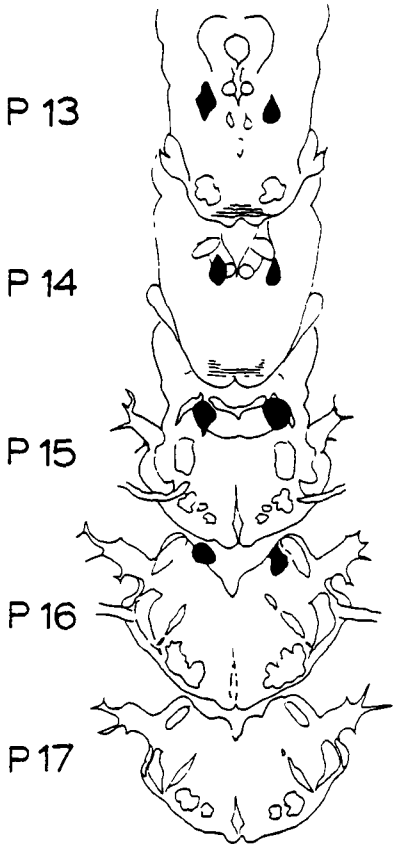
LC fibers project to the ventral part of the spinal cord (Hancock & Fougerousse, 1976 and Pickel et al, 1974) and the somatomotor cord and laminae LV, V and VL (Nygen & Olson, 1977) at least as low as the lumbo-sacral segments (Kuru & Yamamoto, 1964). according to the latter authors, the LC fibers course down to the spinal cord via the lateral reticulospinal tract, cross in the mid lumbar segments and terminate in the intermedio-lateral column of the lumbo-sacral cord, i.e., the spinal vesico-motor center. Comisiong, Hellstrom and Nell (1978) were able to identify catecholaminergicall fluorescent LC terminals clustered near alpha motoneurons in the spinal cord.

Appendix C

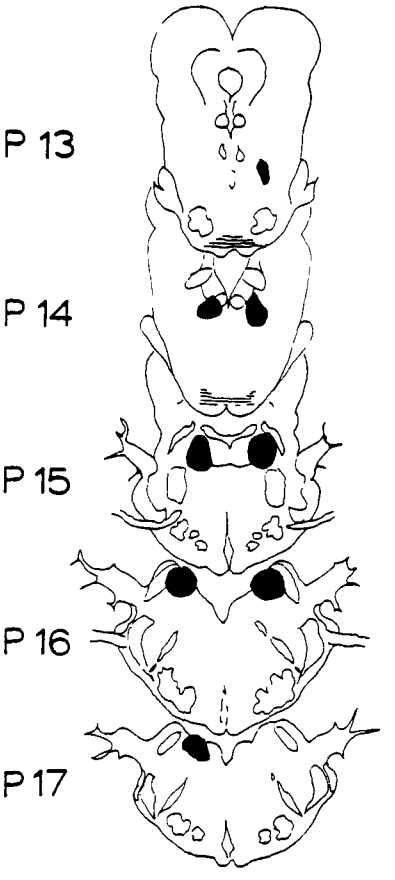
Drawings of the lesions



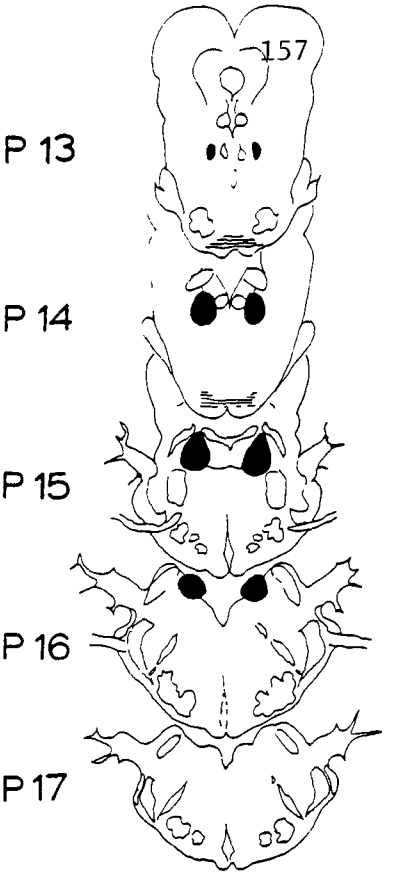
COMPOSITE PRESENTATION OF
LOCUS COERULEUS LESIONS
(N EQUALS 11)



