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ACTIVITY OF NOREPINEPHRINE-CONTAINING LOCUS COERULEUS NEURONS IN BEHAVING RATS ANTICIPATES FLUCTUATIONS IN THE SLEEP-WAKING CYCLE[1]

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Abstract

Spontaneous discharge of norepinephrine-containing locus coeruleus (NE-LC) neurons was examined during the sleep-waking cycle (S-WC) in behaving rats. Single unit and multiple unit extracellular recordings yielded a consistent set of characteristic discharge properties. (*1*) Tonic discharge co-varied with stages of the S-WC, being highest during waking, lower during slow wave sleep, and virtually absent during paradoxical sleep. (*2*) Discharge anticipated S-WC stages as well as phasic cortical activity, such as spindles, during slow wave sleep. (*3*) Discharge decreased within active waking during grooming and sweet water consumption. (*4*) Bursts of impulses accompanied spontaneous or sensory-evoked interruptions of sleep, grooming, consumption, or other such ongoing behavior. (*5*) These characteristic discharge properties were topographically homogeneous for recordings throughout the NE-LC. (*6*) Phasic robust activity was synchronized markedly among neurons in multiple unit populations. (*7*) Field potentials occurred spontaneously in the NE-LC and were synchronized with bursts of unit activity from the same electrodes. (*8*) Field potentials became dissociated from unit activity during paradoxical sleep, exhibiting their highest rates in the virtual absence of impulses.

These results are generally consistent with previous proposals that the NE-LC system is involved in regulating cortical and behavioral arousal. On the basis of the present data and those described in the following report (Aston-Jones, G., and F. E. Bloom (1981) J. Neurosci. *1:* 887-900), we conclude that these neurons may mediate a specific function within the general arousal framework. In brief, the NE-LC system may globally bias the responsiveness of target neurons and thereby influence overall behavioral orientation.

The nucleus locus coeruleus (LC) in the albino rat is a dense collection of norepinephrine (NE)-containing neurons in the dorsorostral pontine tegmentum. Previous studies have demonstrated a uniquely divergent efferent system of NE-containing LC (NE-LC) fibers, innervating the entire neuraxis (Dahlstrom and Fuxe, 1964). In particular, these neurons provide the sole NE innervation of cerebral, cerebellar, and hippocampal cortices (Ungerstedt, 1971; Pickel et al., 1974; Morrison et al., 1978).

The potential significance of this brain system is implicit in the profound clinical, behavioral, and pharmacological effects produced by agents that modify NE activity in brain. Studies in our laboratory (Foote et al., 1975; Segal and Bloom, 1976a, b) and elsewhere (Freedman et al., 1977) have shown that iontophoresis of NE or electrical stimulation of the NE-LC system increases the responsiveness of target neurons to strong or preferred stimuli while decreasing activity elicited by weak inputs, thereby enhancing "signal-to-noise" ratios in target cell impulse activity. Similar manipulations of NE produce profound metabolic changes in target areas, increasing cyclic nucleotide production and altering enzymatic activity (Siggins et al., 1971, 1972; Segal and Bloom, 1974a, b; Gahwiller, 1976).

Several related hypotheses propose that the NE-LC system is involved in initiating or maintaining stages of the sleep-waking cycle (S-WC) (Ramm, 1979; Clark, 1979; Amaral and Sinnamon, 1977; Steriade and Hobson, 1976). Electrophysiological data supporting this view have been obtained only for cat LC (Chu and Bloom, 1974a; Hobson et al., 1975; Sakai, 1980), where NE and non-NE cells are loosely interdigitated (Maeda et al., 1973; Chu and

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Bloom, 1974b; Jones and Moore, 1974). The uncertain neurochemical identity of LC neurons recorded in the cat may underlie the reported physiological heterogeneity and precludes the determination of factors specifically controlling NE neuron discharge. The study of known NE-LC neurons in unanesthetized behaving animals is an essential step in evaluating any hypothesis of NE-LC function. We chose the albino rat for our experimental subject because it appears that every neuron within the compact LC of this species contains NE (Dahlstrom and Fuxe, 1964); thus, with careful histological examination of recording sites, we confidently can report results for known NE-containing LC neurons.

The experiments described in this report were undertaken to determine spontaneous discharge characteristics of NE-LC neurons during natural sleep and waking. In later stages of these studies, observations by Kaufmann and Morrison (1981) also prompted us to investigate low frequency signals (field potentials, FPs) in our LC recordings. We report here that NE-LC neurons in unanesthetized behaving rats alter their discharge as a function of S-WC events. In addition, we found that discharge was diminished during grooming and consumption compared to the otherwise relatively high rates typical of active waking. We also report that FPs occur spontaneously in the NE-LC and typically are synchronized with bursts of unit activity from the same electrodes. In the following report (Aston-Jones and Bloom, 1981), we demonstrate that rat NE-LC neurons also respond to a variety of mild, non-noxious environmental stimuli of many modalities. We then integrate the results of these studies in a theoretical framework and propose specific functions for the NE-LC system in brain and behavioral activity.

Materials and Methods

Surgery

One hundred seventeen male albino rats (300 to 400 gm) were anesthetized with chloral hydrate (350 mg/kg, i.p.) and placed in a stereotaxic instrument using blunt ear bars. The dorsal skull was exposed and drilled to accept five to seven stainless steel jeweler's screws (0–80 threads, 1/16 inch length). Leads for recording the cortical electroencephalogram (EEG) were secured to screws implanted over ipsilateral frontal and occipital cortices, and a grounded reference lead was attached to a third skull screw. A pair of 250-μm-diameter stainless steel wires was sutured bilaterally through dorsal neck muscles to serve as electromyogram (EMG) leads.

Unit electrodes were either single, etched tungsten microelectrodes (Fredrick Haer) insulated with lacquer to provide a 2- to 20-μm length of exposed tip with 1 to 10 megohms impedance (102 rats) or an array of two to four factory-insulated stainless steel microwires (California Fine Wire), 25 or 50 μm in diameter, cut with fine scissors to expose blunt uninsulated tips (15 rats). Unit electrodes were mounted either in a threaded plastic pedestal advancer (designed to prevent electrode rotation; 77 rats) or in a remotely controlled hydraulic microdrive (Trent Wells; 40 rats). The LC was approached at a 15° caudorostral angle to spare the overlying transverse sinus. Impulse activity was monitored with an oscilloscope and loudspeaker during stereotaxic surgery to aid in localizing the LC. The plastic pedestal or hydraulic microdrive base then was anchored to the skull and screws with dental acrylic. Following surgery, animals were caged individually and allowed at least 4 days of recovery before experimental sessions.

Experimental recording environment

The first 83 rats in our studies were allowed to roam freely in a 25 × 30 cm clear Plexiglas chamber during experimental sessions, restricted only by a counterbalanced flexible cable suspended overhead from a pivoted arm to provide electrical connections and to counterweight head-mounted equipment. Thirty-four rats were connected to the same counterbalanced cable but then were suspended a few inches above the cage floor in a restraining harness which allowed free head, tail, and limb movement but prevented body reorientation. Rats were habituated to this harness for a few hours on each of 3 to 7 days before experimental sessions. This procedure resulted in negligible overt stress and restrained or freely moving subjects exhibited apparently equivalent sleep, grooming, and drinking episodes.

