THE BEHAVIORAL PHYSIOLOGY OF LOCUS COERULEUS NEURONS

Thesis by

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"This is dedicated to the one I love."

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ABSTRACT

Factors controlling discharge of known norepinephrine-containing locus coeruleus (NE-LC) neurons were studied in unanesthetized behaving rats, and these neurons' efferent impulse conduction properties were examined in anesthetized rats. Single-unit (SU) and multiple-unit (MU) extracellular recordings in unanesthetized preparations demonstrated the following: (1) Tonic discharge co-varied with stages of the sleep-waking cycle (S-WC), being highest during waking (W), lower during slow-wave sleep (SWS), and virtually absent during paradoxical sleep (PS). (2) Altered discharge predictably anticipated S-WC stages as well as phasic cortical activity such as spindles during SWS. (3) Discharge was reduced 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 behaviors. (5) Discharge was not linked to movement *per se*. (6) Field potentials (FPs) occurred spontaneously in NE-LC recordings, temporally synchronized with bursts of unit activity from the same electrodes during W and SWS, but at highest rates during PS, when discharge was virtually absent. (7) Short-latency (15-50 msec), transient, biphasic unit responses and synchronous FPs were predictably evoked by non-noxious auditory, visual and somatosensory stimuli; individual recordings typically exhibited similar response patterns for each sensory modality. (8) The magnitudes of sensory-evoked response varied as a function of vigilance, such that largest responses occurred for stimuli which awakened animals and least responsiveness was exhibited during uninterrupted sleep. (9) Sensory responsiveness also decreased during

grooming and sweet water consumption. (10) Transiently reduced discharge occurred in response to gustatory stimulation accompanying voluntary consumption of sweet water. (11) SU and MU recordings throughout the nucleus yielded remarkably homogeneous results. (12) Robust phasic discharge was markedly synchronized among neurons in MU populations.

SU recordings of spontaneous and antidromic NE-LC impulse activity in anesthetized rats indicated the following: (1) Impulse conduction velocity fluctuated as a function of basal conduction latency, impulse rate, and number of impulses in a train of activity. (2) Impulse conduction velocity increased briefly, then exhibited a more pronounced, gradual decrease during the same train of activity. (3) Large increases in conduction latency occurred during low-frequency trains of impulse activity. (4) Calculations indicated that these axons may modulate their own impulse flow as a result of ion fluxes associated with spike propagation.

These results are interpreted in light of previous data on the postsynaptic physiology of norepinephrine to indicate that robust activity in the NE-LC system may participate in terminating CNS and behavioral processes which have minimal value in coping with phasic external events, and simultaneously enhance activity within systems primarily concerned with such immediate responses. Conversely, low levels of spontaneous or sensory-evoked NE-LC discharge may enable tonic, endogenously generated vegetative behaviors to proceed. In this way, the NE-LC system may bias global behavioral orientation between the external and internal environments.

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INTRODUCTION

1. Objective

During the past several years, many approaches have been used to analyze the functions of the pontine nucleus locus coeruleus (LC). Anatomical studies, based initially on the technique of Falck and Hillarp (1), have demonstrated a uniquely divergent efferent organization (2) in which fibers from this relatively small number of norepinephrine (NE)-containing neurons project widely throughout the CNS and provide the sole NE input to neo- and paleo-cortices (3,4,5). The potential physiological manifestions of such a ubiquitous brain system utilizing NE as its transmitter agent are implicit in the extensive clinical, behavioral, and pharmacological effects produced by agents which modify the action of NE. Indeed, physiological studies (for example, 6 and 7) have characterized an array of postsynaptic effects of this system. More recent studies (8,9,10,11) have shown that iontophoresis of NE or electrical stimulation of LC can increase signal-to-noise ratios in target cell impulse activity. Similar manipulations of NE levels in LC projection areas produce profound metabolic changes in target neurons, increasing cyclic nucleotide production and altering enzymatic activity (12,13,14). These findings suggest a pervasive function for this system in brain and behavior.

A global hypothesis underlying the present studies, derived from the above data, states that LC discharge helps orient CNS activity towards salient stimuli in the external environment. However, knowledge of the

factors controlling these cells' physiologically normal impulse activity is essential in evaluating any hypothesis of their function. Because a relative paucity of such information has existed, a large number of confusingly diverse hypotheses persist with no unifying theme among them.

The purpose of these investigations was to determine environmental and behavioral factors that influence discharge of known norepinephrine-containing locus coeruleus (NE-LC) neurons, and to examine the propagation of physiological patterns of impulse activity along their axons. Discharge rates and patterns of these neurons were recorded extracellularly in unanesthetized behaving, as well as in anesthetized, rats. Studies in unanesthetized animals sought to measure the relative potencies of the following variables in determining NE-LC activity: a) spontaneously-occurring stages of the sleep-waking cycle (S-WC), b) sensory stimuli of many modalities, c) specific behaviors and motor acts, and d) location within the NE-LC. Anesthetized animals were used to analyze conduction properties of NE-LC axons for patterns of impulse activity characteristic of unanesthetized behaving animals. These studies have provided crucial evidence concerning the physiological function of these neurochemically identified neurons whose postsynaptic influences are anatomically extensive and unusually well characterized.

2. Background

This section summarizes previous neuroanatomical and neurophysiological data concerning possible determinants of LC neuronal activity. Neurophysiological studies reviewed here include only those that have examined discharge of individual LC neurons as a function of sensory or electrical stimulation, pharmacological manipulation, behavioral activity, or vigilance. Other types of information such as behavioral pharmacology of LC or behavioral changes following LC lesions are not reviewed. These approaches may eventually yield hypotheses testable at the cellular level in behaving animals; however, as the following review demonstrates, many more rudimentary straightforward questions must first be answered.