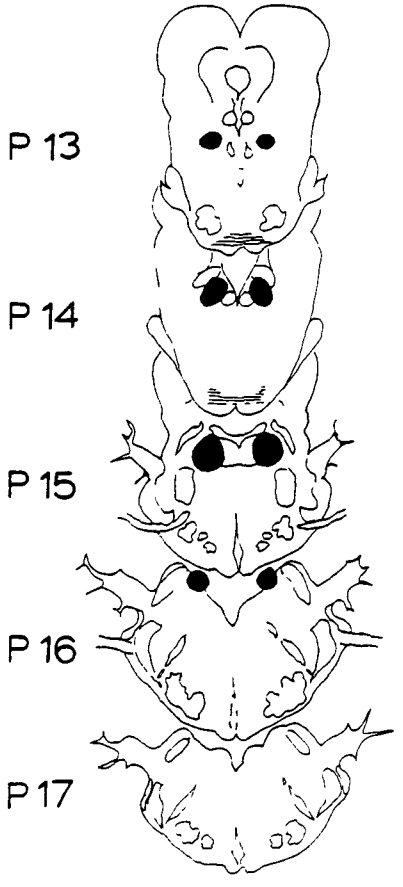
SUBJECT IDENTIFICATION : 06
LESION : LOCUS COERULEUS
GROUP : LOCUS COERULEUS



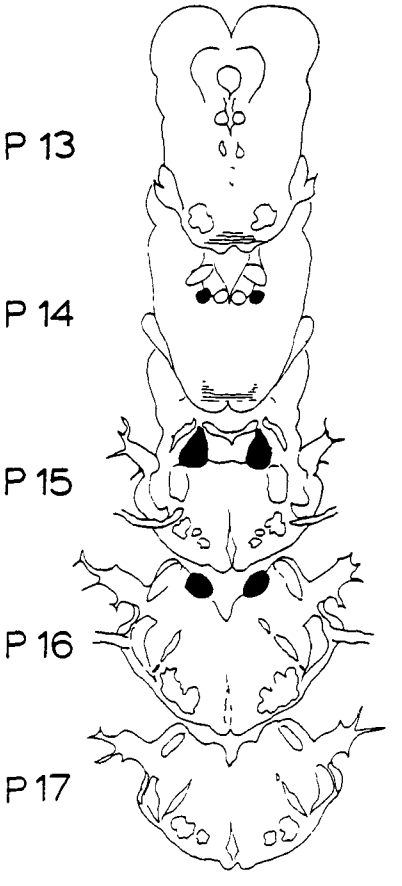
SUBJECT IDENTIFICATION : 14
LESION : LOCUS COERULEUS
GROUP : LOCUS COERULEUS



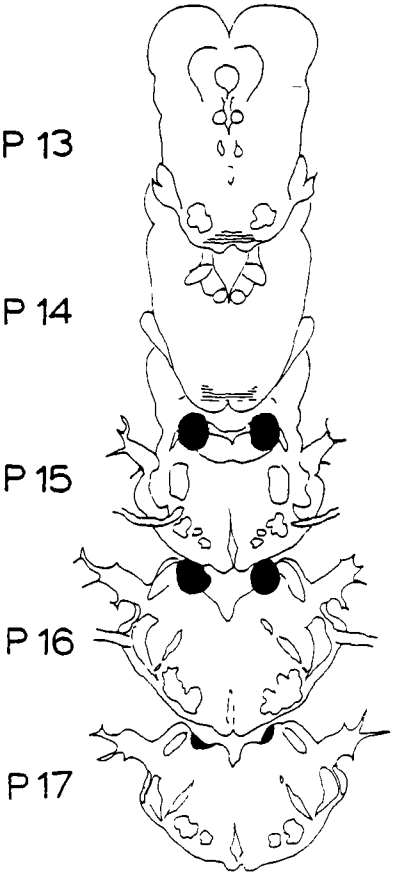
SUBJECT IDENTIFICATION : 35
LESION : LOCUS COERULEUS
GROUP : LOCUS COERULEUS



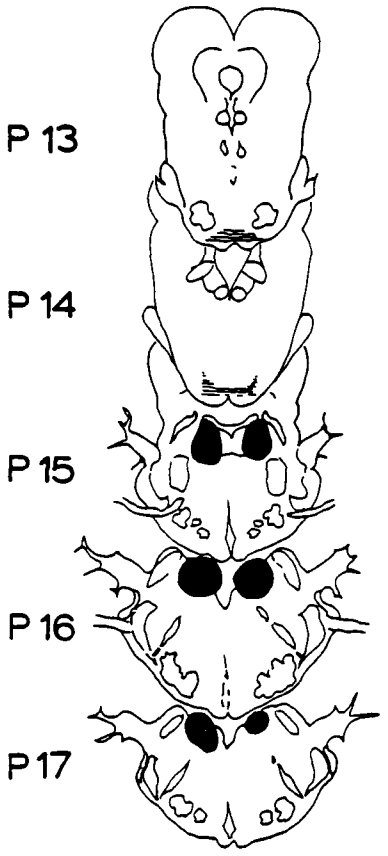
SUBJECT IDENTIFICATION : 25
LESION : LOCUS COERULEUS
GROUP : LOCUS COERULEUS



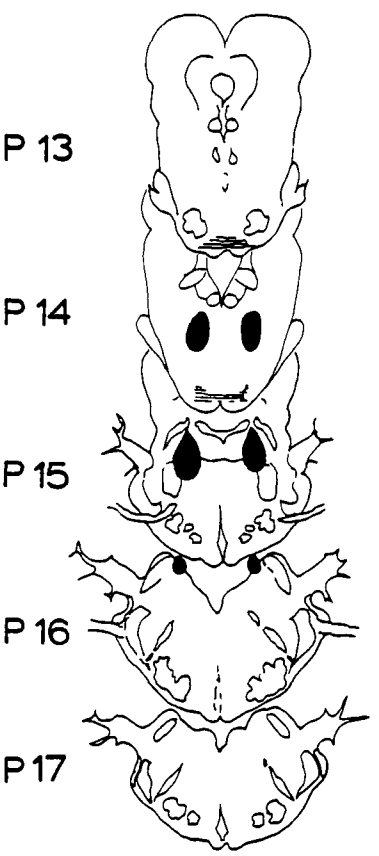
SUBJECT IDENTIFICATION · 08
LESION : LOCUS COERULEUS
GROUP : LOCUS COERULEUS



SUBJECT IDENTIFICATION · 20
LESION · LOCUS COERULEUS
GROUP : LOCUS COERULEUS



SUBJECT IDENTIFICATION : 28
LESION : LOCUS COERULEUS
GROUP : LOCUS COERULEUS



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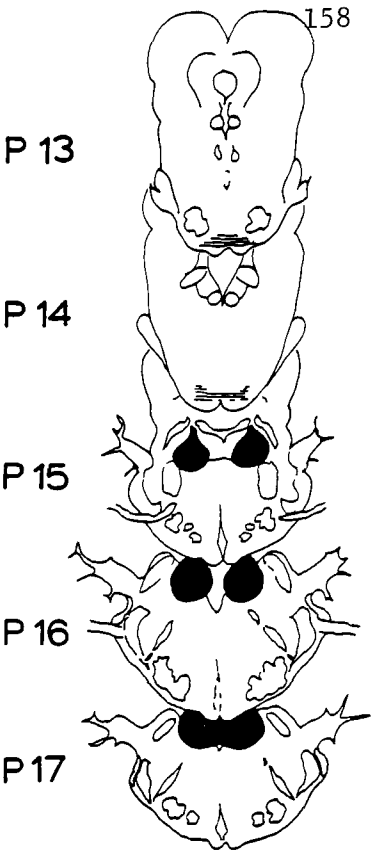
LESION : LOCUS COERULEUS
GROUP : LOCUS COERULEUS



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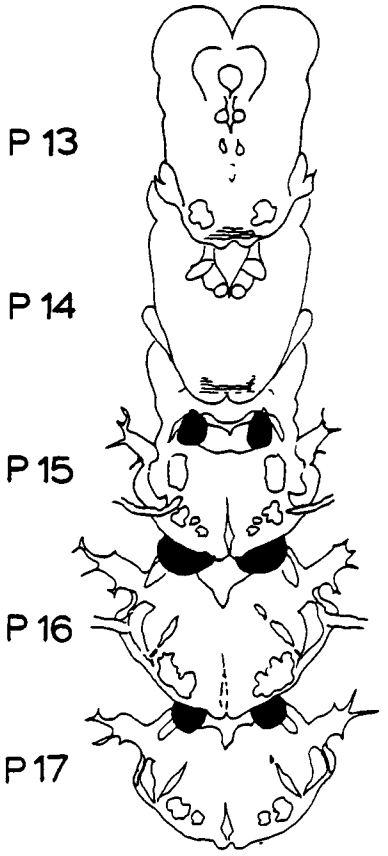
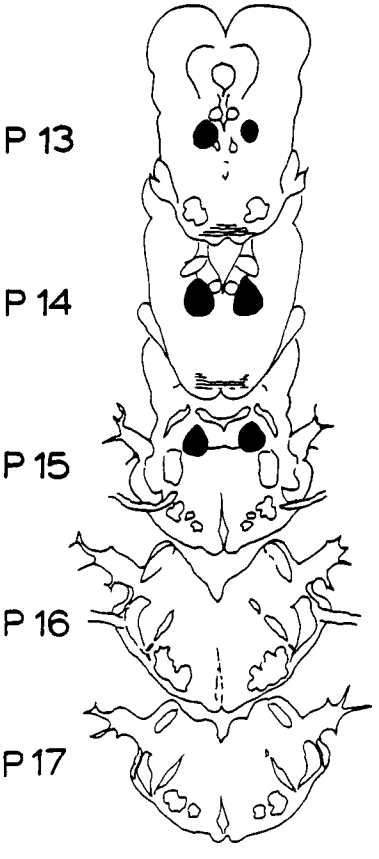
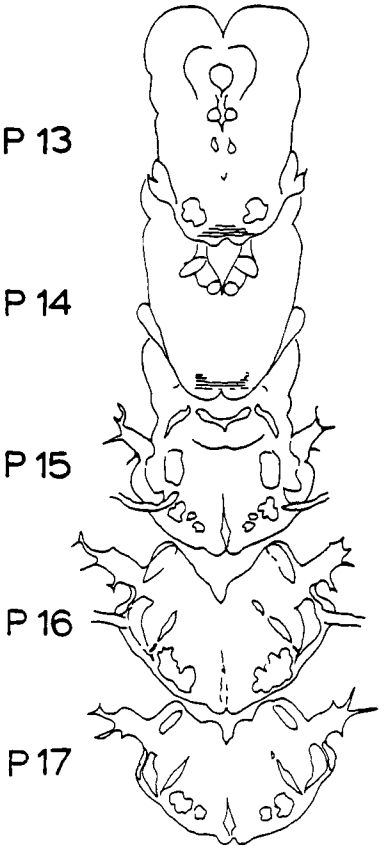
LESION : LOCUS COERULEUS
GROUP : LOCUS COERULEUS