Recording techniques

Unit electrode signals were amplified by a head-mounted, high impedance differential preamplifier and then led through the flexible cable to filters and additional amplifiers. EEG and EMG leads were led through the flexible cable to filters and high impedance differential amplifiers. An open-ended wire was attached to the flexible cable and connected to a high impedance amplifier to generate a movement (MVT) trace. Analog unit electrode, EEG, EMG, MVT, and digitized impulse signals were stored on magnetic tape (Vetter instrumentation recorder) and also displayed on polygraph paper (Brush ink recorder), allowing on- or off-line data analysis.

Single unit (SU) and multiple unit (MU) data were obtained from 600-Hz to 10-KHz bandpass signals. Recordings were either monopolar (tungsten microelectrodes) or differential (microwires, typically 100 to 500 μm apart). SU data were accepted from uniformly superimposing spikes whose amplitudes were at least twice the noise level and exhibited less than 25% variability, allowing reliable discrimination of impulses apparently generated by only one neuron. MU criteria accepted spikes generated by approximately 2 to 10 neighboring neurons, all with amplitudes at least twice the noise level. For gating, analog signals were fed into a spike discriminator which produced digital pulses for impulse waveforms that met minimum as well as maximum voltage criteria and subsequently crossed a third voltage level within a specified time window (0.2 to 0.5 msec wide). Recordings were obtained from sites at least 100 μm apart (about 200 μm for MU data) which exhibited impulse waveforms typical of soma activity. Unfiltered, filtered, and digitized unit electrode signals were monitored continuously on a dual beam storage oscilloscope.

FP traces were 5- to 30-Hz bandpass signals recorded simultaneously with unit activity from the same electrodes. Events in these low frequency traces were scored

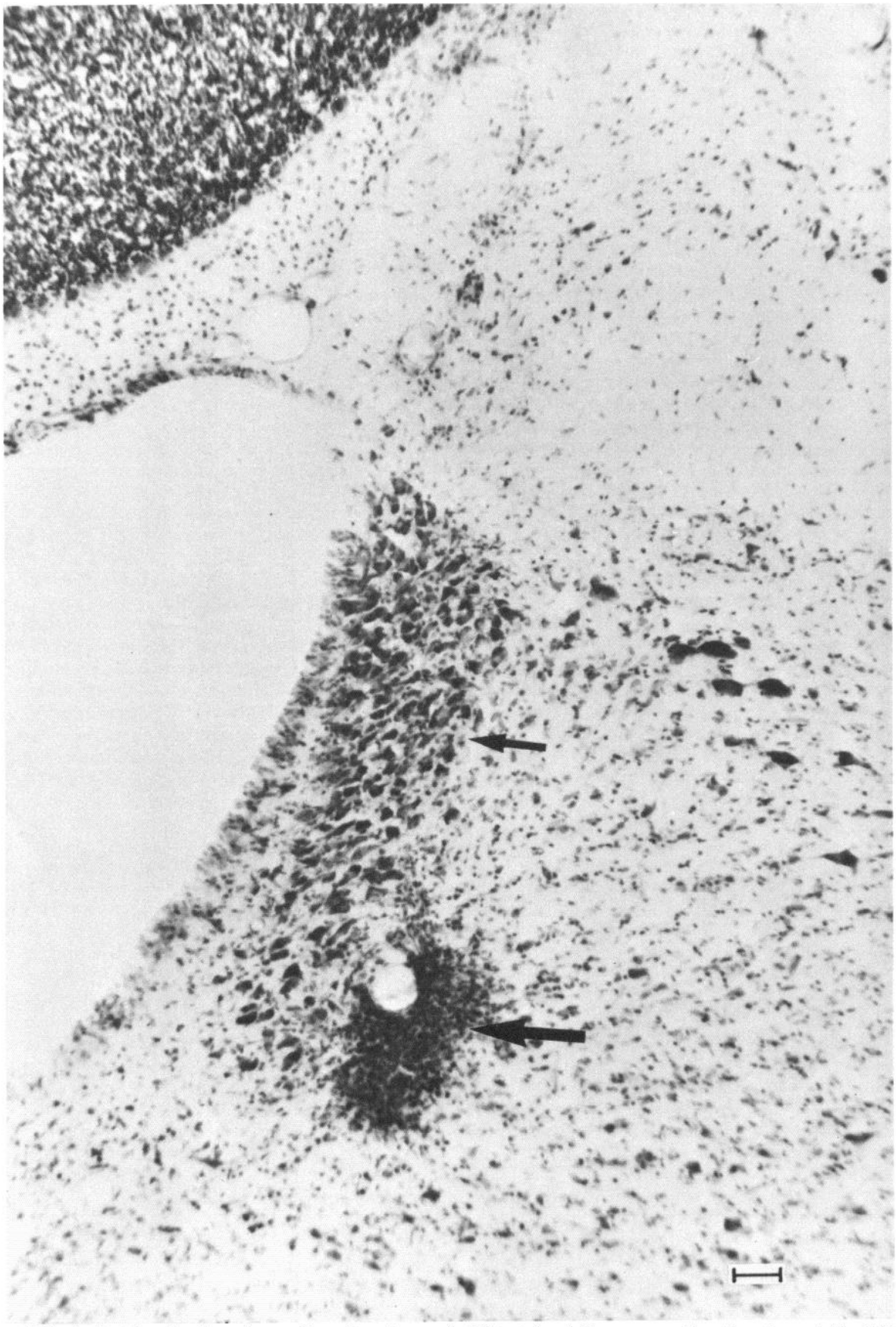

Figure 1. Histological localization of recording sites. A cresyl violet-stained 40-μm coronal section through the NE-LC (*small arrow*) from an experimental rat brain is shown. A gliotic marking lesion (*large arrow*) was made by a recording microelectrode 100 μm ventral to the point where activity characteristic of NE-LC neurons was lost. *Calibration bar* = 100 μm.

(from polygraph records) as FPs if they met the following criteria: (*1*) amplitudes at least twice the noise level; (*2*) 100 to 300 msec duration; (*3*) biphasic (negative-positive) waveforms; (*4*) discrete deflections, isolated from adjacent signals.

S-WC scoring

S-WC events were scored from polygraph records by a trained collaborator blind to unit and FP activities. Generally, the criteria of Timo-Iaria et al. (1970) were used to score five stages of the S-WC. Each stage-epoch consisted of at least 3 sec of uninterrupted activity matching one of the following descriptions (interruptions less than 3 sec in duration were not differentiated from immediately surrounding activity).

Stage I (SI, active waking). The EEG was a low amplitude, high frequency, and aperiodic signal. The EMG maintained high amplitude tonic activity with frequent phasic bursts. This stage was characterized by exploratory, orienting, or "frozen, alert" behavior.

