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NEUROTOXIC *N*-METHYL-D-ASPARTATE LESION OF THE VENTRAL MIDBRAIN AND MESOPONTINE JUNCTION ALTERS SLEEP-WAKE ORGANIZATION

Y. Y. LAI,*§ T. SHALITA,* T. HAJNIK,* J.-P. WU,[t] J.-S. KUO,t L.-G. CHIA[f] and J. M. SIEGEL*

*Department of Psychiatry, School of Medicine UCLA, and VAMC, Sepulveda, CA 91343, U.S.A.

Departments of tMedical Research and Education, and [f]Neurology, Taichung Veterans General Hospital, Taichung, Taiwan

Abstract—The dorsal regions of the midbrain and pons have been found to participate in sleep regulation. However, the physiological role of the ventral brainstem in sleep regulation remains unclear. We used *N*-methyl-D-aspartate-induced lesions of the ventral midbrain and pons to address this question. Unlike dorsal mesencephalic reticular formation lesions, which produce somnolence and electroencephalogram synchronization, we found that ventral midbrain lesions produce insomnia and hyperactivity. Marked increases in waking and decreases in slow wave sleep stage 1 (S1), stage 2 (S2) and rapid eye movement sleep were found immediately after the lesion. Sleep gradually increased, but never returned to baseline levels (baseline/month 1 post-lesion: waking, 30.6±4.58%/62.3± 10.1%; S1, 5.1 ±0.74/3.9± 1.91%; S2, 46.2 ±4.74%/23.1± 5.47%; rapid eye movement sleep, 14.1 ±3.15%/7.2± 5.42%). These changes are comparable in magnitude to those seen after basal forebrain lesions. Neuronal degeneration was found in the ventral rostral pons and midbrain, including the substantia nigra, ventral tegmental area, retrorubral nucleus, and ventral mesencephalic and rostroventral pontine reticular formation.

We conclude that nuclei within the ventral mesencephalon and rostroventral pons play an important role in sleep regulation. © 1999 IBRO. Published by Elsevier Science Ltd.

Key words: substantia nigra, ventral tegmental area, retrorubral nucleus, pons, Parkinsonism, insomnia.

The dorsal regions of the mesencephalic reticular formation (MRF) and pontine reticular formation are known to be involved in sleep regulation. Stimulation of the dorsal MRF, part of the "ascending activating reticular system", induces electroencephalogram (EEG) activation.[44] Electrophysiological studies have shown that the firing rate of neurons in the dorsal MRF increases in waking and rapid eye movement (REM) sleep.[23,60,61] Lesion of the dorsal MRF, using the coagulation technique, produces an increase in sleep in the cat.[26,37] In the pontine reticular formation, microinjection studies have demonstrated that the dorsal pontine tegmentum participates in REM sleep control.[3,18,28] Radiofrequency or electrolytic and neurotoxic kainate lesion at the dorsal mesopontine junction decreases REM sleep, while the number of ponto-geniculo-occipital (PGO) spikes is reported to be increased or decreased, depending on the lesion site.[54,75]

Our previous studies in the decerebrate cat have found that electrical stimulation of the ventral mesencephalon and mesopontine junction produces muscle tone suppression and stepping-like activity between stimulations.[31] Our present study was designed to investigate the role of the ventral portions of the midbrain and mesopontine junction in the regulation of motor activity across the sleep-wake cycle.

§To whom correspondence should be addressed.

Abbreviations: AW, active waking; ChAT, choline acetyltransferase; EEG, electroencephalogram; MPTP, methyl-4-phenyl-l,2,3,6-tetrahydropyridine; MRF, mesencephalic reticular formation; NMDA, *N*-methyl-D-aspartate; PBS, phosphate-buffered saline; PGO, ponto-geniculo-occipital; QW, quiet waking; REM, rapid eye movement; S1, slow wave sleep stage 1; S2, slow wave sleep stage 2; SWS, slow wave sleep; TH, tyrosine hydroxylase.

EXPERIMENTAL PROCEDURES

Eight adult cats (Liberty Research, Waverly, NY) weighing 2.5-3.5 kg (three males and five females) were used in this study. Cats were implanted with electrodes under Nembutal (sodium pentobarbital; 35 mg/kg) anesthesia, using standard techniques.[55] After recovery from surgery, cats were chamber-adapted for at least 24 h. A 24-h baseline control recording was obtained.

After three consecutive 24-h baseline sleep-wake recordings, the animals were re-anesthetized with Nembutal and placed in a stereotaxic instrument. A small hole was made in the skull for needle insertion. Then, 0.5 /ul of 0.5 M *N*-methyl-D-aspartate (NMDA) was injected at A 2, L 3.5, H -4.5 and A 0.5, L 2.5, H -5.5 bilaterally, according to Berman's atlas.[7] Sleep recording was started on day 1 post-NMDA injection and recordings were made every day for seven days after injection. Then, EEG sleep recordings

Table 1. Change in sleep-wake architecture produced by *N*-methyl-D-aspartate lesions in the ventral midbrain and mesopontine junction

Cat	Time	AW	QW	S1	S2	REM	R/S2+R*	R/TST[t]
LC173	Control	31.7	4.6	6.0	46.8	10.9	18.9	17.1
	Day 3	100.0	0	0	0	0	—	—
	M[f] 1	74.5	3.4	5.5	16.6	0	0	0
LC175	Control	23.9	6.0	5.3	52.7	12.2	17.4	18.8
	Day 3	100.0	0	0	0	0	—	—
	M 1	59.6	4.5	4.1	20.8	11.0	30.6	34.6
LC4	Control	36.4	3.4	3.2	42.2	14.8	24.6	26.0
	Day 3	88.7	3.8	3.6	3.8	0	0	0
	W§ 1	62.5	5.0	7.6	24.8	0.1	0.3	0.4
LC5	Control	32.4	2.9	4.3	42.3	17.9	27.8	29.7
	Day 3	91.1	3.4	1.9	3.6	0	0	0
	M 1	64.6	1.9	1.2	26.4	5.9	17.6	18.3
	M 2	56.8	2.3	2.9	25.9	12.1	29.6	31.8
	M 4	55.3	4.3	3.5	25.8	11.0	27.3	29.9
LC7	Control	34.3	2.6	4.7	43.1	15.3	24.2	26.2
	Day 3	65.1	8.2	9.0	12.1	5.7	21.3	32.0
	M 1	50.3	4.5	4.9	28.7	11.7	25.8	29.0
	M 2	49.9	3.0	3.9	31.1	12.1	25.7	28.0
LC8	Control	41.5	5.8	5.5	30.4	16.8	31.9	35.6
	Day 3	82.7	2.6	2.0	11.6	1.1	7.5	8.7
	W 1	80.6	6.0	3.3	9.4	0.6	4.5	6.0
LC3	Control	31.0	10.0	6.9	41.5	10.6	18.0	20.3
	Day 3	27.5	7.1	6.8	49.1	9.6	14.7	16.4
	M 1	22.4	2.4	3.8	56.1	15.3	20.3	21.4
	M 3	22.2	3.0	4.1	54.8	16.0	21.4	22.6
LC6	Control	30.5	3.3	3.8	47.2	15.2	23.0	24.4
	Day 3	22.8	5.9	7.0	56.1	8.2	11.5	12.8
	M 1	20.7	4.6	5.3	51.5	17.8	23.9	25.7

*R/S2+R: the ratio of REM sleep time to the sum of S2 and REM sleep time, expressed as a percentage. [t]R/TST: the ratio of REM sleep time to the total sleep time expressed as a percentage. The total sleep time includes the time spent in S1, S2 and REM sleep. [f]M: month (post-lesion). §W: week (post-lesion).

