Norepinephrine-Containing Neurons: Changes in Spontaneous Discharge Patterns during Sleeping and Waking

Abstract. *Norepinephrine-containing neurons of the locus coeruleus of the cat were recorded with microelectrodes during unrestrained sleeping and waking. The recorded neurons were subsequently defined by combined fluorescence histochemistry of catecholamines and production of microlesions at recording sites. These pontine units show homogeneous changes in discharge patterns with respect to sleep stages, firing slowly during drowsy periods and slow wave sleep and firing in rapid bursts during paradoxical sleep. These data provide a direct correlation between the activity of defined catecholamine-containing neurons and the spontaneous occurrence of sleep stages.*

Histochemical studies have localized the neurons of the brain that contain norepinephrine (NE) to several nuclei in the mesencephalon and brainstem (*1, 2*). One such nucleus [group A6 in the terminology of Dahlstrom and Fuxe (*1*)] appears to be identical to the pontine nucleus locus coeruleus (LC) (*3*) as described by fluorescent cytological studies of rat (*1, 2, 4*), squirrel monkey (*5*), and cat (*6*). Although the cerebellar effects of NE-mediated synapses have been studied in detail for one of the efferent projections of the LC (*7*), such experiments do not clarify what sensory or motor events might regulate the activity of the NE neurons in animal behavior.

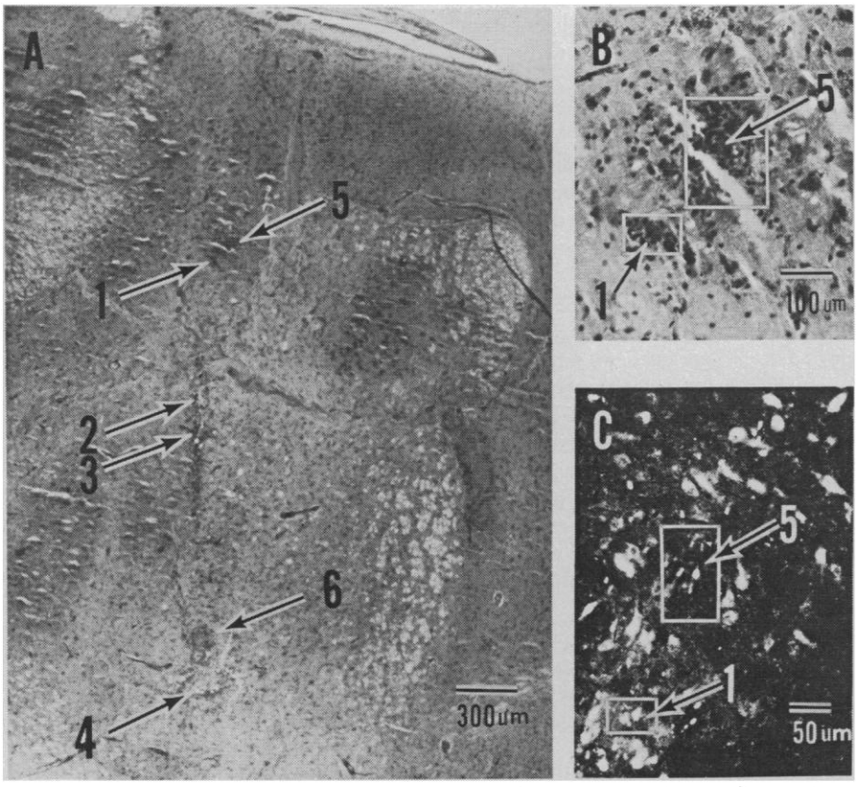

Fig. 1. Photomicrograph of electrolytic lesions in the locus coeruleus (LC) nucleus. (A) Coronal section of the pons at P = 2.0 mm stained by the Nissl method. Arrows 1 to 4 denote four lesions made during one penetration and arrows 5 and 6 denote two lesions made during the last penetration. Note that the progressive increase in lesion size was made as the microelectrode descended. (B) High magnification of LC lesions. Note that the smaller lesion measured about 60 µm in diameter whereas the larger one measured 140 µm. (C) Fluorescent photomicrograph of the two corresponding LC lesions in (A) and (B). As shown in (B), the lesion sites in LC nucleus are indicated by dense cellular aggregates. The typical lesions, especially the larger ones as shown by the fluorescence histochemical method, consist of a central area that is almost devoid of green fluorescence and a peripheral one in which the degenerating neurons shrink into oval or round yellow-greenish fluorescent masses. The normal-looking NE neurons immediately surrounding the lesion usually show high fluorescent intensity.