Stage II (SII, quiet waking). The EEG was about twice the amplitude and more periodic than typical of SI and also lacked spindle activity. The EMG maintained high tonic activity but lacked phasic bursts. Behavioral observations indicated awake, relaxed animals.

Stage III (SIII, light slow wave sleep). The EEG was very periodic but variable in both amplitude and frequency, exhibiting episodes of spindle activity interspersed with shorter epochs of moderate amplitude "slow wave" activity. The EMG was generally lower in amplitude than during SI or SII with no phasic events. Animals were quiescent, with eyes at least partially closed.

Stage IV (SIV, deep slow wave sleep). The EEG was very periodic, low frequency, continuously high amplitude, and occasionally superimposed with exceptionally large spindles. The EMG maintained a low level of activity, and animals were quiescent with eyes at least partially closed.

Stage V (paradoxical sleep, PS). The EEG continuously exhibited pronounced "θ rhythm" (5 to 7 Hz, very periodic), and the EMG lacked tonic activity but contained aperiodic phasic bursts. Animals exhibited phasic twitches in limbs, facial muscles, and vibrissae while otherwise immobile.

Transitions from slow wave sleep (SIII or SIV; SWS) to waking (SI or SII; W) were scored at the onset of abrupt W (3 sec or more in duration) following at least 3 sec of SWS. PS-to-W transitions were scored at EEG θ offset.

Spindles were defined as brief (about 0.4 to 2 sec duration), discrete, highly periodic EEG epochs at least twice the amplitude of adjacent signals during SWS.

Localization of recording sites

At the end of each penetration which yielded acceptable recordings, 5 to 10 μA of cathodal current were passed for 10 to 20 sec through the unit electrode tip, creating a 50- to 200-μm marking lesion (tungsten microelectrodes) or iron deposit (stainless steel microwires). Rats with marking lesions were allowed to survive for 24 to 72 hr and then were perfused under general anesthesia with a 4% solution of paraformaldehyde. This survival period allowed gliosis in the lesion site which provided a clearer and more discrete reference mark than that obtained in freshly lesioned tissue. Rats with iron deposits were immediately anesthetized and then were perfused with a 5% solution of potassium ferrocyanide in 4% formaldehyde to produce a Prussian Blue reaction product. Frozen 40-μm-thick sections were mounted on glass slides, stained for Nissl substance, and examined with a microscope calibrated for precise distance measurements. Recording sites were determined by correlating electrode depths noted during the experiment with histological locations at corresponding distances along the electrode track from glial scar or Prussian Blue reference marks. All unit and FP data in the present report were obtained from such histologically verified sites, as illustrated in Figure 1.

The compact portion of LC was divided histologically into quadrants using criteria similar to those delineated by Grzanna and Molliver (1980). The anterior LC (LC proper) was divided into dorsal (DA) and ventral (VA) components as was the posterior pole of LC (denoted DP and VP, respectively).

SU and MU data were analyzed separately. Data were analyzed independent of the type of unit electrode (etched tungsten microelectrode or stainless steel wire), electrode advancer (plastic pedestal or hydraulic microdrive), or behavioral restraint (freely moving or harness restrained).

Results

Spontaneous discharge during stages of the S-WC. Thirty-three SU recordings were obtained from NE-LC neurons in 22 rats during spontaneously occurring stages of the S-WC. As summarized in Table I, analyses of these data revealed significantly different discharge rates for consecutive stages, with the following order: SI > SII > SIII > SIV > PS. Thus, as illustrated in Figure 2, discharge typically decreased with waning vigilance; in fact, only 2 of 33 cells exhibited any exception to the above order of discharge rates for S-WC stages. Especially striking was the consistently minimal discharge during PS; 4 of 9 SUs in the NE-LC were totally silent throughout PS episodes (mean PS duration for these 4 cells = 226.5 sec).

MU recordings yielded results qualitatively identical to those for SUs (compare Figs. 2 and 3). MU discharge consistently decreased as the S-WC progressed from SI to PS. Twenty MU recordings during 43 PS episodes yielded an average discharge rate of 0.04 ± 0.01 Hz (mean

TABLE I

Spontaneous NE-LC SU discharge rates during S-WC stages

These data show significantly different discharge rates for consecutive stages in the order: SI > SII ($p < 0.0005$, $N = 31$); SII > SIII ($p < 0.0005$, $N = 32$); SIII > SIV ($p < 0.005$, $N = 22$); SIV > PS ($p < 0.05$, $N = 9$). Paired t tests were used.

Stage	Mean Rate ± SEM	Mean Sample Time ± SEM	Number of Cells
	Hz	*sec*	
SI	2.15 ± 0.16	54.0 ± 5.7	31
SII	1.45 ± 0.14	74.0 ± 12.2	32
SIII	0.68 ± 0.12	111.1 ± 9.3	33
SIV	0.22 ± 0.05	46.6 ± 8.0	22
PS	0.02 ± 0.01	187.4 ± 32.6	9