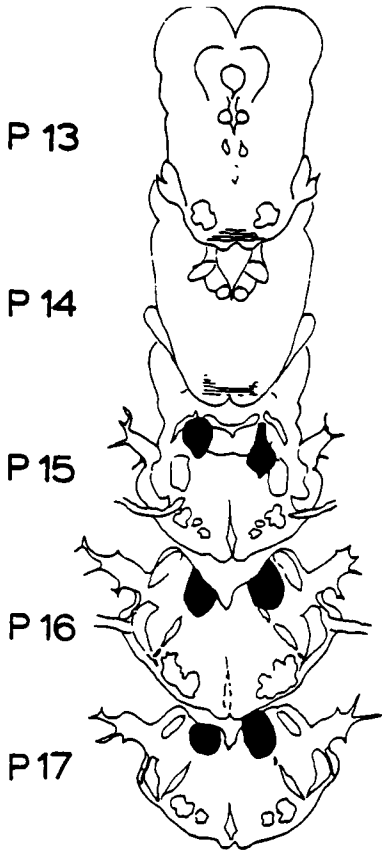


SUBJECT IDENTIFICATION : 34
LESION : LOCUS COERULEUS
GROUP : LOCUS COERULEUS

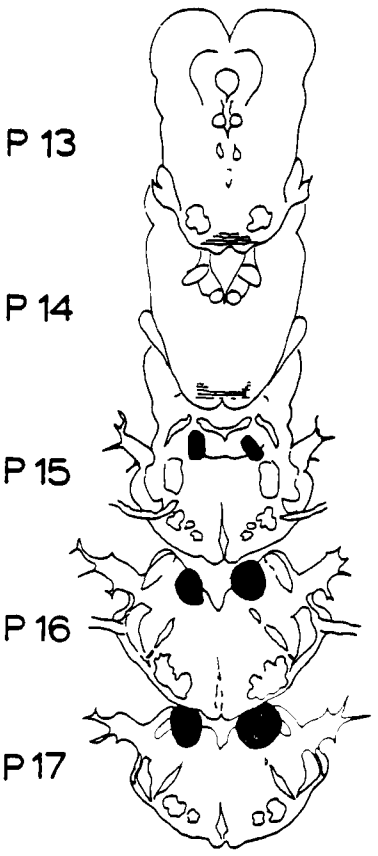


SUBJECT IDENTIFICATION : 36
LESION : ROSTRAL-VENTRAL LOCUS COERULEUS
GROUP : LOCUS COERULEUS

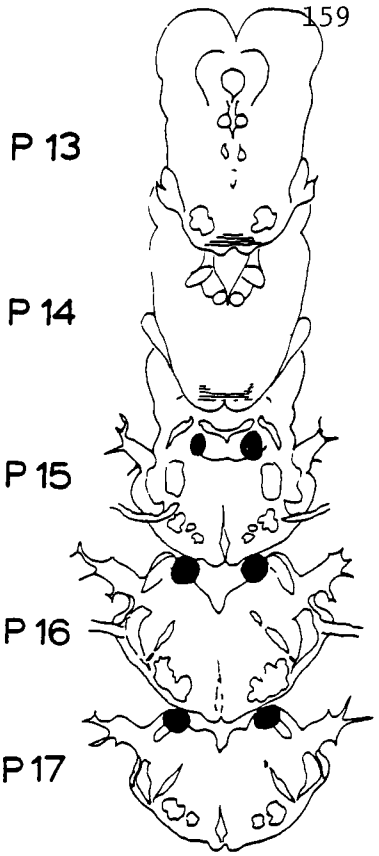




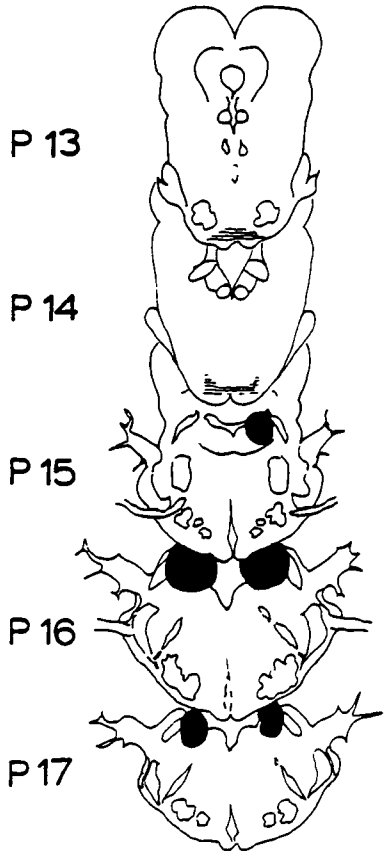
SUBJECT IDENTIFICATION : 11
LESION : CAUDAL LOCUS COERULEUS
GROUP : PARTIAL LOCUS COERULEUS



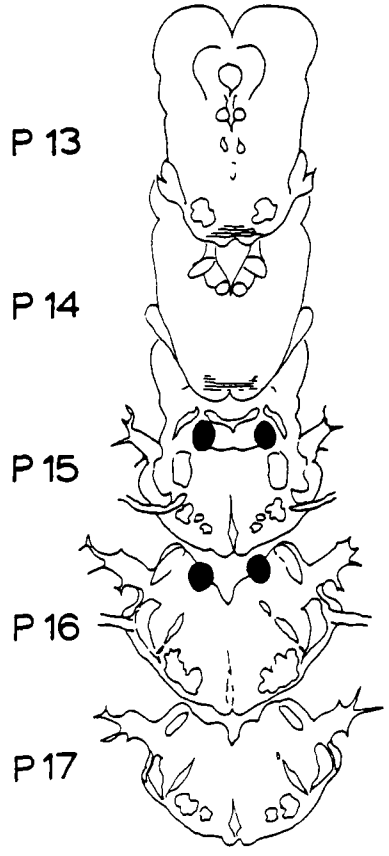
SUBJECT IDENTIFICATION : 12
LESION : CAUDAL LOCUS COERULEUS
GROUP : PARTIAL LOCUS COERULEUS



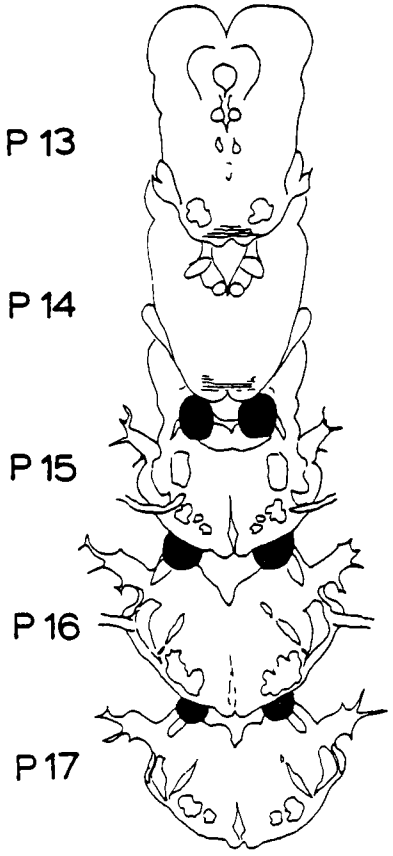
SUBJECT IDENTIFICATION : 22
LESION : CAUDAL LOCUS COERULEUS
GROUP : PARTIAL LOCUS COERULEUS



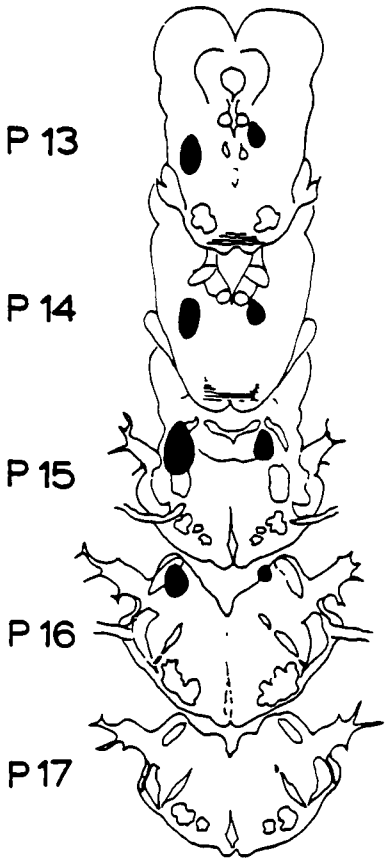
SUBJECT IDENTIFICATION : 33
LESION : CAUDAL LOCUS COERULEUS
GROUP : PARTIAL LOCUS COERULEUS



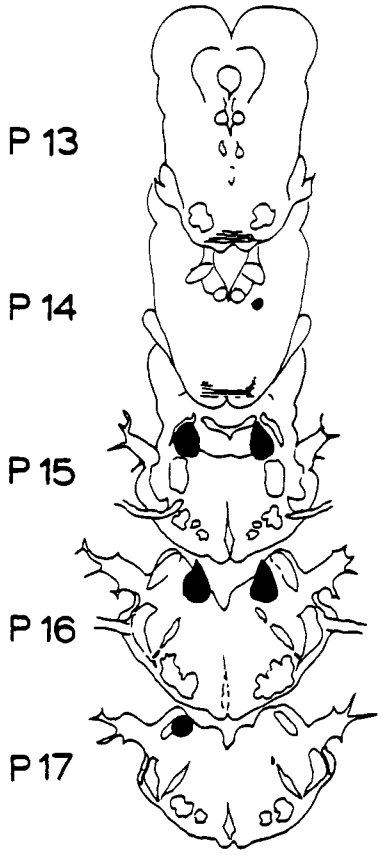
SUBJECT IDENTIFICATION : 41
LESION : CAUDAL LOCUS COERULEUS
GROUP : PARTIAL LOCUS COERULEUS