Table 2. Behavior responses* to the *N*-methyl-D-aspartate lesion of the ventral midbrain and mesopontine junction

Cat	Behavior responses
LC173	Aggressive behavior, adipsia, aphagia, nystagmus, pupillary dilation, hallucination
LC175	Biting hard materials, aggressive behavior, adipsia, aphagia, nystagmus, pupillary dilation, hallucination
LC4	Aggressive behavior, adipsia, aphagia, nystagmus, pupillary dilation, hallucination
LC5	Aggressive behavior, adipsia, aphagia, biting hard materials
LC7	Adipsia, aphagia, nystagmus, pupillary dilation, bite hard materials, hallucination
LC8	Adipsia, aphagia, nystagmus, pupillary dilation, aggressive behavior, hallucination
LC3	No abnormal behavior* after lesion
LC6	Adipsia, aphagia

*A11 cats show hyperactive circling, climbing and walking around the recording chamber after NMDA lesion.

were obtained for two days each week, starting week 2 post-lesion, for the entire period of the experiment.

Data analysis

Five sleep-waking states were scored, active waking (AW), quiet waking (QW), slow wave sleep (SWS) stage 1 (S1), SWS stage 2 (S2) and REM sleep, according to Ursin and Sterman.[71]

Histology

At the end of the study, cats received an overdose of Nembutal (50 mg/kg) and were perfused intracardially with cold (4°C) Ringer's saline, followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline solution (PBS; 4°C) at pH 7.4. Brain tissue was removed and postfixed in 4% paraformaldehyde in 0.1 M PBS at 4°C for 2h and then transferred to 30% sucrose in 0.1 M PBS.

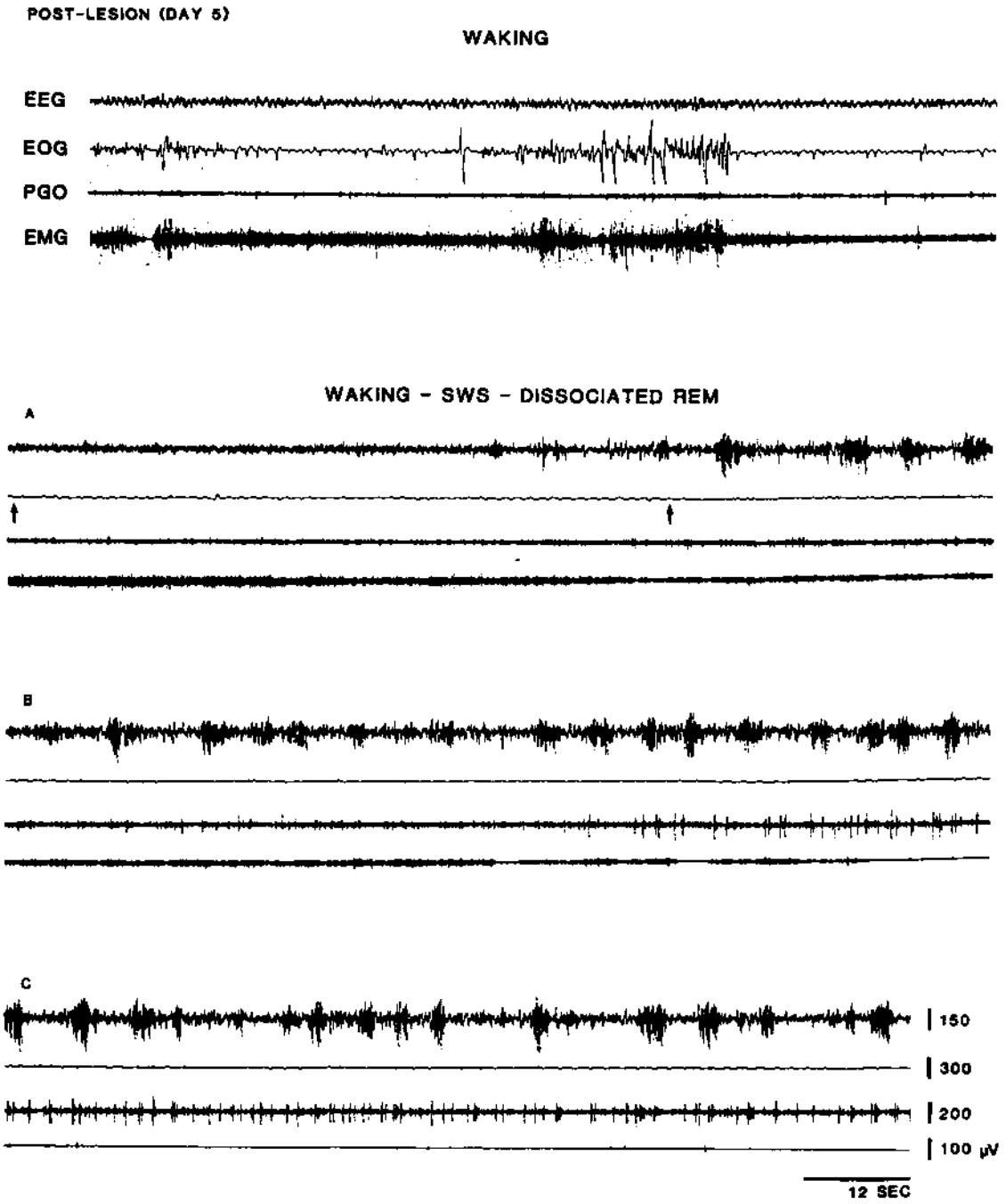

Fig. 1. Polygraphic recording showing abnormal sleep parameters induced by lesion (LC173). In waking, rapid eye movements were found when the cat was active (top panel), while nystagmus appeared when the cat was quiet (between the arrows in A). The lower panels of A-C are a continuous polygraphic recording representing a "dissociated sleep state". (A) The transition from waking to SWS. (B) The change from SWS to a state with EEG synchronization, spindles, isolated PGO spikes and low muscle tone. (C) The dissociated REM sleep state with EEG synchronization, PGO spike bursts and muscle atonia without rapid eye movement. EOG, electro-oculogram; EMG, electromyogram.

Serial coronal sections were cut at 50 /urn. Alternate sections were first processed with either choline acetyltransferase (ChAT) or tyrosine hydroxylase (TH) immunohistochemistry, and then counterstained with Neutral Red.