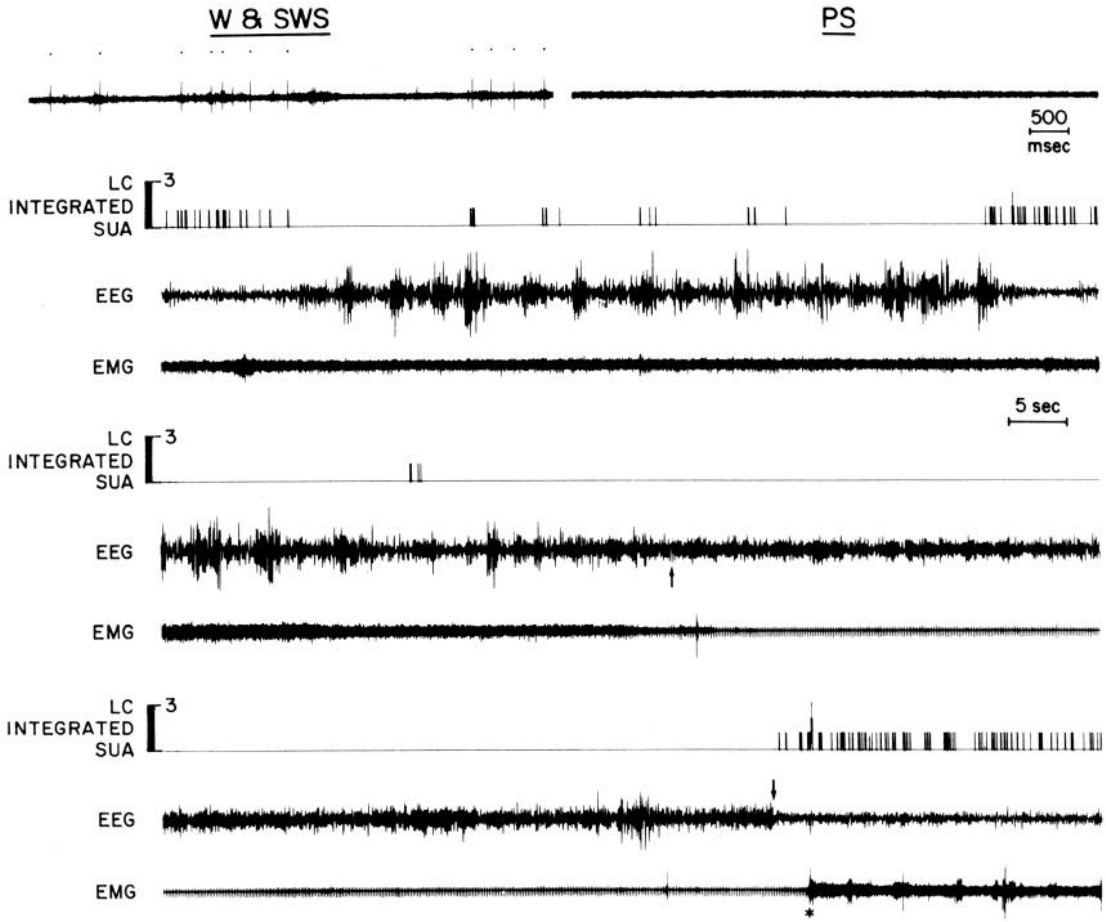

Figure 2. Spontaneous SU activity (*SUA*) during stages of the S-WC. Three epochs from one S-WC for a typical NE-LC single neuron are shown. SWS (high amplitude, low frequency, periodic EEG) contains less discharge than W (low amplitude, aperiodic EEG). Note the altered impulse activity anticipating transitions into and out of SWS and associated with EEG spindles. As is characteristic, discharge is absent during PS (onset at *up arrow*) and returns coincident with EEG-W (*down arrow*) but before EMG-W (*asterisk*). *Top panels* are analog discharge traces taken from epochs (as marked) of one S-WC. *Dots* indicate spikes meeting waveform discriminator criteria. *Upper time calibration bar* refers to *top panels*; *lower bar* refers to all other records.

± SEM); 23 of these PS epochs contained absolutely no impulse activity. Thus, even groups of neighboring NE-LC neurons often failed to discharge during PS. The rare SU or MU impulses that did occur during PS usually were associated with phasic movements or bursts of EMG activity.

Spontaneous discharge during S-WC transitions. As quantitatively demonstrated in Figure 4, S-WC progression was accompanied by characteristic changes in NE-LC activity. In both SU and MU recordings, transitions to SWS and from SWS to PS typically were anticipated by diminished discharge. Conversely, SWS-to-W transitions characteristically were preceded by a burst of impulses (see Figs. 2 and 3). The mean SU discharge rate for the second prior to W (3.35 Hz) was not only higher than for SWS overall (0.54 Hz) but also higher than the SI mean rate (2.14 Hz) ($p < 0.0005$ by paired t tests, $N = 30$ cells scored for a mean of 14.2 SWS-to-W transitions each). This agrees with our repeated observation of robust phasic discharge accompanying W onset. In contrast to all other stage transitions, however, the PS-to-W change was not anticipated by altered NE-LC discharge; rather, discharge remained depressed until EEG θ rhythm vanished and gave rise to a W signal. We also noted that, by the EEG, PS-to-W transitions often preceded, but never followed, the return of tonic EMG activity (see Fig. 4*A*). Accordingly, therefore, when scored by EMG criteria, PS-to-W transitions generally were preceded (0.5 to 2.0 sec) by SU as well as MU activity (see *insets* in Fig. 4).

Spontaneous discharge during EEG spindle activity. While analyzing activity during SWS, we noted that NE-LC neurons often discharged during EEG spindles (see Figs. 2 and 3). Closer examination revealed three consistent relationships (Fig. 5): (*1*) discharge was reduced for the second preceding spindle onsets, (*2*) discharge substantially increased during spindles, and (*3*) discharge then decreased for the second following spindle offsets but remained above average for SWS. Thus, predictable variations in NE-LC activity occurred within periods of low tonic discharge, time locked to spontaneous phasic cortical events.

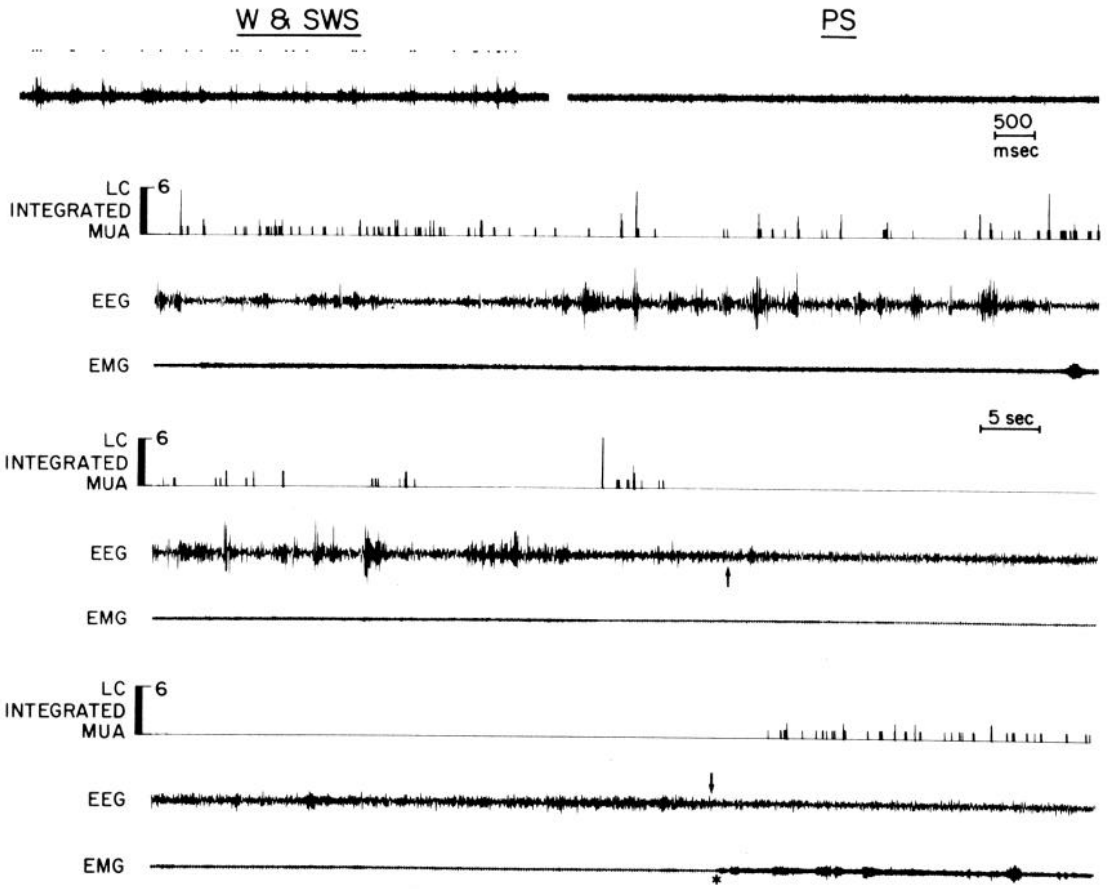

Figure 3. Spontaneous MU activity (*MUA*) during stages of the S-WC. Three epochs from one S-WC for a typical NE-LC MU recording are illustrated as in Figure 2. Note the altered discharge anticipating stage transitions (except PS to W) and in association with EEG spindles. Also note that, in this record, PS-to-W transitions in EEG and EMG activity occur at about the same time and are followed by recovery of MU activity. *Arrows* and *asterisk* are as in Figure 2.