The NE-containing neurons, particularly those of the LC, have been incriminated in the regulation of sleeping and waking behavior in the cat (*8*) on the basis of sleep disturbance that followed destruction of the LC by electrocoagulation (*9*) or by local injection of 6-hydroxydopamine (*10*), or that followed treatment with drugs to alter NE synthesis or storage (*11*). The development of techniques that permit microelectrode recording from single neurons in unrestrained, unanesthetized animals (*12*) offers a direct means for investigating the potential correlation between activity of LC neurons and sleep stages. We now report that the activity patterns of individual NE-containing LC neurons of the cat are closely correlated with periods of quiet but attentive wakefulness and with phases of rapid eye movement (REM) sleeping.

Two types of experiments were performed. Five cats, some initially treated with a monoamine oxidase inhibitor to enhance neuronal NE content (*1*), were used to map neurons exhibiting catecholamine fluorescence by the Falck-Hillarp technique (*13*). The distribution of these cells was correlated with consecutive sections stained by the Nissl method and with other surveys of the cat pontomesencephalic nuclei (*14*). These studies (*15*) show that NE-containing neurons in the cat pons are distributed within the LC and locus subcoeruleus nuclei and the medial and lateral parabrachial nuclei. The NE neurons of the cat LC appeared to be more diffusely situated than the catecholamine neurons of other mammals (*1, 2, 4, 5*). Within a given section (10 to 15 µm thick), clusters of six to ten fluorescent neurons were found to be scattered throughout the LC region, although frequently separated from each other by a few hundred micrometers. We explored this region (P = 2.0 to 3.0 mm, L = 2.5 to 3.0 mm, D = −2.0 to −3.0 mm) with microelectrodes and correlated the discharge activity of single LC neurons with electroencephalogram (EEG), electromyogram (EMG), electrooculogram (EOG), and behavioral observations.

In the microelectrode recording experiments, more than 300 brainstem neurons were studied in 18 cats. Each cat showed good behavioral adaptation to the experimental chamber during the 2 to 3 weeks that followed implanta-

tion of recording macroelectrodes and a specially designed pedestal (*16*) through which a remotely controlled hydraulic microadvancer could be attached for stereotaxic exploration with microelectrodes (*17*). Epoxy-coated, stainless steel microelectrodes (tip, 2 to 3 μm) were used to record units and to make small electrolytic lesions (*18*) that marked the recording sites for either the fluorescence histochemical or the Prussian blue reactions (Fig. 1) (*19*). Although some cytological details were lost by combining these two techniques, both approaches proved necessary to establish that the patterns of unit electrical activity recorded during defined EEG stages arose from within the NE fluorescent cell groups of the LC, because the mongrel cats used in this study frequently showed considerable variability in the fine stereotaxic location of these NE-containing neurons.

For unit recording, action potentials were displayed on an oscilloscope and, simultaneously with EEG, EOG, and EMG, were recorded on a Grass polygraph and on a seven-channel magnetic tape for later data analysis. Mean discharge rates and interspike interval histograms were calculated with a PDP-12 computer. Usually only one or two satisfactorily recorded LC units could be obtained in each penetration. All of the analyzed spikes were well-isolated electrically (amplitudes, 0.4 to 3.5 mv), and exhibited stability of their spike size for 5 to 10 minutes before polygraph and tape recordings were initiated.

All neurons which did not meet these electrophysiological criteria or whose content of NE could not be supported by subsequent histochemical identification were eliminated from analysis. This left us with 24 units, each observed for more than 30 minutes during which the animal exhibited episodes of quiet, drowsy, awake behavior; slow wave sleep; and paradoxical sleep with rapid eye movements and ponto-geniculo-occipital (PGO) spikes. A subgroup of ten histochemically identified neurons were observed during these three stages plus a fourth type of behavior that was termed attentive waking. We wanted to define two waking states because of the clear-cut difference in unit and animal behavior. For instance, during quiet waking or drowsiness the cats assumed relaxed postures, usually in a crouch, paying little attention to the environment. In contrast, the cats in the attentive waking state were constantly looking around and adjusting their postures.