For immunohistochemistry, sections were incubated in 10% normal goat serum for 1 h. Then, tissue was incubated in either monoclonal ChAT antibody (1:1000), raised from rat-mouse hybrid cells for cholinergic immunohistochemistry, or TH antibody (1:5000), raised from the rabbit for catecholaminergic immunohistochemistry, for 24-48 h at 4°C. Tissue sections were processed with goat anti-rat immunoglobulin G for ChAT or goat anti-rabbit immunoglobulin G for TH for 1 h. The sections were then incubated

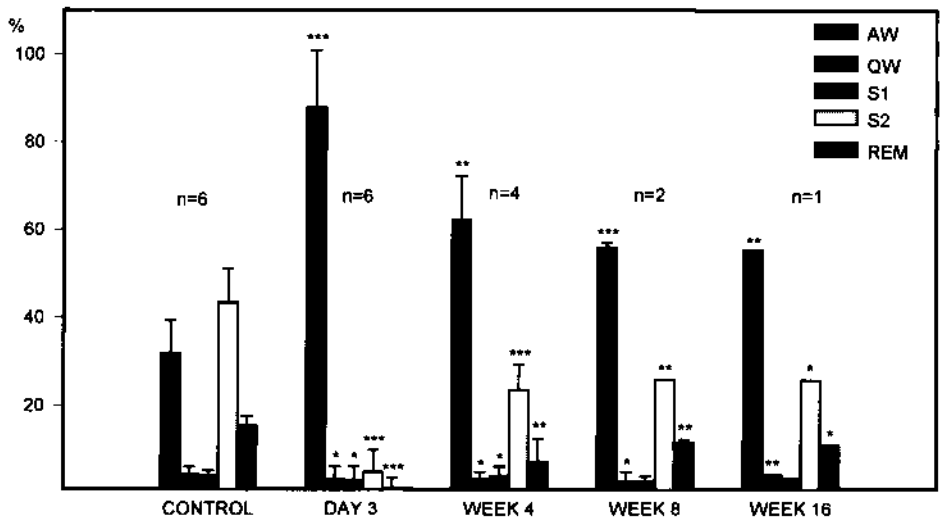

Fig. 2. Percentage of time spent in each state during the 24-h recording period before and after lesion. The data are for cats LC173, LC175, LC4, LC5, LC7 and LC8. A significant change in each sleep-wake state occurred on day 3 post-lesion. All stages of the sleep-wake cycle except S1 remained statistically different from the baseline control value over the entire four-month period of the experiment. Statistical significance: *P<0.05; **P<0.01; ***P<0.001.

in rat or rabbit peroxidase-antiperoxidase for another hour. The final product was visualized with 0.05% 3,3'-diaminobenzidine and 0.02% H_2O_2. Tissue sections were rinsed with 2% normal goat serum in 0.01 M PBS for TH, or in 0.05 M Tris-buffered saline for ChAT immunohistochemistry between the incubations. All incubations, except in the primary antibody, were performed at room temperature. The sections were then transferred to slides and stained with Neutral Red.

Lesion areas and cholinergic as well as catecholaminergic neurons were plotted with a DiLog 4000+ microscope interface.

Materials

NMDA was from Tocris Cookson (St Louis, MO), primary antibodies for ChAT and TH from Boehringer Mannheim Biochem. (Indianapolis, IN), goat anti-rat and goat anti-rabbit immunoglobulin Gs and rat and rabbit peroxidase-antiperoxidase from Jackson ImmunoResearch Lab. Inc. (West Grove, PA).

RESULTS

Baseline control recording of sleep

The percentage of AW, QW, S1, S2 and REM sleep in a 24-h baseline recording from each animal is shown in Table 1.

Behavioral responses to lesion

Cats were able to stand and walk without motor disturbances on day 2 post-lesion. Hyperactive circling, climbing and walking around the recording chamber were seen on day 2 post-lesion and lasted for the entire period of the experiment in all animals. Table 2 summarizes the behavioral results of the NMDA lesion. Three cats were observed to bite hard materials (hardwood, metals) starting day 2 post-lesion and lasting for one week. Aggressive behavior, such as hissing and attacking, was also seen in five of eight cats starting on day 3 post-lesion. This aggressive behavior was found to be correlated with the period of absence of REM sleep after NMDA lesion and disappeared after the appearance of the first REM sleep period. Force feeding was required during the first week after the lesion in seven of eight animals. After the first week post-lesion, all animals were able to eat and drink by themselves. Nystagmus (Fig. 1) was seen in five of the eight animals when they were quietly sitting and drowsy on day 2 post-lesion. At the same time, these five animals also showed abnormal pupillary dilation, which appeared during "hallucinatory" behavior in waking. During these "hallucinatory" behaviors, they stared ahead and would ignore objects or individuals they had previously attended to and appeared to interact with objects in the empty recording chamber. These behaviors lasted for four to five days. Two animals died, one on day 6 (LC4) and one on day 7 (LC8) post-lesion. The remaining six animals lost weight in the first week after the lesion, and completely regained their weight by week 3 post-lesion.

Effect of ventral midbrain-mesopontine junction lesion on sleep

Though three of eight animals showed "dissociated REM sleep" (see below) during the first week after lesion, the polygraphic signs of the sleep states returned to the baseline condition by day 6 post-