Spontaneous discharge during waking behavior. During active waking, two behaviors were associated with decreased NE-LC activity. (*1*) Spontaneous discharge was observed to decrease during grooming episodes (in 5 of 5 recordings exhibiting good stability and negligible artifact). However, as illustrated in Figure 6, diminished discharge was not characteristic of intense motor activity per se. (*2*) Decreased discharge also was observed in 9 of 10 SU and 5 of 5 MU recordings during voluntary consumption of a 5% aqueous glucose solution. This relationship is quantitatively documented in the following paper (Aston-Jones and Bloom, 1981).

Conversely, other waking behaviors were associated consistently with increased NE-LC discharge: bursts of impulses accompanied orienting, startle, awakening, and other responses to sudden interruption of ongoing behavior. Similar movements in the absence of such abrupt changes in behavioral state did not correspond to altered NE-LC discharge (see Fig. 7).

Field potentials. Low frequency components of unit electrode signals from the NE-LC contained spontaneous, large (150- to 500-μV), biphasic (negative-positive) FPs. During SWS and W, FPs typically were time locked to spontaneous unit activity simultaneously recorded from the same electrodes as seen in Figure 8. In contrast, during PS, a marked dissociation between FPs and unit activity was evident so that the highest tonic rate for FPs occurred in the virtual absence of impulses (shown for one sample recording in Fig. 9). Also, FPs during PS fluctuated substantially in size, often exhibiting smaller amplitudes than during SWS or W; otherwise, FP waveforms were similar in all S-WC stages.

Topographical specificity of discharge properties. In order to detect possible topographical distinctions within the NE-LC, each histologically confirmed SU recording was assigned to its corresponding quadrant of the nucleus (see "Materials and Methods"). Spontaneous discharge rates generally varied little by quadrant; however, VP neurons tended to fire more slowly during W than other NE-LC neurons (Table II).

Some topographical specificity also was found for neurons located near an edge of the NE-LC (less than about 50 μm either inside or outside of the boundaries of the compact nucleus). Edge neurons discharged significantly faster during W than cells located more centrally; firing rates during SWS and PS exhibited similar tendencies but failed to yield statistically significant differences (see Table III).

Whereas 35 of 42 (83%) NE-LC SU recordings yielded predominantly slow tonic spontaneous discharge, only 14

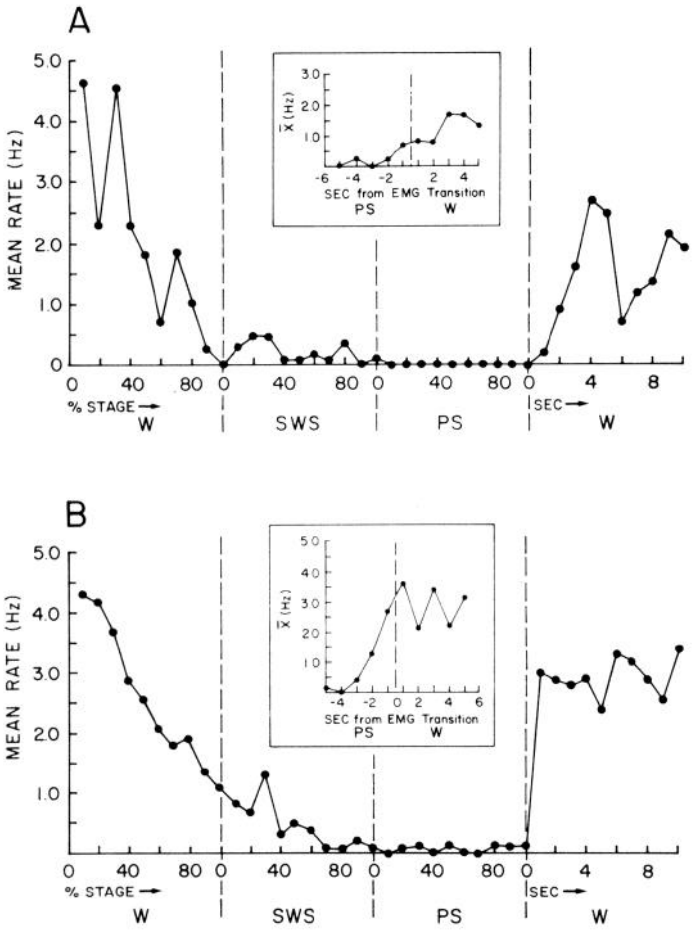

Figure 4. NE-LC discharge rate during S-WC progression. Mean discharge rates for 6 SU (*A*) and 9 MU (*B*) recordings during epochs normalized for the percentage of S-WC stage completion are plotted consecutively for complete S-WCs (mean rates for W following PS are plotted in 1-sec epochs and are not normalized relative to other data). Note that when PS-to-W transitions are judged by the EEG (*main plots*), cellular activity does not anticipate the transition; however, enhanced discharge does anticipate these same transitions scored by EMG criteria (*insets*).

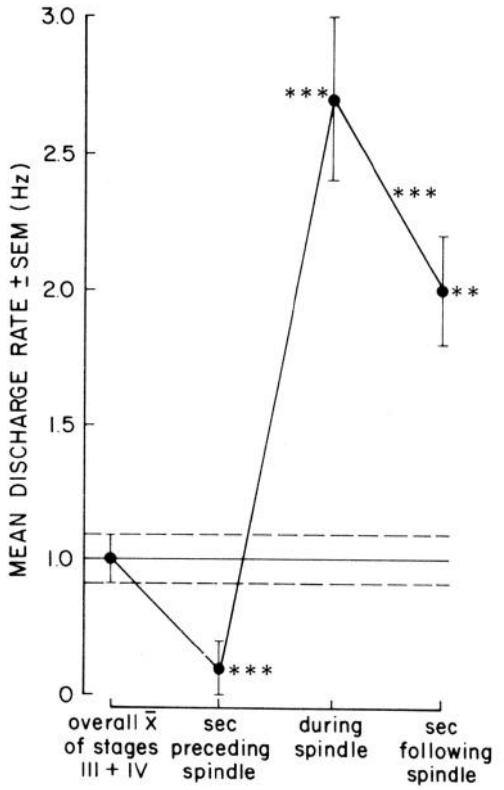

Figure 5. NE-LC discharge during EEG spindles. Mean SU discharge rate during SWS overall is compared to epochs immediately preceding, during, and immediately following EEG spindles. Corresponding rates differ as follows: 1-sec epochs preceding spindles < SWS overall (***$p < 0.0005$), spindle epochs > SWS overall (***$p < 0.0005$), 1-sec epochs following spindles < spindle epochs (***$p < 0.0005$) but > SWS overall (**$p < 0.005$); $N = 28$ cells; paired t tests were used.

of 91 (15%) non-LC pontine cells (50 to 2000 μm distant) exhibited similar activity. As shown in Table IV, the NE-LC also yielded a higher proportion of neurons whose mean discharge rates were greater during W than during SWS and were slowest during PS compared to other nearby pontine sites.