The discharge patterns of NE-containing LC neurons were relatively homogeneous with respect to sleeping and waking behavior. In general, LC cells were tonically active slow units except for periods of bursting discharges during paradoxical sleep. The discharge patterns of a representative unit are shown in Fig. 2. In the drowsy state, the unit fired regularly and slowly at 4.8 per second as shown by a relatively narrow distribution of interspike intervals (Fig. 2B). Slow wave sleep was associated with slightly decreased but more irregular neuronal discharge at 3.8 per second. Bursts of firing, usually associated with EEG synchronization in other types of brainstem neurons, were not frequent, and usually occurred in doublets. During paradoxical sleep, however, the neuronal discharge became irregular and was closely related to PGO monophasic waves and eye movements. Preceding the regularly occurring PGO monophasic potentials were bursts of two to five spikes, which were usually followed either by a second burst during the falling phase of the PGO waves or by a silent period of about 200 msec (Fig. 2, A3). Frequently the bursts were still associated with PGO spikes when an isolated ocular twitch did not occur simultaneously. During the REM episodes, the bursting discharges occurred in rapid successions (far right in Fig. 2, A3). The bursting is clearly described by the very short interspike intervals followed by a broader group of longer

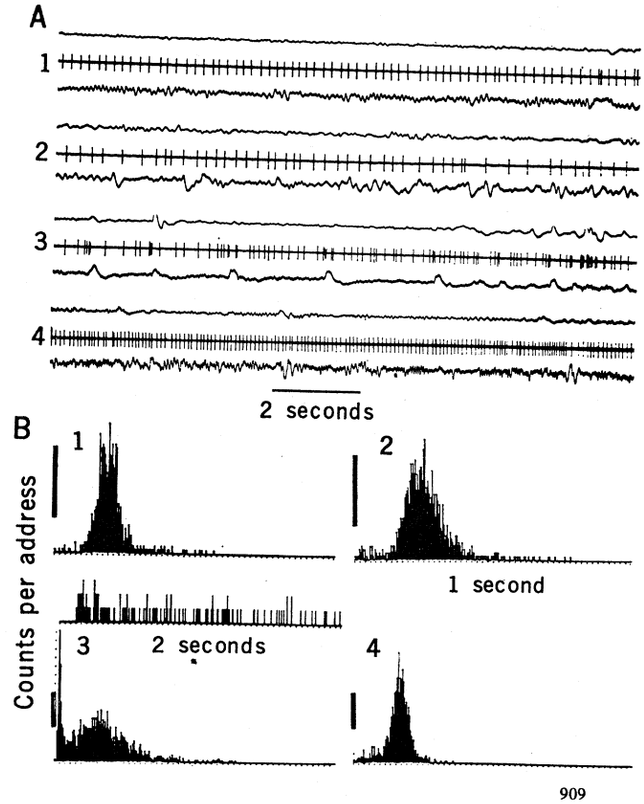

Fig. 2. Single neuron activity recorded from the LC nucleus during four behavioral states. The unit was observed for 55 minutes and the data were presented in accord with the sequence of observation. (A) Unit activity (middle trace), EOG (upper trace), and EEG (lower trace) are simultaneously shown to reveal their relation and the behavioral state. The EEG was recorded through bipolar depth electrodes in the visual cortex. Both EOG and EEG were slightly attenuated. The PGO monophasic waves are the upward deflections of 150 to 200 msec in the EEG trace that occurred regularly during paradoxical sleep; (1) denotes quiet wakefulness, (2) slow wave sleep, (3) paradoxical sleep, and (4) attentive waking state. Note that unit activity precedes and bears an almost one-to-one relation to PGO waves in paradoxical sleep. Episodes of REM's (far right in part 3) were associated with several bursts of unit spikes. In the attentive waking state, movement artifacts are seen superimposed on the low-voltage, fast activity EEG trace and eye movements are also present. (B) Interspike interval histogram for the same four periods of recording shown in (A). Upper histogram in part 3 shows the distribution of interburst intervals. Note that unit firing during paradoxical sleep is irregular and that bursts of spikes associated with PGO monophasic waves and REM episodes produce a distinct peak at the far left of the interspike internal histogram for paradoxical sleep (part 3). The histograms are composed of at least 1000 spikes. For all interspike interval histograms, the vertical bar indicates 20 counts per address and the abscissa is 1 second; for the interburst interval histogram, the bar of part 3 indicates 2 counts per address, and the entire abscissa is 2 seconds.

intervals (Fig. 2, B3). The rapid bursting during REM episodes are illustrated by the distribution of interburst intervals, showing clusters of intervals at 200 to 400 msec (upper histogram in Fig. 2, B3). In other LC cells the relation between unit bursts and PGO monophasic waves was not maintained throughout the entire period of paradoxical sleep. The frequency of occurrence for unit bursts increased approximately by a factor of 100 from slow wave sleep to paradoxical sleep. The firing rate in the attentive wakefulness phase was even higher, ranging from 6 to 12 per second.