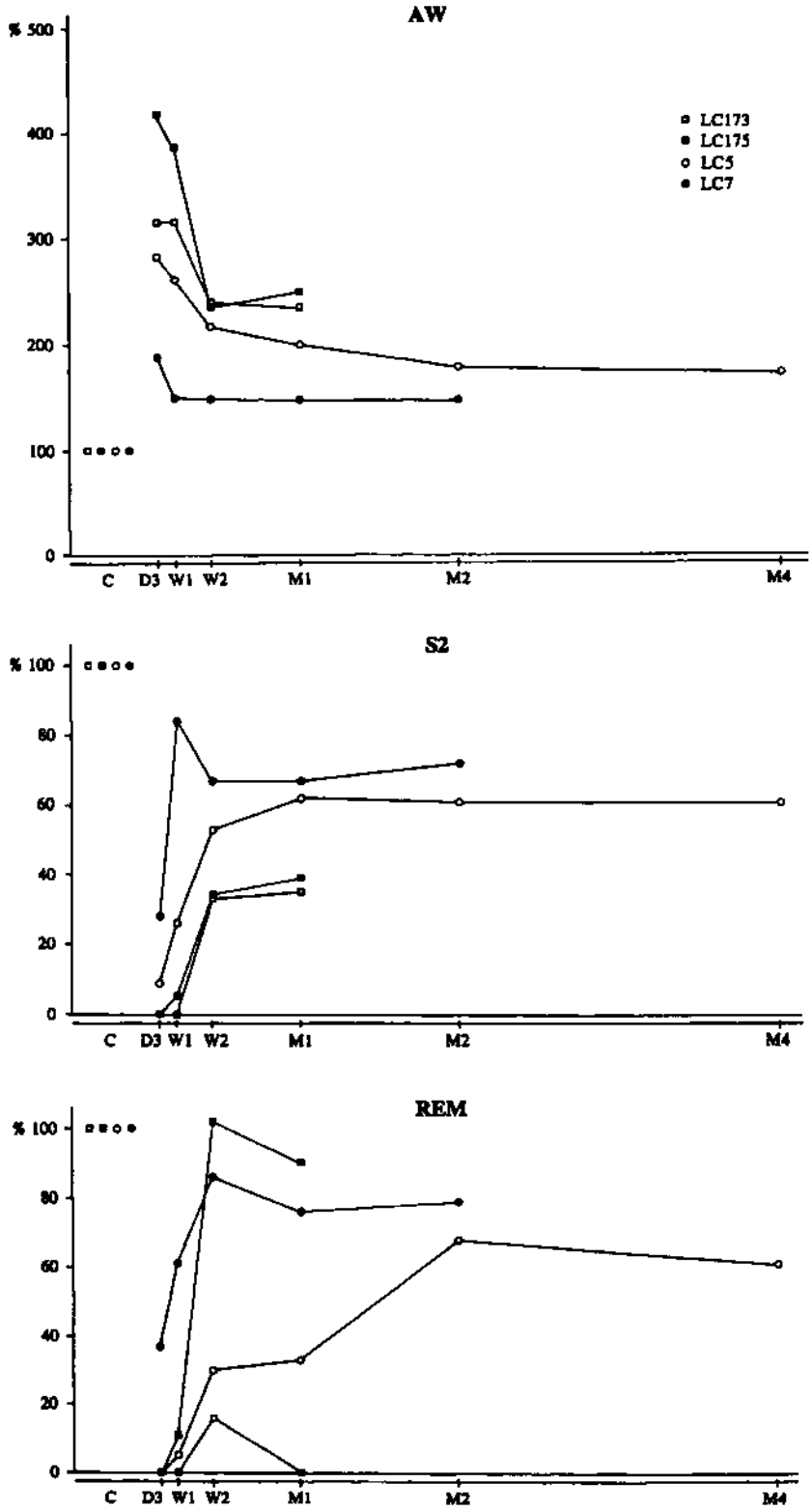

Fig. 3. Relative percentage change of AW, S2 and REM sleep after lesion compared to baseline value. Data were taken from the animals which survived for longer than one month after lesion. The baseline control value of each animal was expressed as 100%. The abscissa represents the time before (C) and after lesion. Each stage of the sleep-wake cycle gradually recovered, but never returned to the baseline level. The change of AW and S2 reached a plateau two weeks after lesion, while the change in REM sleep was inconsistent. C, baseline control; D3, day 3 post-lesion; M1, month 1 post-lesion; M2, month 2 post-lesion; M4, month 4 post-lesion; Wl, week 1 post-lesion; W2, week 2 post-lesion.

Table 3. Percentage, number and average duration of episodes in active waking, slow wave sleep stage 2 and rapid eye movement sleep before and after *N*-methyl-D-aspartate lesion* in a 24-h recording

Cat	Time	AW			S2			REM		
		%	Nt	D[f]	%	N	D	%	N	D
LC173	Control	32	100	4.5	47	175	3.8	11	34	4.6
	Day 3	100	1	1420	0	0	0	0	0	0
	M§1	75	271	3.9	17	262	0.9	0	0	0
LC175	Control	24	162	2.1	53	220	3.4	12	25	6.8
	Day 3	100	1	1420	0	0	0	0	0	0
	M 1	60	126	6.7	21	148	2.0	11	16	9.5
LC4	Control	36	103	5.0	42	154	3.9	15	46	4.6
	Day 3	89	120	10.5	4	42	1.3	0	0	0
	W‖ 1	63	*211*	3.2	25	196	1.8	0	0	0
LC5	Control	32	148	3.1	42	162	3.7	18	33	7.8
	Day 3	91	479	2.7	4	71	0.8	0	0	0
	M 1	65	68	13.4	26	56	6.6	6	20	4.3
	M 2	57	87	9.3	26	75	4.9	12	26	6.6
	M4	55	108	7.3	26	119	3.1	11	50	3.1
LC7	Control	34	132	3.7	43	175	3.5	15	39	5.6
	Day 3	65	402	2.3	12	245	0.7	6	14	6.1
	M 1	50	123	5.8	29	127	3.2	12	35	4.7
	M2	50	136	5.2	31	130	3.4	12	33	5.2
LC8	Control	42	184	3.2	30	144	3.0	17	40	5.9
	Day 3	83	82	14.3	12	150	1.1	1	13	1.2
	W 1	81	201	5.7	9	111	1.2	1	8	1.1

*Data from LC3 and LC6 are not included.
[t]N: number of the episode.
[f]D: average duration of the episode (min).
§M: month (post-lesion).
‖W: week (post-lesion).

lesion in all animals. The amplitudes of the EEG, electro-oculogram, electromyogram and PGO spikes were not changed by NMDA lesion.

Insomnia was seen starting day 1 post-lesion and lasted for the entire period of the experiment in six of eight animals. Lesion locations in the two animals not showing insomnia differed from those producing insomnia (see below). Five of these six animals had a lesion on both sides of the ventral midbrain and mesopontine junction, while the other had a lesion extending over both sides of the ventral midbrain and one side of the mesopontine junction. Although sleep gradually recovered in these six animals, it never returned to the baseline level over the four-month period of the experiment (Figs 2, 3, Table 1). Though significant changes in QW and S1 were found after the lesions (Fig. 2), this change was variable across the animals and showed no correlation with the time post-lesion (Table 1). However, consistent and time-dependent changes in S2 and AW were found after the lesion (Fig. 3). S2 was greatly decreased during the first three days post-lesion, then gradually recovered and reached a plateau at two weeks after the lesion (Fig. 3, Table 1). The reduction of S2 was found to result from a decrease in both number and duration of episodes (Table 3). Active waking was greatly increased, then there was a gradual decrease, reaching a plateau two weeks after the lesion (Fig. 3, Table 1). The increase in active waking was due to an increase in either the number or duration of episodes (Table 3).

REM sleep also decreased after bilateral ventral midbrain and mesopontine junction lesions in these six animals. Like SWS, REM sleep gradually recovered after lesion, but it never returned to the baseline level (Fig. 3, Table 1). The degree of decrease in REM sleep varied between animals (Fig. 3, Table 1). The proportion of REM sleep time as a percentage of total sleep time (the total time spent in S1, S2 and REM sleep) or to S2 and REM sleep time was decreased on day 3 post-lesion (except in cat LC7); it recovered at various post-lesion times across the animals (Table 1). The decrease in REM sleep was due to a decrease in the number and duration of episodes in five of six animals (Table 3); however, an increase in duration and decrease in the number of episodes was responsible in one animal (LC175). Normal sleep morphology was disrupted during the first week after lesion in three of the six animals (LC173, LC4 and LC7). These animals had SWS that was characterized by EEG synchronization with spindles and a decrease in basal muscle tone.

However, REM sleep was replaced by a "dissociated sleep state" (Fig. 1). The dissociated sleep state had both SWS and REM sleep components. The SWS components included EEG synchronization with spindles and without rapid eye movement, while REM sleep components included PGO spike bursts and muscle atonia. The phenomenon of dissociated REM sleep lasted for four to five days. By day 6 post-lesion, REM sleep with all the normal aspects of EEG desynchronization, rapid eye movement, PGO spike bursts and muscle atonia reappeared.