Discussion

This study has revealed a set of characteristic properties for NE-LC neurons not established in previous work. Corresponding attributes were observed on a broad time scale ranging from transient cortical events to tonic behavioral states. Although NE-LC neurons discharged in a slow tonic manner overall, mean rates changed significantly as a function of naturally occurring stages of the S-WC. Tonic discharge was highest during waking, slower within SWS, and nearly absent preceding and throughout PS. These alterations in discharge anticipated transitions into and out of SWS but were approximately simultaneous with waking after PS. In addition, there was a systematic triphasic relationship between NE-LC activity and EEG spindles during SWS: discharge decreased before spindle onset, increased substantially during spindling, and subsequently declined after spindle offset. Thus, predictable changes in NE-LC discharge anticipated phasic, as well as tonic, spontaneous cortical EEG signals.

Consistent fluctuations in NE-LC activity also were observed for certain waking behaviors. Discharge decreased during grooming and consumption of sweet water; in contrast, bursts of impulses accompanied orienting responses to spontaneous or sensory-evoked interruptions of such ongoing behaviors. NE-LC discharge was not found to vary with any specific isolated motor act but only with organized patterns of movements forming holistic behaviors such as orienting, grooming, or consumption.

We also observed spontaneous, low frequency long duration events (FPs) in NE-LC recordings. During W and SWS, these FPs typically were synchronized with bursts of impulses from the same electrodes. Interestingly, however, FPs exhibited their highest tonic rates during PS, in the virtual absence of impulses. Consistent with the possibility that NE-LC activity was involved in generating these FPs, cells in MU populations fired in

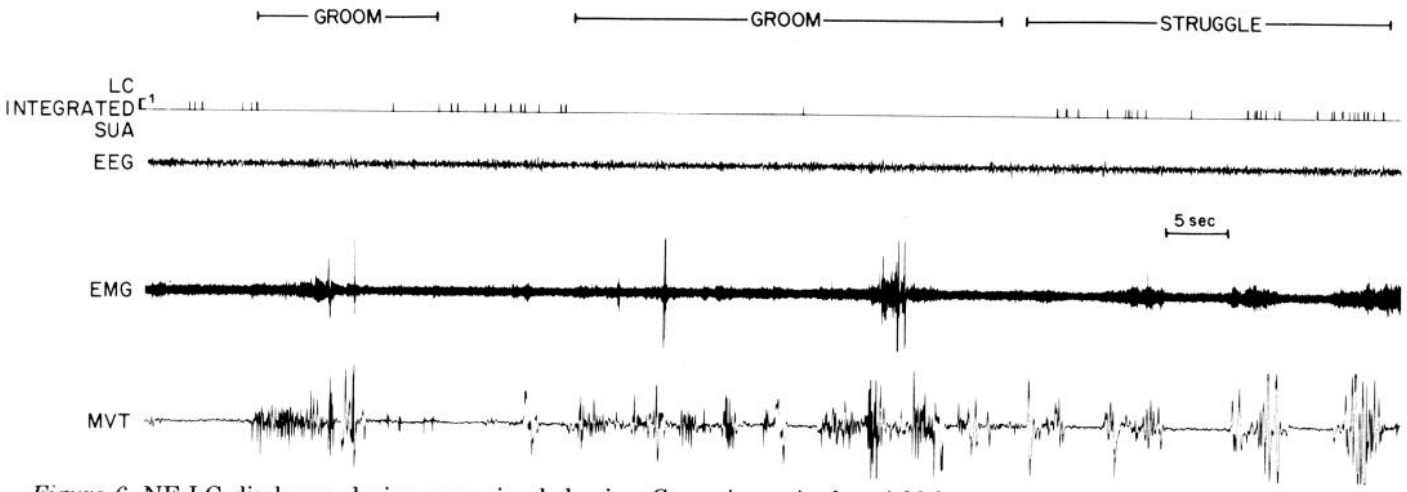

Figure 6. NE-LC discharge during grooming behavior. Grooming episodes yield less spontaneous discharge than adjacent epochs of active waking, including hand-held struggling. *SUA*, SU activity.

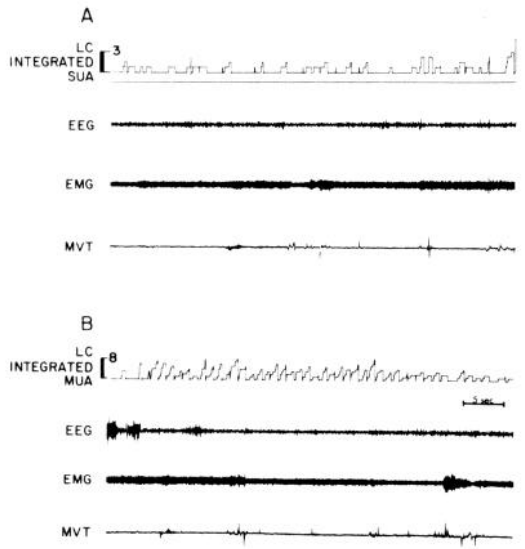

Figure 7. NE-LC discharge during motor activity. Illustrated in *A* for SU recording and in *B* for MU recording, discharge does not correspond to intense movement per se (see in *EMG* and *MVT traces*). Note that bursts of impulses sometimes occur before, after, or during movements. *SUA*, SU activity; *MUA*, MU activity.

marked synchrony during phasic epochs of robust discharge, and discharge characteristics were predominantly uniform throughout the nucleus. From these results, we conclude that NE-LC neurons may function homogeneously as a group. Considering the present data overall, we propose that this system may serve to facilitate transitions between global behavioral states.

Previous reports of LC discharge during the S-WC, all in the cat, have indicated a physiologically heterogenous cell population. Chu and Bloom (1974a, b) concluded that the majority of LC neurons (in recordings localized near a group of fluorescent cell bodies) exhibited phasically intense discharge during PS. Hobson et al. (1975), on the other hand, reported that most of their LC recordings yielded "REM-off" cells, neurons that virtually ceased discharging prior to and during PS. These two studies were performed in a species (cat) whose LC contains interdigitated NE and non-NE neurons, precluding neurochemical identification of recorded cells. This anatomical heterogeneity may explain the corresponding physiological heterogeneity in cat LC recordings. Our results, obtained in a species whose LC permits the study of NE-containing neurons specifically, indicate that NE-LC neurons homogeneously exhibit a characteristic set of discharge properties. The strong similarities between the present results and those for cat REM-off LC cells support previous suggestions (McCarley and Hobson, 1975; Jones et al., 1979; Foote et al., 1980) that this latter subpopulation represents NE-LC neurons in that species. Additional support for this proposal is provided by more recent cat studies (Sakai, 1980), reporting that the percentages of REM-off cells in different LC areas were directly proportional to the corresponding densities of fluorescent cell bodies. Moreover, experiments in our laboratory (Foote and Bloom, 1979; Foote et al., 1980) have shown that the discharge of known NE-LC neurons in behaving squirrel monkeys during W and SWS resembles that described here for behaving rats. Thus, the present results may reveal discharge properties common to NE-LC neurons in many species.