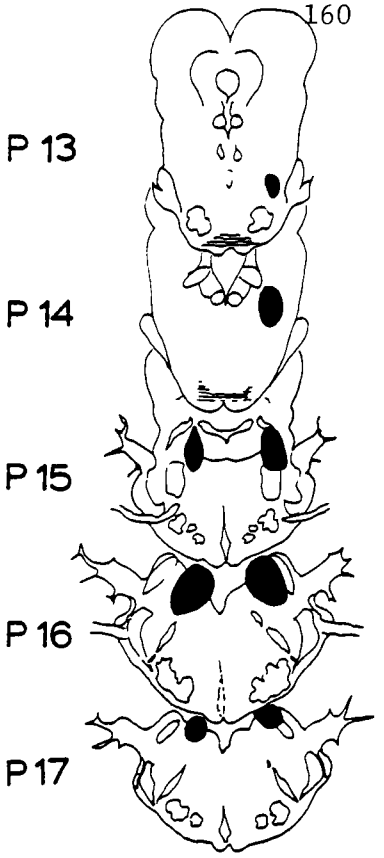
SUBJECT IDENTIFICATION : 38
LESION : DORSAL-CAUDAL LOCUS COERULEUS
GROUP : PARTIAL LOCUS COERULEUS



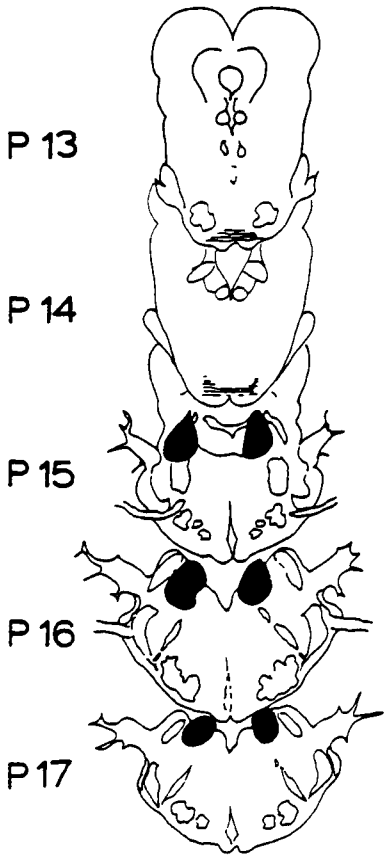
SUBJECT IDENTIFICATION : 16
LESION : LATERAL LOCUS COERULEUS
GROUP : PARTIAL LOCUS COERULEUS



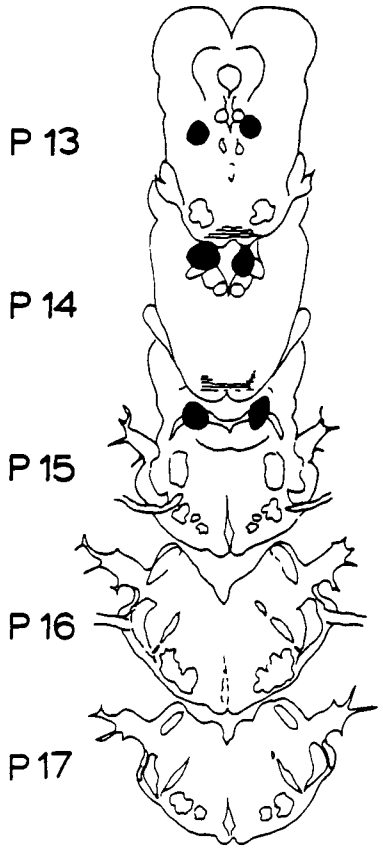
SUBJECT IDENTIFICATION : 15
LESION : LATERAL LOCUS COERULEUS
GROUP : PARTIAL LOCUS COERULEUS



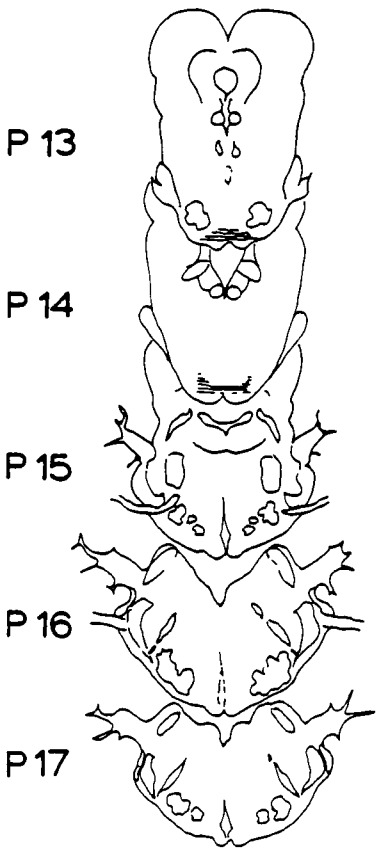
SUBJECT IDENTIFICATION : 40
LESION : LATERAL-CAUDAL LOCUS COERULEUS
GROUP : PARTIAL LOCUS COERULEUS

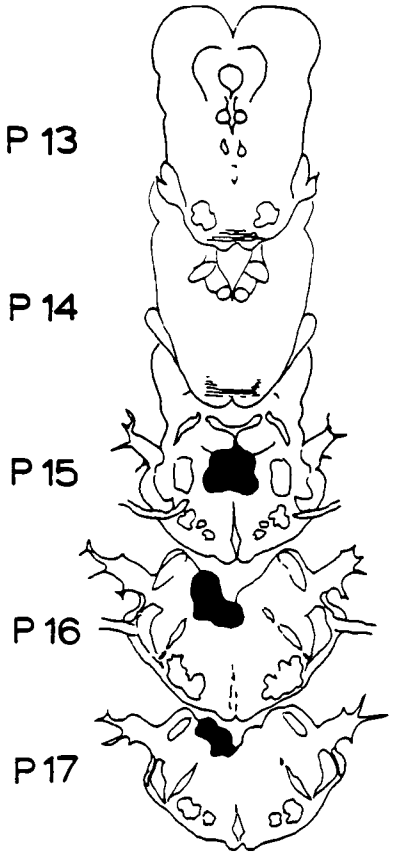


SUBJECT IDENTIFICATION : 27
LESION : LATERAL-CAUDAL LOCUS COERULEUS
GROUP : PARTIAL LOCUS COERULEUS

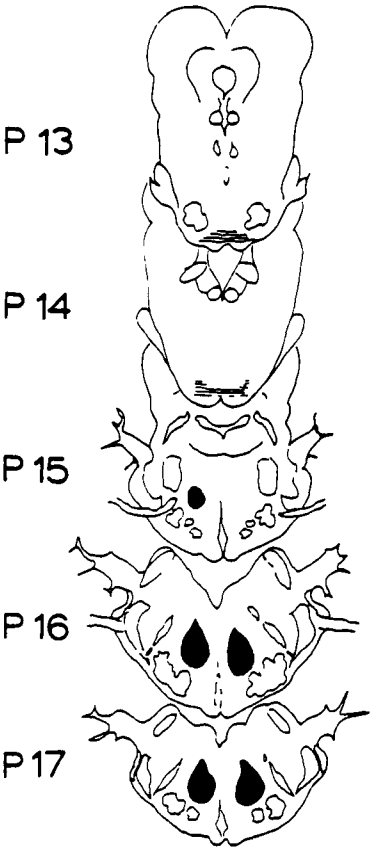


SUBJECT IDENTIFICATION : 09
LESION : DORSAL LOCUS COERULEUS
GROUP : PARTIAL LOCUS COERULEUS

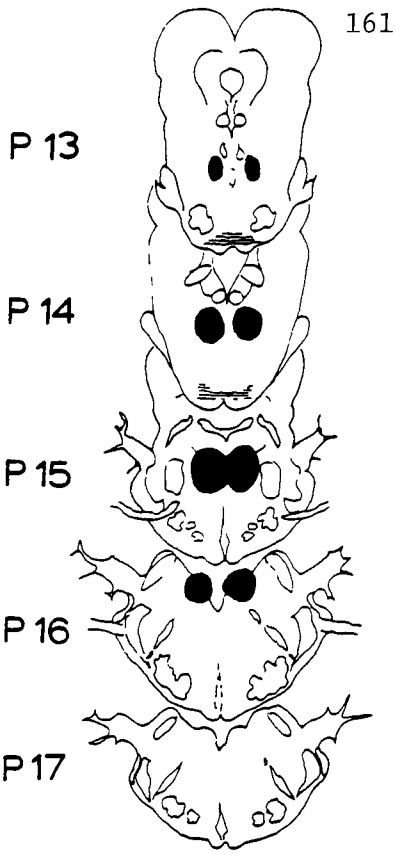




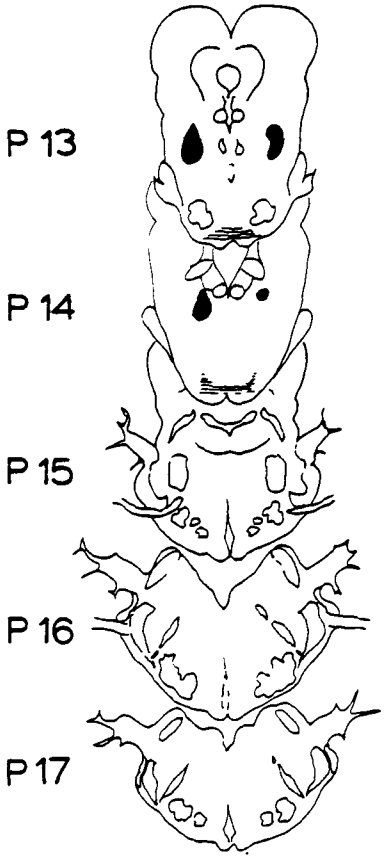
SUBJECT IDENTIFICATION : 10
LESION : N. RETICULARIS PONTIS CAUDALIS
GROUP : RETICULAR