In the remaining two animals (LC3 and LC6), a slight increase of SWS and decrease in waking was found after the lesions (Table 1). Histological examination found that lesion locations in these animals differed from those that produced insomnia (Fig. 4B; also see "Histology" section below).

Histology

The six cats in which insomnia was produced by NMDA lesion had bilateral lesions of the ventral mesencephalon (Figs 4, 5), including the ventral MRF, retrorubral nucleus, ventral tegmental area, substantia nigra pars compacta, reticulata and lateralis, while at the ventral mesopontine junction level, symmetrical bilateral (LC175, LC4, LC5, LC7, LC8) or unilateral (LC173) lesion was found (Fig. 4). In the two animals that did not have insomnia, one (LC3) had a unilateral lesion in the ventral mesopontine junction, whilst the other (LC6) had a lesion in the dorsal midbrain on one side and the ventral midbrain on the other side (Fig. 4B). This cat (LC6) also had an asymmetrical lesion on both sides of the ventral mesopontine junction area. ChAT immunohistochemistry demonstrated that cholinergic neurons in the dorsolateral tegmental nucleus (Fig. 6c, j) and 70% of the ChAT-positive neurons in the pedunculopontine nucleus were not damaged (Figs 4, 6f, g). Noradrenergic neurons in the locus coeruleus complex were undamaged (Figs 4, 6h, i). However, more than 80% of dopaminergic neurons in the ventral mesencephalon were removed (Table 4, Fig. 6a, c).

DISCUSSION

We found that bilateral neurotoxic NMDA lesion of the ventral portions of the midbrain and mesopontine junction induced insomnia and hyperactivity. Unilateral lesions induced a slight increase in sleep and hyperactivity in the chronic cat. In the animals in which lesions induced insomnia, significant decreases in SWS and REM sleep and an increase in active waking were found immediately after the lesion. Although sleep gradually increased with time, SWS amounts did not return to the baseline level. This lesion-induced insomnia does not appear to be due to hyperactivity, since the two animals with unilateral lesions showed increased sleep and hyperactivity.

A variety of effects on sleep have been observed after midbrain lesions. Electrolytic coagulation lesions in the ventral mesencephalon induce a "comatose" state without waking in the cat[26,37] and monkey.[14] Neurotoxic ibotenate lesions that include both the dorsal and ventral MRF produced a very short-lasting (8-12h) increase in waking. However, the long-term sleep-waking cycle is not changed after lesions of both the dorsal and ventral MRF.[11] In contrast, our present study finds that a lesion restricted to the ventral midbrain induces a persistent and profound insomnia. The differences between our findings and those of Denoyer *et al.*[11] appear to be due to the lesion locus, since the dorsal MRF, which participates in waking, was also damaged in their study. The difference in results between prior electrolytic lesions and our chemical lesion could be due to damage to the descending fibers originating in the tuberomammillary nucleus and the adjacent posterior hypothalamus that are related to arousal[72,74] and that descend to the brainstem through the ventral midbrain area.[36]

Neurons in the ventral midbrain and rostroventral pons have been shown to contain dopamine,[10,25,76] glutamate[24,32] and GABA.[13] However, NMDA injection reduces the numbers of dopaminergic as well as non-dopaminergic cells. Therefore, further studies will be needed to determine the transmitters utilized by the ventral midbrain cells whose loss caused the profound insomnia we observed.

Pharmacological studies using agonists and antagonists have shown that dopaminergic mechanisms are involved in the control of waking. Systemic administration of dopamine D_1 receptor agonists induces arousal and suppresses REM sleep in the rat.[67,68] The selective D_2 receptor agonist, quinpirole, also produces EEG arousal in rabbits.[46] RO 41-9067, a D_2 agonist, produces a decrease in REM sleep and an increase in waking when given to rats during the light period. When given in the dark period it produced an increase in REM sleep and a decrease in waking.[48] EEG desynchronization and increases in waking have been found after L-DOPA administration in the rabbit.[46] In contrast, a highly variable change in the sleep-waking pattern has been found in L-DOPA-treated parkinsonian patients. Improved sleep,[2] unaltered sleep-waking pattern[27] and suppressed REM sleep[1] had been reported.

In contrast to the result of the pharmacological studies described above, results from extracellular recording and lesion studies have shown that the activity of dopaminergic neurons of the substantia nigra changes very little across the sleep cycle.[42,59,69] In contrast, non-dopaminergic neuronal activity in the substantia nigra pars reticulata is significantly increased in waking and REM sleep ralative to SWS values.[42] The activity of ventral tegmental area