As well as demonstrating many properties previously reported for REM-off neurons in cat LC, the present study revealed an interesting characteristic of NE-LC discharge during PS-to-W transitions not described in studies of cat LC. Although discharge of corresponding cells in both species anticipated the return of tonic EMG activity following PS, additional observations in the rat disclosed that increased discharge did not precede the onset of characteristic waking signals in the EEG. This result contradicts the previous proposal (McCarley and Hobson, 1975) that NE-LC discharge serves to terminate PS episodes; in the rat at least, other brain activity precedes or coincides with the return of NE-LC discharge at the end of PS. Therefore, although the NE-LC may have a role in terminating peripheral atonia, we conclude that discharge in these neurons is regulated by systems more directly responsible for the control of PS phenomena. As the most apparent change in rat EEG during this transition is the loss of θ rhythm, probably generated by

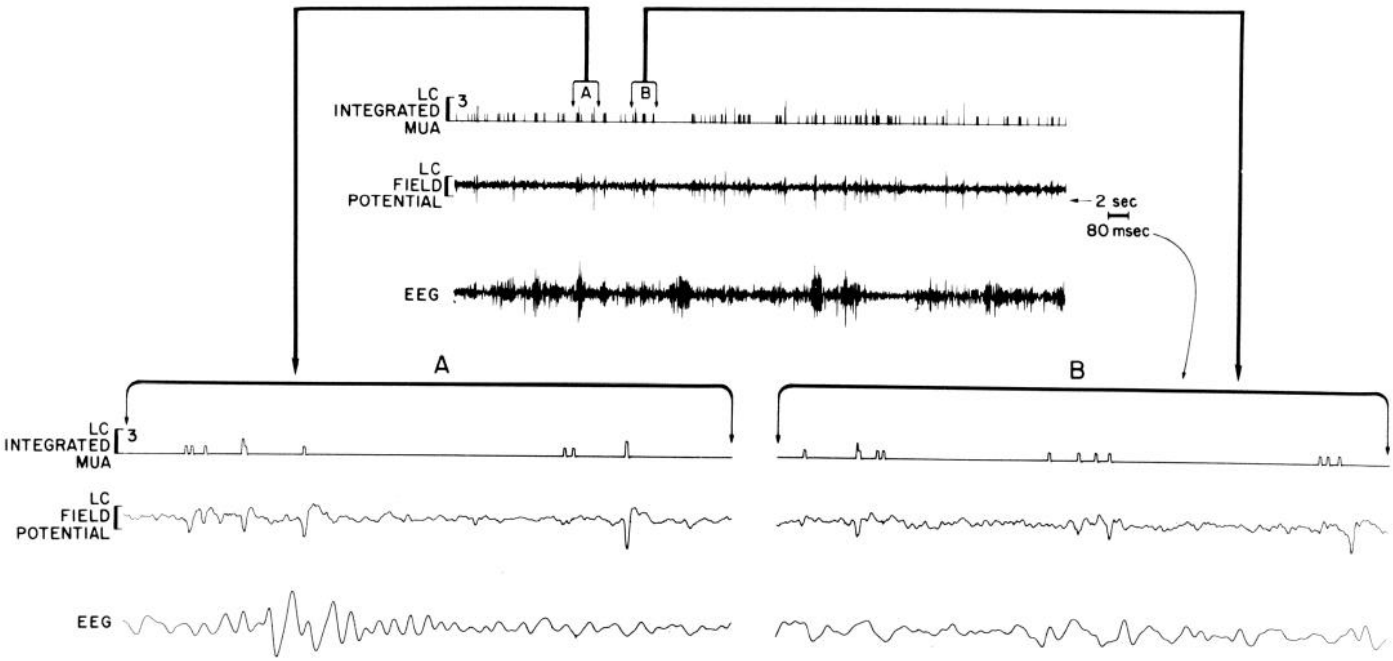

Figure 8. Spontaneously occurring FPs synchronized with unit activity in the NE-LC. The *upper set of traces* illustrates tonic spontaneous activity. *Bracketed epochs* are presented at a higher time magnification in the *lower sets of traces*. Note the temporal synchrony between FPs and phasically increased unit activity apparent in high resolution records. Differential recordings are separated into FP and unit traces as described under "Materials and Methods." FP calibration = 100 μV. *MUA*, MU activity.

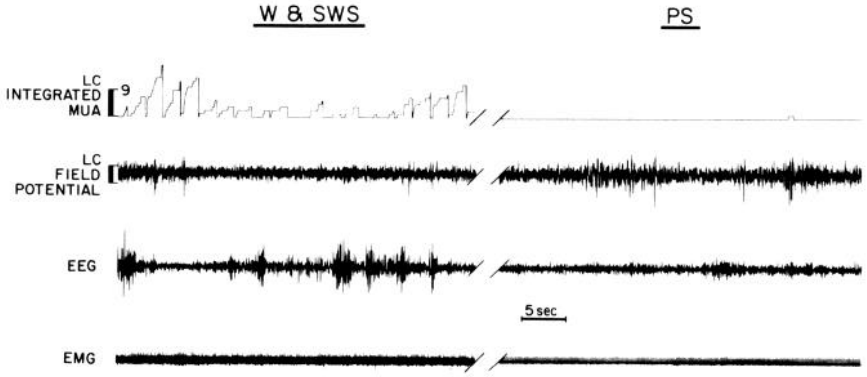

Figure 9. Spontaneously occurring FPs and unit activity during W and SWS versus during PS. Two epochs from one S-WC for an NE-LC MU recording are shown, illustrating concurrent FPs and impulses during W and SWS and their dissociation during PS. Recordings are as in Figure 8. FP calibration = 100 μV. *MUA*, MU activity.

TABLE II

Spontaneous SU discharge rates in NE-LC quadrants during S-WC stages

Rates (mean ± SEM) for the indicated number of cells examined in each quadrant. Mean rates for S-WC stages do not differ significantly across quadrants ($p > 0.1$ by one-way analyses of variance). However, the mean W (SI or SII) rate for VP neurons is less than the corresponding rate for the other quadrants ($p < 0.05$ by t tests).