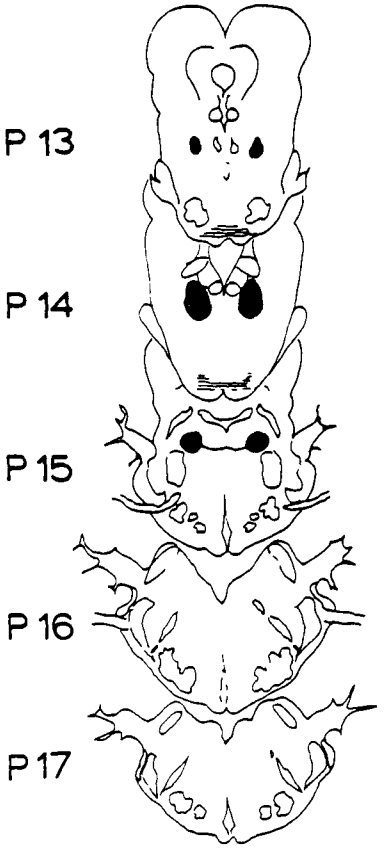
SUBJECT IDENTIFICATION : 24
LESION : N. RETICULARIS PONTIS CAUDALIS
GROUP : RETICULAR



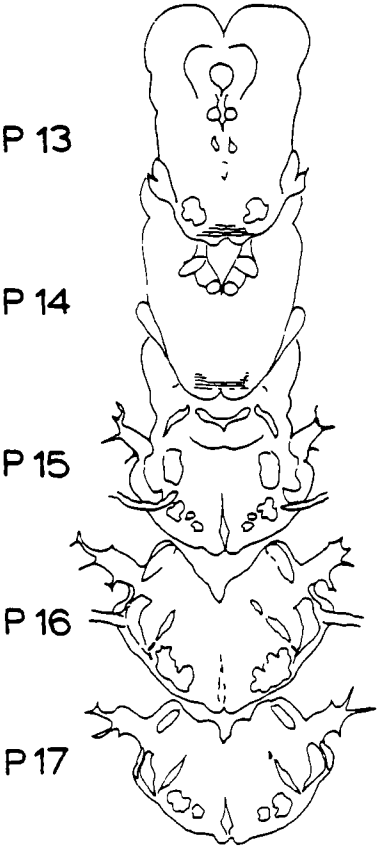
SUBJECT IDENTIFICATION : 31
LESION : N.R.P. ORALIS AND CAUDALIS
GROUP : RETICULAR

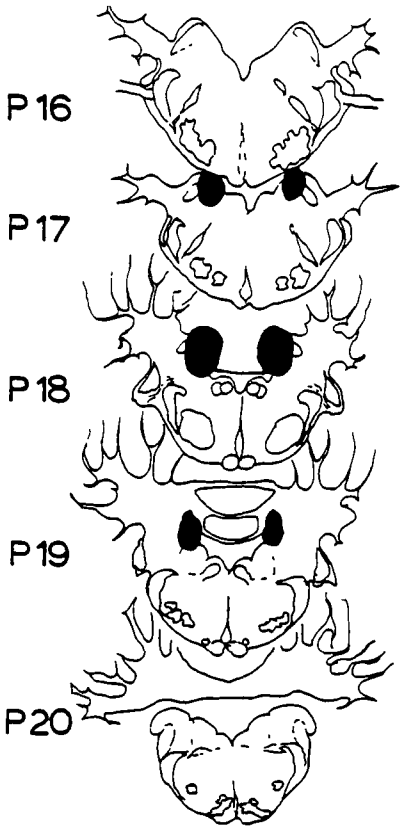


SUBJECT IDENTIFICATION : 17
LESION : N. RETICULARIS PONTIS ORALIS
GROUP : RETICULAR

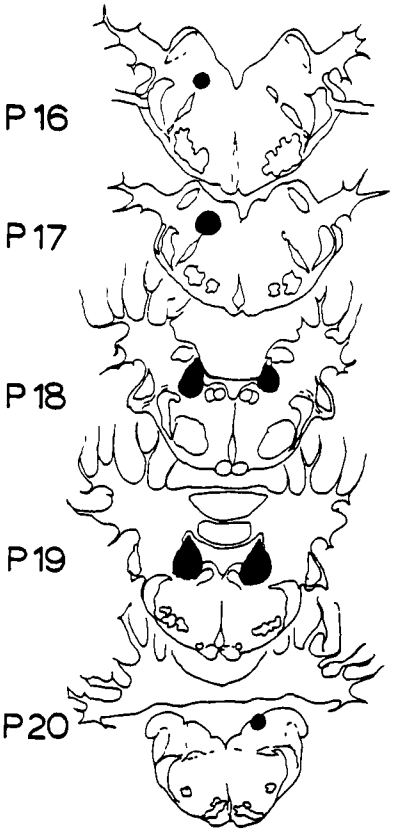


SUBJECT IDENTIFICATION : 18
LESION : N. RETICULARIS PONTIS ORALIS
GROUP : RETICULAR

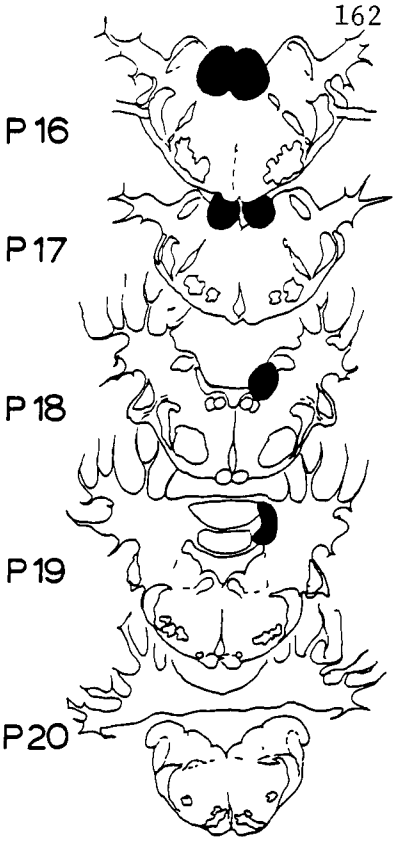




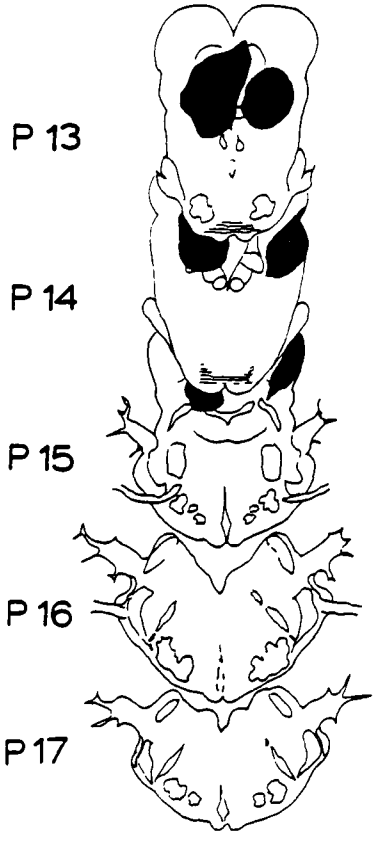
SUBJECT IDENTIFICATION : 05
LESION : MEDIAL VESTIBULAR NUCLEUS
GROUP : NON RETICULAR