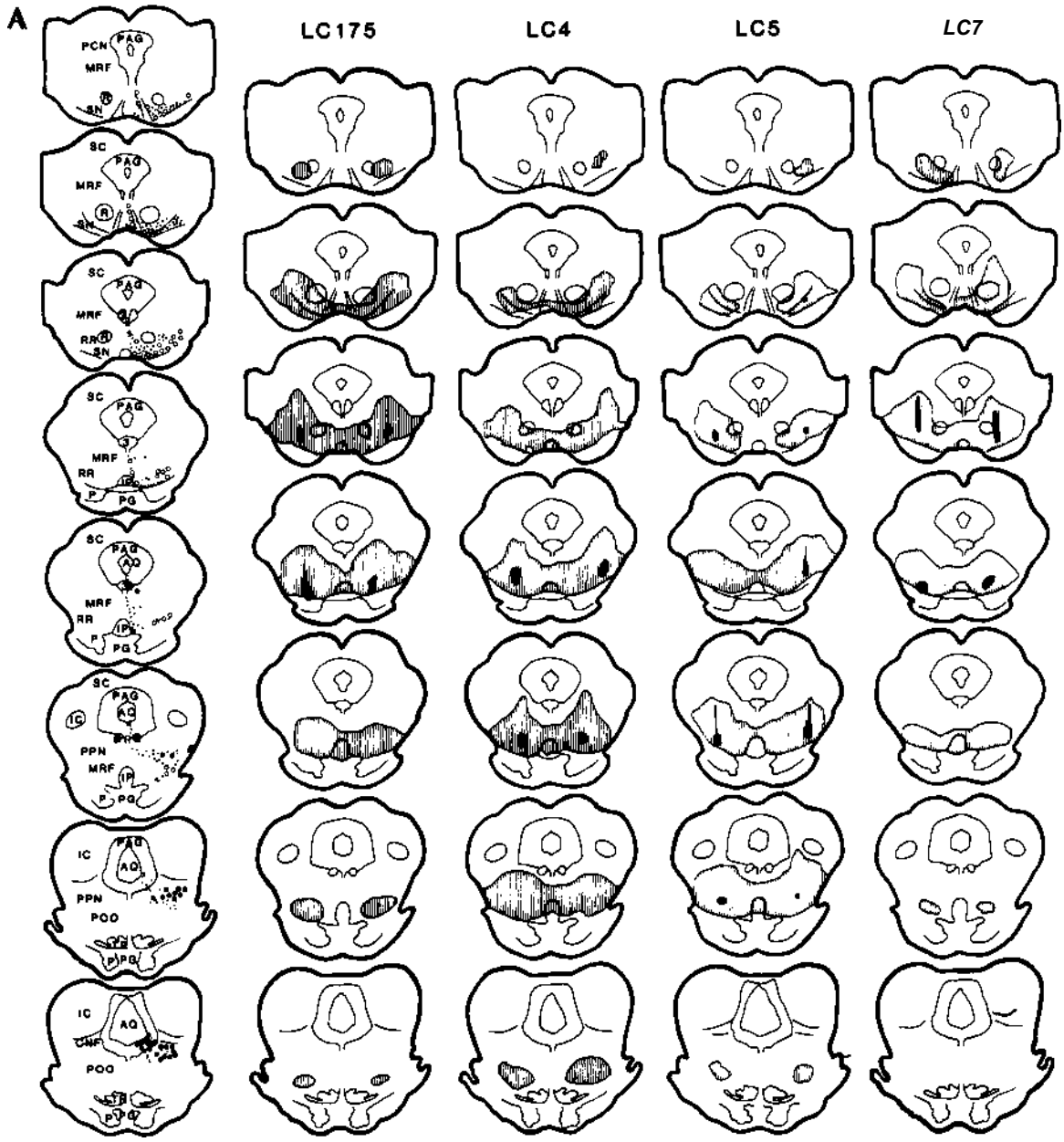

Fig. 4A.

non-dopaminergic neurons has been found to decrease in SWS and increase in REM sleep.[33] These non-dopaminergic neurons have been identified as GABAergic.[50,58] Neurotoxic lesions which specifically target the dopaminergic system have not been found to produce changes in sleep-waking pattern. The sleep-waking pattern is not changed after intraventricular injection of 6-hydroxydopamine.[43] Methyl-4-phenyl-l,2,3,6-tetrahydropyridine (MPTP), a neurotoxic chemical that selectively damages dopaminergic neurons, has been shown to suppress REM sleep and increase SWS immediately after intraperitoneal injection in the cat.[47] The MPTP effect on the sleep-waking cycle lasted for six to nine days. This was followed by a gradual return to baseline levels of REM sleep and SWS on day 9 post-MPTP treatment. In contrast, we found decreases in SWS and changes in sleep-waking pattern that never returned to baseline level in our NMDA-lesioned animals which lasted for the four-month duration of the experiment. We suggest that the dopaminergic system may not play a critical role in sleep regulation. However, a modulatory effect of the dopaminergic system on sleep cannot be ruled out, since dopamine has been found to enhance GABA activity in the hypothalamus *in vitro*.[5]

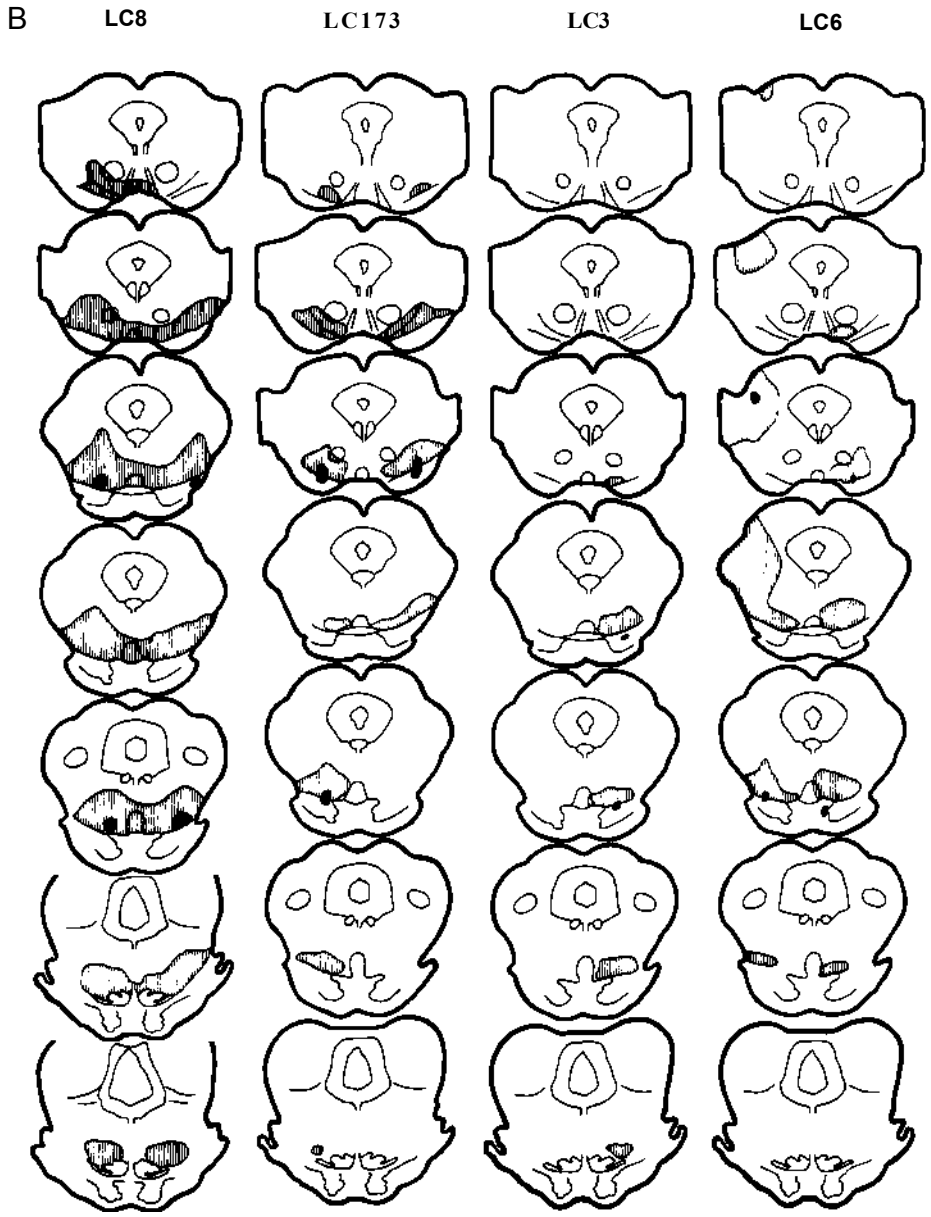

Fig. 4B.

Fig. 4. Histological mapping showing the lesion areas. The leftmost row gives anatomical labels and plots the dopaminergic (open circles) and cholinergic (filled circles) neurons. The small and large symbols represent one and 10 neurons, respectively. The lined areas represent the location of neuronal degeneration produced by NMDA injection. The black sites represent the center of injection (gliosis). AQ, aqueduct; CNF, cuneiformis nucleus; IC, inferior colliculus; IP, interpeduncle nucleus; MRF, mesen-cephalic reticular formation; P, pyramidal tract; PAG, periaqueductal gray; PCN, nucleus of the posterior commissure; PG, pontine gray; PPN, pedunculopontine nucleus; PRF, pontine reticular formation; R, red nucleus; RRN, retrorubral nucleus; SC, superior colliculus; SNC, substantia nigra pars compacta; SNR, substantia nigra pars reticulata; TR, tegmental reticular nucleus; 3, oculomotor nucleus; 4, trochlear nucleus.