The entire group of 24 LC units showed comparable mean discharge rate (per second) during quiet waking (4.5 $\pm$ 1.1, S.E.) and slow wave sleep (4.2 $\pm$ 1.1) (*20*). The unit activity during paradoxical sleep was higher (10.0 $\pm$ 1.3) but similar to that in the attentive waking state (11.6 $\pm$ 1.9, $N =$ 10). During transition from slow wave sleep into paradoxical sleep, 40 percent of LC neurons slightly increased their activity, whereas 60 percent of them showed either a slight decrease or no change in firing rates. During paradoxical sleep, about 60 percent of LC units exhibited bursting behavior that was closely related to PGO spikes, or eye movements, or both. The burst discharge was more commonly seen in neurons in the medial part of the LC nucleus. During transition from paradoxical sleep to waking, 37 percent of LC neurons continued to increase their firing activity, 45 percent decreased, and 18 percent did not have apparent changes. Even those neurons whose activity decreased during this transition phase still fired faster than in quiet waking and slow wave sleep. Nevertheless, differences in neuronal discharge between various sleep periods were far less obvious from comparisons of mean discharge rate than from comparisons of interspike interval histograms.

From these experiments it appears that LC neurons become relatively inactive once the cat begins to ignore its surroundings. The inactive state of LC cells in quiet waking continues to persist through slow wave sleep until the cat goes into paradoxical sleep or else awakens and becomes attentive again. The enhanced neuronal activity in the attentive waking state may be related either to the maintenance of the state (*9*) or to behaviors that occur only during that state. Unfortunately the present recording technique has not yet allowed us to correlate unit activity with other important spontaneous behaviors such as drinking, eating, exploration, aggression, or fear.

In any event, our data indicate that NE-containing LC neurons do change their discharge patterns with sleep-waking behavior, becoming more active in attentive wakefulness and exhibiting bursting discharges in paradoxical sleep.

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16. The attachment pedestal was secured stereotaxically to the posterior skull at an angle of 35° to the horizontal plane. The dura under the pedestal was removed and replaced with silastic membrane of 0.15 mm in thickness. The artificial dura made microelectrode penetrations possible even several months after the surgery. Because of the relatively large size of the pedestal, only one side of the pons was explored.
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18. By a modification of the method used by K. T. Brown and K. Tasaki [*J. Physiol. London* **158**, 281 (1961)], microscopic lesions of 40 to 60 μm in the LC nucleus were produced by passage of 1.0- to 5.0-μa d-c current for 5 seconds through a constant current stimulator. At the end of the microelectrode descent, a large lesion easily visible to the naked eyes was produced by passing 20 to 30 μa for 20 to 30 seconds. A maximum of four microelectrode penetrations was made in each cat.
19. For Prussian blue reaction and histological staining, the freeze-dried brain tissue, whose paraffin was removed, was immersed for 5 minutes in 10 percent formalin-Ringer solution which contained 3 percent potassium ferrocyanide and 3 percent potassium ferricyanide. The blue spots thus formed were counterstained for 15 to 20 minutes with 0.1 percent nuclear fast red solution or Nissl stain.
20. The statistical difference between slow wave sleep and either paradoxical sleep or attentive waking is 0.05 to 0.02 in P value (t-test), whereas the difference between quiet waking and either paradoxical sleep or attentive waking is 0.1.
21. We thank Y. Sheu for his contribution in the recording setup, B. J. Hoffer for comments on the manuscript, and S. Pekarik for secretarial assistance.

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Diazacholesterol Myotonia: Accumulation of Desmosterol and Increased Adenosine Triphosphatase Activity of Sarcolemma

Abstract. *Myotonia induced in rats by 20,25-diazacholesterol is accompanied by accumulation of desmosterol in serum, fragmented sarcoplasmic reticulum, and sarcolemma. Activities of (Na^+,K^+)- and Ca^{2+}-stimulated adenosine triphosphatases of the sarcolemma are increased, but not the Mg^{2+}-stimulated adenosine triphosphatase. The altered sterol composition of the sarcolemma may cause this type of myotonia by decreasing the chloride conductance of the membrane.*

20,25-Diazacholesterol, an inhibitor of the cholesterol synthesis at the step of the reduction of the 24-25 double bond in the side chain, induces myotonia in humans as well as in animals (*1*). The myotonia observed is similar to that of myotonia congenita and myotonic dystrophy of humans which are characterized by an impaired relaxation of skeletal muscles after contraction.

Certain features of experimental myotonia, myotonia congenita, and myotonic dystrophy suggest that a basic abnormality is located in the muscle cell membrane, which is hyperirritable and characterized by curare-resistant bursts of repetitive depolarization after mechanical or electrical stimulation [see (*2*) and (*3*)]. The accumulation of desmosterol during development of 20,25-diazacholesterol-induced myotonia in the