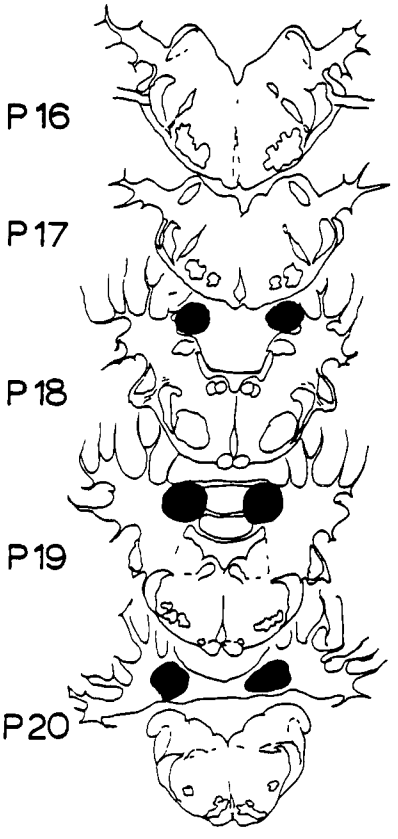
SUBJECT IDENTIFICATION : 13
LESION : VESTIBULAR NUCLEI
GROUP : NON RETICULAR



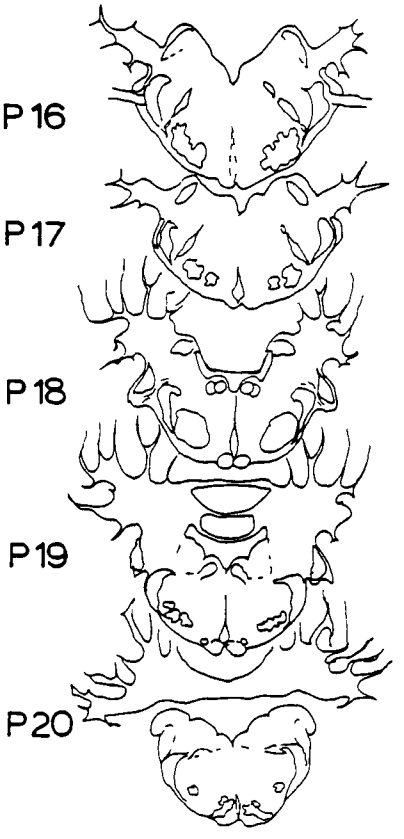
SUBJECT IDENTIFICATION : 26
LESION : CENTRAL GREY MATTER
GROUP : NON RETICULAR



SUBJECT IDENTIFICATION : 01
LESION : COLLICULI
GROUP NON RETICULAR



SUBJECT IDENTIFICATION : 02
LESION : FASTIGIAL N. OF CEREBELLUM
GROUP : NON RETICULAR



Appendix D

Precise description of lesions

The descriptions of the lesions in this appendix include only the set of subjects with bilateral lesions. Subjects with markedly assymetric lesions were entirely omitted from the Results section and are also ommitted here. They are presented in the same order as Appendix A, according to the groups used in the statistical analysis. The description of each lesion proceeds from the rostral to the caudal.

Locus Coeruleus group

(LEFT)	SUBJECT: LC 6	(RIGHT)
Part of the Brachium Conjunctivum.		
Central Tegmental Tract.		Central Tegmental Tract.
Part of the Central Grey Area around the fourth ventricle.		
Locus Coeruleus.		Locus Coeruleus.

(LEFT)	SUBJECT: LC 14	(RIGHT)
		Part of Lateral Lemniscus and Nucleus Reticularis. Pontis Oralis.
Nucleus Sub-Brachialis.		Nucleus Sub-Brachialis.
Locus Coeruleus.		Locus Coeruleus.
Nucleus Triangularis.		Nucleus Triangularis.
Medial portion of Nucleus of Bechterev.		Medial portion of Nucleus of Bechterev.
Radix Mesencephalica Nervi Trigemini.		Radix Mesencephalica Nervi Trigemini.

(LEFT)	SUBJECT: LC 35	(RIGHT)
Locus Coeruleus.		Locus Coeruleus.

(LEFT)	SUBJECT: LC 25	(RIGHT)
Part of Area Cuneiformis.		Part of Area Cuneiformis.
Locus Coeruleus.		Locus Coeruleus.

(LEFT)	SUBJECT: LC 8	(RIGHT)
Locus Coeruleus.		Locus Coeruleus. Ventral part of Parabrachial Nucleus.

(LEFT)	SUBJECT: LC 20	(RIGHT)
		Parts of Brachium Conjunctivum and Parabrachial Nucleus. Part of Central Grey Area medial to the Inferior Colliculus.
Locus Coeruleus. Medial part of Cerebellar Vermis. Radix Mesencephalica Nervi Trigemini.		Locus Coeruleus. Medial part of Cerebellar Vermis. Radix Mesencephalica Nervi Trigemini.

(LEFT)	SUBJECT: LC 28	(RIGHT)
Locus Coeruleus.		Locus Coeruleus.
Locus SubCoeruleus.		
Stratum Griseum Centrale.		Stratum Griseum Centrale.
Radix Mesencephalica		Radix Mesencephalica
Nervi Trigemini.		Nervi Trigemini.
Large part of Deiter's		Large part of Deiter's
Subnucleus-B.		Subnucleus-B.
Nucleus Triangularis.		Nucleus Triangularis.

(LEFT)	SUBJECT: LC 39	(RIGHT)
Locus Coeruleus.		Locus Coeruleus.

(LEFT)	SUBJECT: LC 37	(RIGHT)
Locus Coeruleus.		Locus Coeurleus.
Small medial part of		Small medial part of
Nucleus of Bechterev.		Nucleus of Bechterev.
Nucleus Supragenualis.		Nucleus Supragenualis.
Nucleus Triangularis.		Nucleus Triangularis.

(LEFT) SUBJECT: LC 34 (RIGHT)

Locus Coeruleus.
Posterior part of
Brachium Conjunctivum.

Locus Coeruleus.
Posterior part of
Brachium Conjunctivum.

(LEFT) SUBJECT: LC 36 (RIGHT)

Small portion of
Subnucleus Dissipatus.
Nucleus Pedunculo-
Pontini Tegmenti.
Nucleus Ventralis
Tegmenti Guddeni.
Part of decussation
of Brachium Conjunctivum.
Locus Coeruleus.
Small dorsal part of
Nucleus Reticularis
Pontis Caudalis.

Small portion of
Subnucleus Dissipatus.
Nucleus Pedunculo-
Pontini Tegmenti.
Nucleus Ventralis
Tegmenti Guddeni.
Part of decussation
of Brachium Conjunctivum.
Locus Coeruleus.
Small dorsal part of
Nucleus Reticularis
Pontis Caudalis.

Partial Locus Coeruleus Group

(LEFT)	SUBJECT: PLC 11	(RIGHT)
Medial part of Brachium Conjunctivum.		
Ventral part of Central Gray Area.		
Small ventral portion of Inferior Colliculus.		
Nucleus Triangularis.		Nucleus Triangularis.
Part of Deiter's Nucleus.		Part of Deiter's Nucleus.
Dorsal -caudal part of Locus Coeruleus.		Dorsal-caudal part of Locus Coeruleus.
Genu of VIIth Nerve.		Genu of VIIth Nerve.
Nucleus of VIth Nerve.		Nucleus of VIth Nerve.

(LEFT)	SUBJECT: PLC 22	(RIGHT)
		Anterior part of Locus Coeruleus.
Caudal Locus Coeruleus.		Caudal Locus Coeruleus.
Part of Vermis of Cerebellum.		Part of Cerebellar Vermis.

(LEFT) SUBJECT: PLC 12 (RIGHT)

Portion of Sub-brachial	Portion of Sub-brachial
Central Grey.	Central Grey.
Dorsal part of Nucleus	Dorsal part of Nucleus
Reticularis Pontis Oralis.	Reticularis Pontis Oralis.
Small medial portion of	Small medial portion of
rostral Locus Coeruleus.	rostral Locus Coeruleus.
Radix Mesencephalica	Radix Mesencephalica
Nervi Trigemini.	Nervi Trigemini.
Medial part of Nucleus	Medial part of Nucleus
of Bechterev.	of Bechterev.
Caudal Locus Coeruleus.	Caudal Locus Coeruleus.
Nucleus Triangularis.	Nucleus Triangularis.

(LEFT) SUBJECT: PLC 33 (RIGHT)

	Small part of rostral Locus Coeruleus.
Caudal Locus Coeruleus.	Caudal Locus Coeruleus.
Nucleus Triangularis.	Nucleus Triangularis.
Medial part of Nucleus	Medial part of Nucleus
of Bechterev.	of Bechterev.