GABAergic mechanisms have been shown to be important in sleep regulation. Increases in GABA release during SWS had been seen in the posterior hypothalamus,[45] an area related to the control of behavioral arousal.[40,41,66] Microinjection of musci-mol, a GABA agonist, into the posterior hypothalamus induces hypersomnia in the cat.[35] GABAergic neurons projecting to the posterior hypothalamus have been identified in the ventral midbrain, including the MRF, ventral tegmental area, substantia nigra pars compacta and retrorubral nucleus,[13] as well as in the basal forebrain.[20] Thus, neuronal

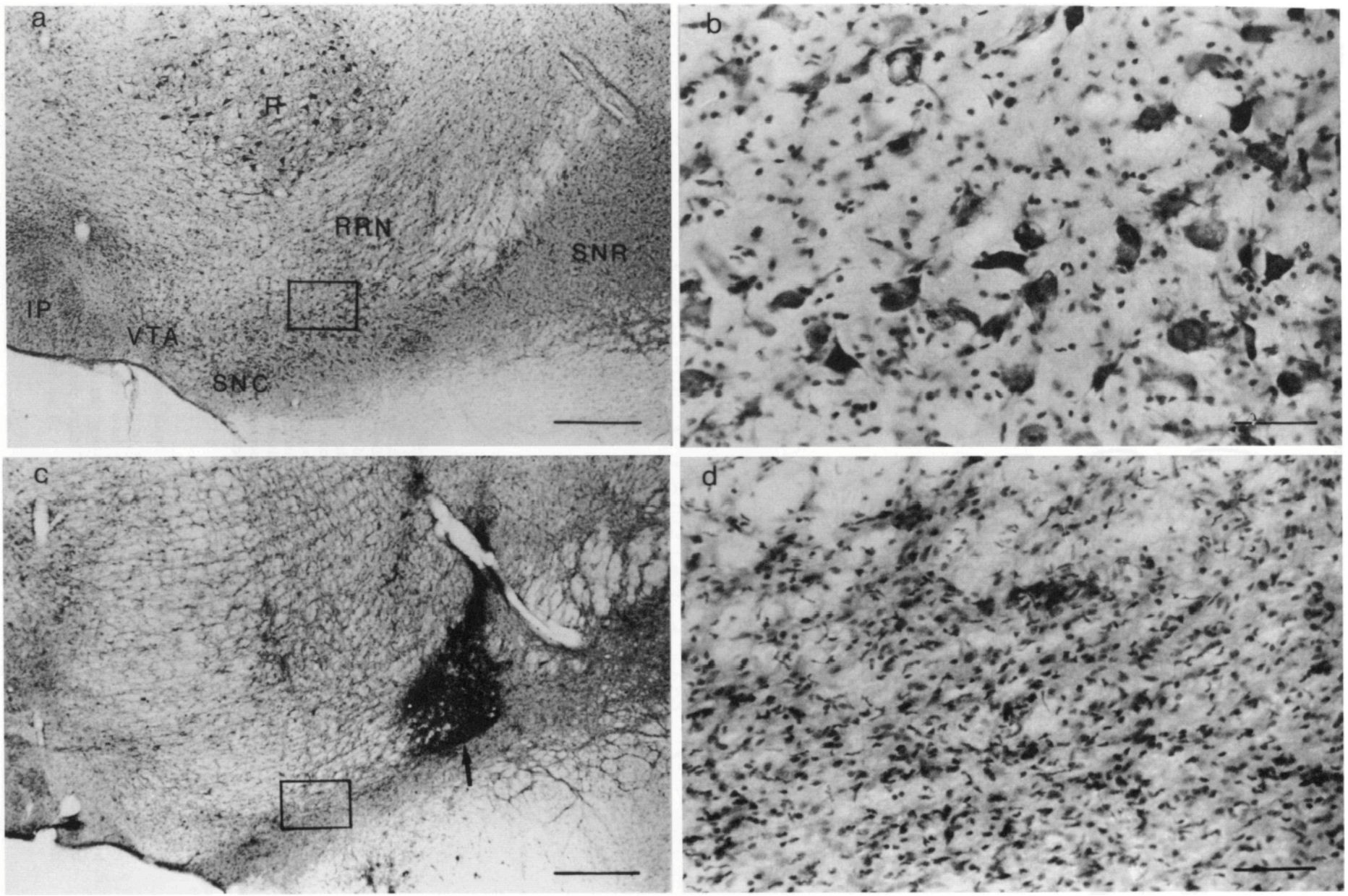

Fig. 5.

Table 4. Percentage of dopaminergic neuron degeneration in each cat

	Total	RRN	SNC	SNR	SNL	VTA
LC173	64.5	63.1	81.3	59.0	25.9	28.1
LC175	83.9	90.8	89.4	81.7	49.2	67.6
LC4	83.3	93.5	87.0	76.4	52.4	67.5
LC5	78.1	94.0	87.6	83.7	51.4	34.0
LC7	81.2	89.4	89.5	69.5	40.1	60.2
LC8	83.3	86.8	85.7	88.5	64.7	74.8

RRN, retrorubral nucleus; SNC, substantia nigra pars compacta; SNL, substantia nigra pars lateralis; SNR, substantia nigra pars reticulata; VTA, ventral tegmental area.

degeneration in the ventral midbrain after NMDA lesion in our present study may have resulted in decreased GABA release in the posterior hypothalamus, which in turn produced insomnia.

Neurons in the ventral midbrain and mesopontine junction participating in sleep may also act through the basal forebrain. Neurons in the ventral midbrain and mesopontine junction have been found to project to the basal forebrain.[4,8,12,16,17,49,57,73] Neurons from the basal forebrain have projections to the ventral mesencephalon.[9,19,51,56] Electrical stimulation in the basal forebrain elicits sleep with EEG synchronization,[62,63] while lesion in this area produces insomnia.[40,65] Our cytotoxic lesion in the ventral midbrain and mesopontine junction produced a permanent decrease in SWS comparable in magnitude and duration to that seen after basal forebrain lesion.[52,65] We suggest that interactions between the basal forebrain and ventral midbrain play a critical role in the control of sleep duration.

The aphagia and adipsia seen in our present study were similar to those seen previously with midbrain lesions. Adipsia and aphagia were found in rats after bilateral electro-coagulation in the ventral mid-brain or 6-hydroxydopamine-induced dopaminergic neuron degeneration.[70] In the rat, electrolytic lesion of the MRF produced hyperactivity and hypo-dipsia.[22] Bilateral electrolytic lesions in the ventral MRF also produced locomotor hyperactivity in the rat.[15,34,64]

The aggressive behavior found in the animals which did not have REM sleep after lesion in the present study could be due to REM sleep deprivation, since it disappeared after the appearance of the first REM sleep. This result is consistent with the report of Hicks *et al.*[21] who found increases in aggressiveness after REM sleep deprivation in the rat. Hallucinatory-like activity seen in our NMDA-injected animals was similar to that reported by Kitsikis and Steriade in the cat.[29] They found a hallucinatory-type behavior in the cat after kainate injection into the midbrain. Hallucination has also been found in Parkinson's disease[30] and parasomnia overlap disorder[53] patients. Furthermore, the phenomenon of dissociated states of sleep seen in our present animal study was also seen in human sleep disorder patients.[38,39]

Neuronal degeneration in the ventral midbrain is present in parkinsonian patients. Sleep fragmentation with frequent arousal, decrease in SWS stages 3 and 4, and decrease in REM sleep have been reported in drug-free parkinsonian patients.[6,27] Our present study found that sleep-wake architecture in bilateral ventral midbrain- and mesopontine junction-lesioned cats is similar to that of parkinsonian patients.