LC Quadrant	Stage				
	SI	SII	SIII	SIV	PS
			Hz		
DA	2.15 ± 0.28 ($N = 11$)	1.67 ± 0.29 ($N = 12$)	0.88 ± 0.27 ($N = 12$)	0.13 ± 0.04 ($N = 3$)	0.04 ± 0.01 ($N = 3$)
VA	2.29 ± 0.32 ($N = 4$)	1.50 ± 0.22 ($N = 4$)	0.73 ± 0.30 ($N = 4$)	0.14 ± 0.09 ($N = 2$)	($N = 0$)
DP	2.41 ± 0.30 ($N = 11$)	1.46 ± 0.19 ($N = 11$)	0.48 ± 0.09 ($N = 11$)	0.24 ± 0.07 ($N = 8$)	0.01 ± 0.01 ($N = 3$)
VP	1.47 ± 0.32 ($N = 5$)	0.83 ± 0.29 ($N = 5$)	0.30 ± 0.09 ($N = 5$)	0.11 ± 0.03 ($N = 5$)	0.00 ± 0.00 ($N = 3$)

TABLE III

Spontaneous NE-LC discharge rates during S-WC stages for SUs localized near an edge of LC versus those located centrally

Rates (mean ± SEM) for the indicated number of cells are listed for each group. The mean W (SI or SII) rate for edge cells is greater than non-edge cells ($p < 0.005$ by t tests). Neither the mean SWS (SIII or SIV) nor the PS rates differ significantly ($0.05 < p < 0.1$ by t tests).

LC Recording Location	Stage				
	SI	SII	SIII	SIV	PS
			Hz		
Edge	2.68 ± 0.25 ($N = 12$)	1.81 ± 0.22 ($N = 12$)	0.78 ± 0.22 ($N = 13$)	0.28 ± 0.10 ($N = 11$)	0.02 ± 0.01 ($N = 6$)
Non-edge	1.82 ± 0.19 ($N = 19$)	1.23 ± 0.17 ($N = 20$)	0.62 ± 0.14 ($N = 20$)	0.15 ± 0.03 ($N = 10$)	0.00 ± 0.00 ($N = 3$)

TABLE IV

Proportions of pontine SUs with spontaneous discharge during the S-WC in the order characteristic of NE-LC neurons

Number of cells qualitatively exhibiting order per number of cells examined. The right column summarizes observations for cells recorded through complete S-WCs; the center column gives data for cells studied only during W and SWS.

Recording Location	W > SWS	W > SWS > PS
LC	24/24	9/9
Pons, 50–250 μm from LC	2/6	1/3
Pons, 300–2000 μm from LC	2/6	0/4

hippocampal structures located near the neocortical surface, the distinction between brain and peripheral events marking PS-to-W transitions may be observed in other species most easily with in-depth hippocampal EEG recordings.

The present data are consistent with the popular view that the NE-LC system participates in controlling cortical and behavioral arousal. However, our results may help to discriminate among several specific hypotheses within this general arousal framework. From lesion studies in the cat, Jouvet (1972) concluded that, in addition to maintaining tonic behavioral and cortical arousal, NE-LC neurons may be executive elements for PS, so that this stage would be critically dependent upon their robust discharge. However, in the present study, NE-LC neurons markedly decreased their discharge prior to and throughout PS, directly opposite to the pattern required for hypothetical neurons which execute PS phenomena. Our results support the proposal of McCarley and Hobson (1975) that NE-LC neurons play a critical, but permissive, role in the generation of PS, enabling that state by virtually ceasing to discharge.

The consistent anticipation of most tonic EEG periods by NE-LC discharge in both the rat and the monkey (Foote et al., 1980) indicates that this system may participate in adjusting cortical activity, yielding heightened cortical arousal subsequent to robust discharge. The altered discharge preceding and accompanying EEG spindles in the rat is consistent with this possibility, indicating that the NE-LC system also may influence phasic fluctuations in cortical activity. Thus, decreased NE-LC discharge may enable cortical spindling some 500 to 1000 msec later, whereas increased discharge during spindles may aid in spindle termination. Therefore, spindles could be viewed as brief reductions in cortical arousal, perhaps partially resulting from changes in NE-LC discharge.

PS provides a prominent exception to the typical relationship between NE-LC activity and cortical arousal. Previous studies have established that the cortical EEG of many species during PS resembles that of aroused waking. We found that, during this "paradoxical" state, NE-LC neurons emitted virtually no impulses, in marked contrast to their activity during aroused waking and to discharge typical of most other central nervous system (CNS) neurons during PS.

Another notably unique feature of NE-LC activity during PS is the dissociation that occurs between FPs and unit discharge. In all other stages of the S-WC, phasic unit activity typically accompanied FPs, much like the relationship between FPs and unit activity elsewhere in nervous tissue (Steriade and Hobson, 1976). This relationship is consistent with the classical interpretation that such low frequency long duration events reflect extracellular currents produced by synchronous responses in a nearby group of neighboring cells. Thus, concerted depolarization in many NE-LC neurons may generate a local current sink, resulting in a negative FP deflection; conversely, synchronous hyperpolarization may yield a positive deflection. We postulate that the dissociation between FPs and unit activity during PS results from a strong tonic inhibition of NE-LC neurons preventing soma discharge. The robust FP activity during PS may reflect intense concerted excitatory postsynaptic potentials in NE-LC dendrites and somas similar to those reported for other CNS neurons during PS, while the cells do not reach firing thresholds due to simultaneous active inhibitory input. Therefore, NE-LC neurons may be influenced by inhibitory afferents similar to those generating strong tonic inhibition of motoneurons during PS (Chase, 1980). This proposed mechanism suggests a CNS correlate of peripheral atonia and implies that NE-LC discharge is incompatible with PS.

Varying levels of tonic activity in such inhibitory afferents could underlie certain present results for waking and SWS as well. Decreased NE-LC discharge during grooming and consumption may reflect enhanced afferent inhibition associated with those waking behaviors; similar tonic inhibition may serve to dampen NE-LC excitability during SWS. In addition, the following paper (Aston-Jones and Bloom, 1981) demonstrates that sensory-evoked responses in the NE-LC vary in intensity as a function of behavioral state, similar to spontaneous activity reported here. Thus, inhibitory afferents may modulate phasic as well as tonic excitability of NE-LC neurons.

Previous concepts of NE-LC function (Ramm, 1979; Clark, 1979; Amaral and Sinnamon, 1977; Steriade and

Hobson, 1976) have focused on tonic regulatory roles, emphasizing mean discharge levels over long time intervals. In the present study, significant phasic fluctuations in NE-LC discharge consistently accompanied awakening, EEG spindling, and orienting behavior and was indicated further by pronounced FP activity. As described in the accompanying paper (Aston-Jones and Bloom, 1981), NE-LC neurons also exhibit robust phasic activity in response to mild sensory stimuli. Thus, one predominant feature of the set of characteristic properties for NE-LC neurons is their phasic alterations in discharge. We propose (Aston-Jones and Bloom, 1981) that, in addition to any possible roles in tonic regulation, the NE-LC system operates phasically, facilitating transitions between global CNS modes and behavioral states.

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