(LEFT) SUBJECT: PLC 41 (RIGHT)

Caudal Locus Coeruleus.	Caudal Locus Coeruleus.
Small dorsal part of	Small dorsal part of
Nucleus Triangularis.	Nucleus Triangularis.
Deiter's Subnucleus Alpha.	Deiter's Subnucleus Alpha.

(LEFT) SUBJECT: PLC 38 (RIGHT)

Locus Coeruleus.	Dorsal-caudal Locus Coeruleus.
Area Cuneiformis.	Area Cuneiformis.
Medial portion of	Medial portion of
Brachium Conjunctivum.	Brachium Conjunctivum.
Small part of Cerebellar	Small part of Cerebellar
Vermis.	Vermis.

(LEFT) SUBJECT: PLC 16 (RIGHT)

Part of Lateral Lemniscus.	Small dorsal portion of Nucleus Reticularis Pontis Oralis.
Nucleus Sub-brachialis.	
Lateral part of Locus	Locus Coeruleus.
Coeruleus.	

(LEFT) SUBJECT: PLC 15 (RIGHT)

Lateral part of Locus Coeruleus.	Locus Coeruleus.
Nucleus Sub-brachialis.	
Medial portion of Brachium Conjunctivum.	
Radix of Mesencephalic V.	Radix of Mesencephalic V.
Medial portion of Nucleus of Bechterev.	Nucleus Triangularis.
Part of Deiter's Subnucleus (alpha).	Subnucleus Deiter's (alpha).

(LEFT) SUBJECT: PLC 40 (RIGHT)

	Part of Lateral Lemniscus.
Small part of Stratum Griseum Centrale.	Ventral Parabrachial Nucleus.
Small dorsal part of Nucleus Reticularis Pontis Caudalis.	
Locus Coeruleus.	Lateral-caudal part of Locus Coeruleus.
Radix Mesencephalica Nervi Trigemini.	Part of Stratum Griseum Centrale.
Small part of Nucleus of Bechterev.	Small part of Nucleus of Bechterev.

(LEFT)	SUBJECT: PLC 27	(RIGHT)
Rostral part of Brachium Conjuctivum and Parabrachial Nucleus.		
Lateral-caudal Locus Coeruleus.		
		Caudal Locus Coeruleus.
		Radix of Mesencephalica Nervi Trigemini.
Part of Nucleus of Bechterev.		Stratum Griseum Centrale.
Part of Genu of Nerve VII.		Small part of Deiter's
Part of Nerve VI.		Subnucleus (gamma).
Part of Nucleus Triangularis.		Small part of Nucleus Triangularis.

(LEFT)	SUBJECT: PLC 9	(RIGHT)
Small part of Nucleus Cuneiformis.		Small part of Nucleus Cuneiformis.
		Stratum Griseum Centrale.
		Nucleus Dorsalis Tegmenti Guddeni.
Dorsal Locus Coeruleus.		Dorsal Locus Coeruleus.
Medial part of Brachium Conjunctivum.		Medial part of Brachium Conjunctivum.

Reticular Group

(LEFT)	SUBJECT: R 10	(RIGHT)
Medial Londitudinal Fasciculus.		Medial Longitudinal Fasciculus.
Medial-dorsal portion of Nucleus Reticularis Pontis Caudalis.		Medial-dorsal portion of Nucleus Reticularis Pontis Caudalis.
Rostral-dorsal portion of Nucleus Reticularis Gigantocellularis.		Rostral-dorsal portion of Nucleus Reticularis Gigantocellularis.
Deiter's Nucleus.		
Nucleus Triangularis.		
Supragenual Nucleus.		
Genu of Nerve VII.		
Nucleus of Nerve VI.		

(LEFT)	SUBJECT: R 24	(RIGHT)
Large ventral part of Nucleus Peticularis Pontis Caudalis.		Large ventral part of Nucleus Reticularis Pontis Caudalis.
Large part of Nucleus Reticularis Gigantocellularis.		Large part of Nucleus Reticularis Gigantocellularis.

(LEFT)	SUBJECT: R 31	(RIGHT)
Small portion of Nucleus Reticularis Pontis Oralis. Large dorsal part of Nucleus Reticularis Pontis Caudalis.		Small portion of Nucleus Reticularis Pontis Oralis. Large dorsal part of Nucleus Reticularis Pontis Caudalis.

(LEFT)	SUBJECT: R 17	(RIGHT)
Nucleus Reticularis Pontis Oralis.		Nucleus Reticularis Pontis Oralis.

(LEFT)	SUBJECT: R 18	(RIGHT)
Caudal-dorsal part of Nucleus Reticularis Pontis Oralis. Rostral-dorsal part of Nucleus Reticularis Pontis Caudalis.		Caudal-dorsal part of Nucleus Reticularis Pontis Oralis. Rostral-dorsal part of Nucleus Reticularis Pontis Caudalis.

Non-Reticular Group

(LEFT)	SUBJECT: NR 5	(RIGHT)
Medial part of Brachium Conjunctivum.		Medial part of Brachium Conjunctivum.
Dorsal part of Nucleus Triangularis.		Dorsal part of Nucleus Triangularis.
Lateral portions of the Cortex of the Cerebellar Vermis.		Lateral portions of the Cortex of the Cerebellar Vermis.

(LEFT)	SUBJECT: NR 13	(RIGHT)
Dorsal part of Nucleus Reticularis Pontis Caudalis.		
Part of Deiter's Nucleus (alpha)		Part of Deiter's Nucleus (alpha)
Part of Nucleus Triangularis.		Part of Nucleus Triangularis.
Medial part of Nucleus of Bechterev.		

(LEFT)	SUBJECT: NR 26	(RIGHT)
Fasciculus Longitudinalis Medialis.		Fasciculus Longitudinalis Medialis.
Part of the 4th ventricle.		Part of the 4th ventricle.
Part of Cerebellar Vermis.		Part of Cerebellar Vermis.
Medial part of Stratum Griseum Centrale.		Medial part of Stratum Griseum Centrale.
Dorsal part of Genu of Nerve VII.		Nucleus Triangularis.
Nucleus Supragenualis.		

(LEFT)	SUBJECT: NR 1	(RIGHT)
Medial-ventral part of Inferior Colliculus.		Entire Inferior Colliculus.
Periventricular Central Gray Area.		Periventricular Central Gray Area.
Part of Central Tegmental Tract.		Dorsal part of Brachium of Inferior Colliculus.

(LEFT)	SUBJECT: NR 2	(RIGHT)
Small part of Cortex of Cerebellar Vermis.		Small part of Cortex of Cerebellar Vermis.
Part of Intermediate Nucleus of Cerebellum.		Part of Intermediate Nucleus of Cerebellum.
Fastigial Nucleus of Cerebellum.		Fastigial Nucleus of Cerebellum.

Appendix E

Assumptions for Repeated Measures Analysis of Variance

State	Normality	Homogeneity	Symmetry
W	met	not met	met
D	met	met	met
SW	met	met	met
PS	met	not met	met
P	met	met	met
TI	met	not met	not met

Note. Tests of assumptions were made by looking up the standard errors of measurement of kutosis and symmetry in the t-table, by Bartlett's Box test of homogeneity of variance and by Bartlett's test of symmetry of the variance-covariance matrix (see BMD-P, 1977).

Appendix F

Mean Times Spent in Each State and Standard Deviations

WAKING (means)

Groups	1 Day Pre-Lesion	5 Days Post-Lesion	14 Days Post-Lesion
LC	109.94	124.72	131.44
PLC	129.27	098.36	121.95
R	117.12	148.87	145.50
NR	119.87	118.62	128.00

WAKING (SDs)

LC	32.04	21.22	39.77
PLC	24.73	29.14	19.36
R	31.66	19.93	11.36
NR	17.58	17.12	31.57

Note. Time is in minutes.