CONCLUSIONS

The changes in sleep-wake architecture produced by neurotoxic lesion of the ventral midbrain and mesopontine junction areas suggest that the ventral brainstem plays a critical role in the regulation of sleep. Two mechanisms by which the ventral midbrain and mesopontine junction may control sleep are suggested by the present study. (1) The ventral midbrain and mesopontine junction may act through its innervation of the posterior hypothalamus. (2) They may act through their reciprocal connections with the basal forebrain. It is possible that both mechanisms are active simultaneously.

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Fig. 5. Microphotographs showing neuronal degeneration in the ventral midbrain. (a) Microphotograph taken from an unlesioned animal, (b) High-magnification microphotograph taken from the area shown in a. (c) Microphotograph taken from cat LC175. Gliosis of NMDA injection site is indicated by the arrow, (d) High-magnification microphotograph taken from the area shown in c. No neurons were found in this area. VTA, ventral tegmental area. Other abbreviations are as defined in Fig. 4. Scale bars=500 μm (a, c), 50 μm (b, d).

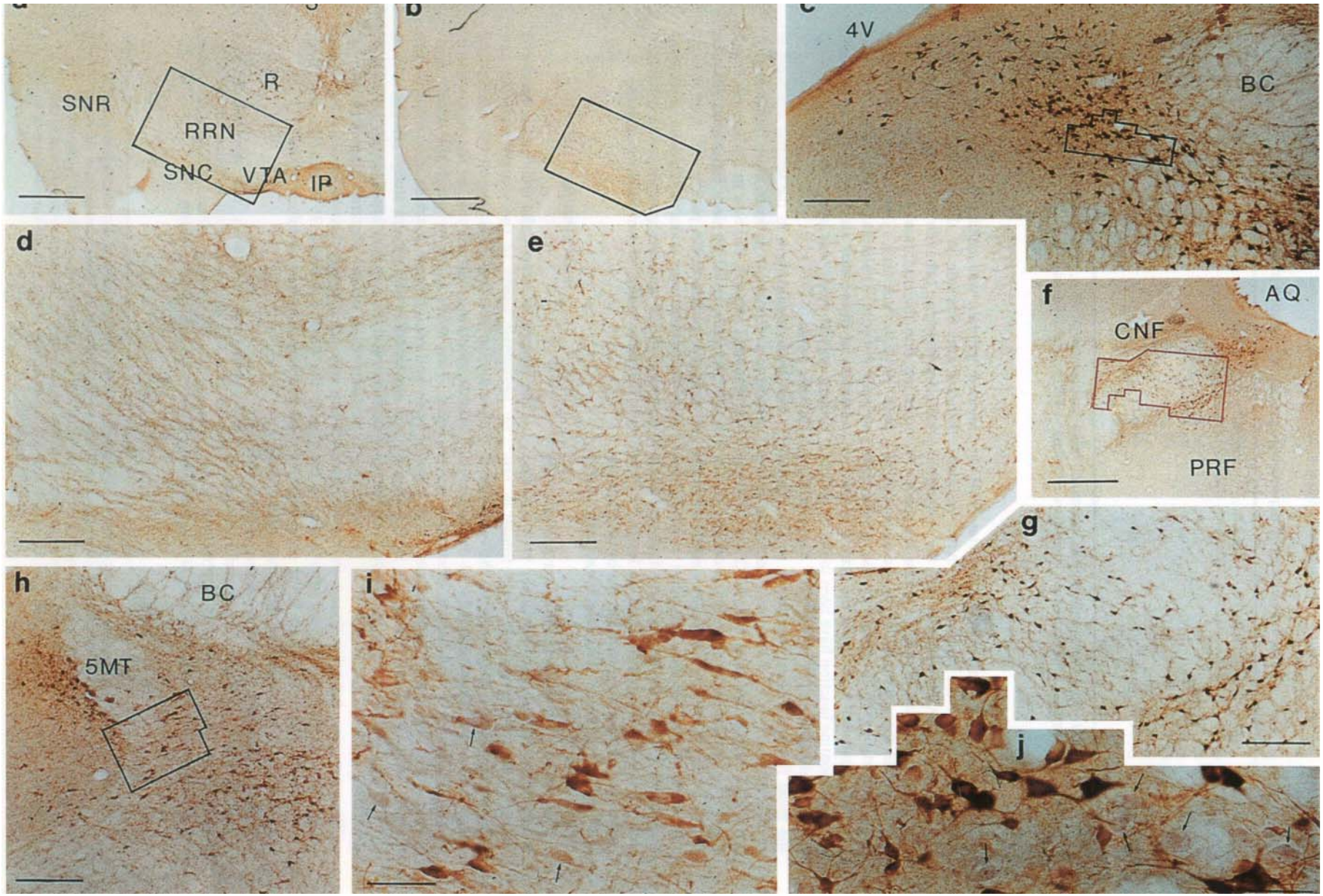

Fig.6.

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Fig. 6. Microphotographs showing degenerated and intact neuronal groups. All microphotographs, except b and e, which were taken from the control unlesioned animal, were taken from the lesioned cat EC5. TH immunohistochemistry double stained with Neutral Red was done on sections a, d, h and i, while ChAT immunohistochemistry double stained with Neutral Red was done on sections c, f, g and j. TH immunohistochemistry was done on the control animal, as shown in b and e. (a, b) Eow-magnification microphotographs taken from lesioned and control cats, respectively, (c) Microphotograph taken from the dorsolateral tegmental nucleus (EDT) area, (d, e) Higher magnification microphotographs taken from the areas shown in a and b, respectively. No dopaminergic neurons were seen in d. (f) Microphotograph taken from the rostral pons at the level of the dorsolateral tegmental nucleus and pedunculopontine nucleus (PPN). (g) Higher magnification of microphotograph taken from the area shown in f. Cholinergic neurons, except those located in the ventrolateral portion of the pedunculopontine nucleus, were undamaged, (h) Eow-magnification microphotograph taken from the area of the locus coeruleus. (i) High-magnification microphotograph taken from the area shown in h. Neurons single stained with Neutral Red are indicated by arrows, while the rest of the neurons are double labeled with TH immunohistochemistry. (j) High-magnification microphotograph taken from the area shown in c. Neurons single stained with Neutral Red are indicated with arrows, while the rest of the neurons are double labeled with ChAT immunohistochemistry. BC, brachium conjunctivum; 4V, fourth ventricle; 5MT, mesencephalic trigeminal tract. Other abbreviations are as defined in Figs 4 and 5. Scale bars = 1000 urn (a, b, f), 250 urn (c-e, g, h), 50 urn (i, j).

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