DROWSINESS (means)

Groups	1 Day	5 Days	14 Days
LC	38.05	45.15	43.41
PLC	36.35	49.18	37.91
R	33.25	43.75	39.50
NR	24.87	53.12	36.75

DROWSINESS (SDs)

LC	11.17	17.31	09.84
PLC	09.43	16.81	09.99
R	08.50	07.42	19.63
NR	07.21	13.64	20.03

SLOW WAVE SLEEP (means)

Groups	1 Day	5 Days	14 Days
LC	183.15	162.94	139.89
PLC	165.40	186.68	159.09
P	177.50	144.87	138.50
NR	187.50	170.50	166.12

SLOW WAVE SLEEP (SDs)

LC	34.76	33.44	26.91
PLC	26.52	31.54	25.36
R	29.59	23.45	09.22
NR	20.78	08.95	40.69

PARADOXICAL SLEEP (means)

Groups	1 Day	5 Days	14 Days
LC	28.83	07.55	06.66
PLC	28.50	14.85	23.40
R	32.12	04.37	21.75
NR	27.75	16.87	27.87

PARADOXICAL SLEEP (SDs)

LC	11.33	13.73	11.47
PLC	15.97	13.34	13.90
R	08.55	04.60	27.45
NR	13.65	12.23	19.70

PHASIC ACTIVATION (means)

Groups	1 Day	5 Days	14 Days
LC	00.00	19.61	38.55
PLC	00.00	10.90	17.63
R	00.00	18.12	14.75
NR	00.00	00.85	01.25

PHASIC ACTIVATION (SDs)

LC	00.00	15.45	15.09
PLC	00.00	16.45	21.59
R	00.00	21.91	12.74
NR	00.00	01.75	02.50

TONIC IMMOBILITY (means)

Groups	1 Day	5 Days	14 Days
LC	085.50	066.87	96.12
PLC	107.00	231.00	30.25
R	130.50	472.75	68.00
NR	079.00	029.00	43.50

TONIC IMMOBILITY (SDs)

LC	042.50	078.39	62.11
PLC	117.46	380.52	33.93
R	137.90	855.42	04.51
NR	090.87	030.75	26.31

Note. Time is in seconds.

APPENDIX G

Time spent in each state for each recording by each subject in minutes.

	State	Pre-lesion	Post-lesion 5 Days	Post-lesion 14 Days
		RECORDING		
ID= 01	W	12.5	22.0	19.0
	D	15.0	24.5	18.0
	SW	17.0	23.0	16.0
	PS	8.5	7.0	3.0
	P	0.0	0.0	3.0
ID= 02	W	14.5	22.5	0.0
	D	18.0	19.0	0.0
	SW	16.5	20.0	0.0
	PS	3.5	6.0	0.0
	P	0.0	0.0	0.0
ID= 05	W	14.0	38.5	20.0
	D	16.0	40.5	17.0
	SW	20.5	37.5	26.0
	PS	2.5	0.5	10.5
	P	0.0	0.0	0.0
ID= 06	W	18.5	9.5	17.5
	D	23.5	20.5	37.5
	SW	23.0	19.0	43.0
	PS	12.5	0.0	0.0
	P	0.0	14.0	38.0
ID= 08	W	21.5	22.5	36.5
	D	19.5	26.0	28.0
	SW	24.5	32.5	33.5
	PS	3.0	0.0	0.0
	P	0.0	19.0	35.0
ID= 09	W	22.5	17.5	20.0
	D	20.5	19.5	18.0
	SW	22.0	23.0	22.0
	PS	6.5	7.0	6.0
	P	0.0	0.0	0.0
ID= 10	W	15.0	19.5	22.5
	D	13.0	19.0	20.0
	SW	15.5	19.0	21.0
	PS	10.5	4.0	7.0
	P	0.0	6.5	3.5

RECORDING

	State	Pre-lesion	Post-lesion 5 Days	Post-lesion 14 Days
ID= 11				
	W	16.5	28.0	27.5
	D	21.5	29.0	26.5
	SW	19.0	21.0	24.5
	PS	3.0	0.0	3.0
	P	0.5	4.5	19.0
ID= 12				
	W	18.5	24.5	8.0
	D	16.0	25.0	17.0
	SW	20.5	28.0	19.5
	PS	6.0	5.5	7.5
	P	0.0	0.0	11.0
ID= 13				
	W	26.0	31.0	26.5
	D	27.0	23.5	24.5
	SW	28.5	25.0	30.0
	PS	8.0	5.0	12.5
	P	0.0	0.0	0.0
ID= 14				
	W	13.5	11.0	21.5
	D	12.0	13.0	29.0
	SW	10.5	11.5	21.0
	PS	6.5	1.0	0.0
	P	0.0	0.0	15.5
ID= 15				
	W	13.0	19.5	27.5
	D	17.0	14.5	21.0
	SW	16.0	21.0	34.5
	PS	11.0	10.0	11.5
	P	0.0	1.0	10.0
ID= 16				
	W	22.5	24.5	34.0
	D	23.5	28.0	33.0
	SW	21.5	23.0	27.5
	PS	5.5	0.0	0.0
	P	0.0	18.5	32.5
ID= 17				
	W	21.5	18.5	14.5
	D	21.0	15.5	20.0
	SW	22.5	16.5	19.5
	PS	9.0	1.0	10.0
	P	0.0	0.0	0.0

	State	RECORDING Pre-lesion	Post-lesion 5 Days	Post-lesion 14 Days
ID= 18	W	16.0	16.5	15.0
	D	18.0	15.0	19.5
	SW	19.5	18.5	17.0
	PS	6.5	0.0	2.5
	P	0.0	6.0	5.0
ID= 20	W	29.5	19.0	20.0
	D	29.0	27.5	24.5
	SW	29.5	22.5	27.5
	PS	4.5	3.0	9.0
	P	0.0	2.5	10.5
ID= 22	W	23.5	26.5	20.5
	D	24.5	31.5	23.0
	SW	22.0	22.0	24.5
	PS	7.0	13.0	13.0
	P	0.0	0.0	0.0
ID= 24	W	22.0	20.0	0.0
	D	19.5	24.0	0.0
	SW	21.5	24.5	0.0
	PS	11.0	0.0	0.0
	P	0.0	15.0	0.0
ID= 25	W	26.0	24.0	22.5
	D	20.0	23.5	24.0
	SW	28.0	27.5	25.0
	PS	13.0	8.0	11.5
	P	0.0	17.0	23.0
ID= 26	W	19.0	21.0	20.5
	D	16.5	25.5	15.5
	SW	22.0	27.0	21.0
	PS	5.5	9.5	2.5
	P	0.0	2.5	0.0
ID= 27	W	23.5	16.5	20.5
	D	20.5	27.0	19.0
	SW	23.5	26.5	28.5
	PS	4.5	1.5	8.5
	P	0.0	10.5	5.0

	State	Pre-lesion	RECORDING Post-lesion 5 Days	Post-lesion 14 Days
ID= 23				
	W	31.0	14.5	27.5
	D	29.5	30.0	28.0
	SW	30.5	24.5	25.5
	PS	6.0	6.5	1.0
	P	0.0	9.5	12.5
ID= 31				
	W	26.0	23.5	39.5
	D	25.5	30.0	31.0
	SW	24.0	20.5	33.5
	PS	6.5	4.5	0.0
	P	0.0	27.5	15.0
ID= 32				
	W	25.0	12.0	19.5
	D	24.5	17.5	25.0
	SW	23.0	19.5	24.5
	PS	1.5	7.0	2.0
	P	0.0	11.5	7.0
ID= 34				
	W	13.5	24.0	0.0
	D	13.5	25.5	0.0
	SW	16.0	21.5	0.0
	PS	4.5	9.5	0.0
	P	0.0	0.5	0.0
ID= 35				
	W	15.5	8.0	19.5
	D	22.5	13.0	20.0
	SW	20.5	11.0	16.0
	PS	5.0	0.0	0.0
	P	0.0	3.5	8.5
ID= 36				
	W	22.5	17.5	20.0
	D	29.5	26.5	17.0
	SW	22.0	24.5	25.0
	PS	7.0	0.0	0.5
	P	0.0	15.5	13.5
ID= 37				
	W	29.0	20.5	0.0
	D	30.5	22.5	0.0
	SW	29.0	20.0	0.0
	PS	5.5	1.0	0.0
	P	0.0	5.0	0.0

	State	RECORDING Pre-lesion	Post-lesion 5 Days	Post-lesion 14 Days
ID= 38	W	21.5	26.0	21.5
	D	20.0	30.0	23.0
	SW	18.5	25.0	21.0
	PS	6.5	4.0	4.5
	P	0.0	0.0	8.0
ID= 39	W	22.0	17.0	18.0
	D	19.5	17.5	27.5
	SW	22.5	29.0	28.0
	PS	4.0	0.0	0.0
	P	0.0	21.5	18.0
ID= 40	W	20.0	15.0	19.0
	D	23.0	27.0	29.0
	SW	21.5	24.5	33.5
	PS	8.0	0.5	5.0
	P	0.0	14.0	25.5
ID= 41	W	14.5	22.0	29.5
	D	19.5	2[illegible].5	29.5
	SW	15.5	20.5	23.0
	PS	6.5	5.0	9.5
	P	0.0	0.0	0.0