Anatomy: Afferents to LC. Data from three neuroanatomical techniques indicate that locus coeruleus neurons receive an extremely rich array of inputs. First, in two studies (15,16) HRP was injected into LC and the locations of retrogradely labelled cells determined. According to these reports, several forebrain and brainstem sites project into, or adjacent to, LC. Areas containing substantial numbers of labelled cells in the rat included the bed nucleus of the stria terminalis, central nucleus of the amygdala, several hypothalamic areas, central grey, and the lateral reticular nucleus; labelled neurons were also observed in the fastigial nuclei and the marginal zones of the spinal dorsal horns. Second, "terminal" labelling within or adjacent to LC has been reported following injections of radioactively labelled amino acids into various telencephalic and brainstem nuclei, including the ventromedial nucleus of the

hypothalamus (17), nucleus cuneiformis (18), dorsal raphe nucleus (19), preoptic area (20,21), and anterior hypothalamus (22). Third, immunocytochemical techniques have revealed reactive "terminals" within or adjacent to LC for β-endorphin (23), Substance P (24), tryptophan hydroxylase (25), and phenylethanolamine-N-methyltransferase (26). Except for projections from contralateral NE-LC (16), caudal NE (16,27) and epinephrine (26) cell groups, and midbrain raphe (16,25), these data do not yet overlap sufficiently to permit combined anatomical and histochemical characterization of LC afferents.

Physiology: Anesthetized and Paralyzed Preparations. NE-LC neurons in anesthetized rats discharge spontaneously in a slow tonic fashion (28-34, 36-40). The only sensory stimuli reported to affect this pattern are strongly noxious; painful pressure applied to the tail or paws evoke biphasic (excitatory-inhibitory) responses in discharge (28). Mild body strokes, bright flashes, or loud auditory stimuli fail to reliably elicit responses (28, unpublished observations). Although most of these studies have used chloral hydrate anesthesia (28-37), there have also been a few using urethane anesthesia (38-40), gallamine-induced paralysis (32,35), or halothane anesthesia (unpublished observations). The basal firing rate of NE-LC neurons varies with these different treatments: Mean rates of 1.1 Hz, 2.6 Hz, 6-30 Hz and 2.4 Hz were seen with chloral hydrate, urethane, gallamine and halothane, respectively; increased sensitivity to somatosensory stimuli was reported for unanesthetized paralyzed preparations (35). Electrical stimulation of peripheral (vagal, splanchnic and sciatic nerves) and central (bed nucleus of the stria terminalis,

olfactory bulb, preoptic region, ventromedial nucleus of the hypothalamus, ventral tegmentum, and central grey) sites has been reported to produce converging, orthodromic excitatory and inhibitory influences onto NE-LC neurons (30,40).

NE-LC neurons have been antidromically activated by electrical stimulation in many of their target areas (30,38-41). This technique not only helps identify these cells, but also permits analysis of various physiological properties. NE-LC axons in rat conduct impulses slowly, at a velocity of .4 - 1.3 m/sec (30,38-40). Antidromic stimulation reliably elicits inhibitory responses at slightly longer latencies than driven spikes, even in cases where the recorded cell is not one that is antidromically activated. This post-excitatory pause in discharge, similar to that seen in response to orthodromic electrical or noxious peripheral stimulation, has been pharmacologically altered in a manner consistent with recurrent collateral inhibition among NE-LC neurons (30).

Numerous studies have described effects of various pharmacological agents on NE-LC discharge in anesthetized rats. Systemically administered amphetamine (29,32), morphine (35), clonidine (34,37), or tricyclic antidepressants (36) slow discharge rates, while systemic piperoxane, an alpha-adrenergic antagonist, increases impulse activity (28). More direct tests indicate that iontophoretic NE, epinephrine, isoproterenol, GABA (28), clonidine (28,37), and opiates (33) typically reduce firing in NE-LC neurons, while iontophoretically delivered piperoxane (28), Substance P (31), and acetylcholine (33) increase activity.

Data such as the above on NE-LC discharge in anesthetized animals have led some investigators to propose that this system is primarily involved with pain, anxiety or fear (42-44). The pharmacological result that NE-LC neurons are sensitive to opiates is consistent with such a conclusion, while the other drug treatments lead to no clear physiological or behavioral hypotheses.

Physiology: Unanesthetized, Nonparalized Animals. There have been few published studies of LC discharge in nondrugged animals: Three laboratories have reported LC discharge rates as a function of the sleep-waking cycle in cat, and there has been one report on LC neuronal activity in behaving monkeys.

Hobson and McCarley and collaborators (46,47) reported that a majority of cells in the cat LC region exhibited the distinctive property of lower discharge rates during paradoxical sleep (PS) than during waking or slow-wave sleep (SWS). These cells exhibited slow regular discharge during waking, reduced activity during SWS, and only occasional impulses during PS. However, other LC cells did not possess this "REM-off" property, and some REM-off cells were located outside LC. Hobson and McCarley have not reported on LC discharge during the waking state as a function of vigilance or sensory stimulation.

Sakai (48) reported on 108 REM-off neurons in the cat LC region. Cytoarchitectonic areas containing many NE-fluorescent neurons exhibited higher percentages of REM-off cells than areas containing few NE neurons; however, many REM-on cells were recorded in the same areas as REM-off

cells. No description was given for REM-off neurons during waking, except that their mean discharge rate = 0.5 - 2.5 Hz.

The third description of impulse activity in cat LC was by Chu and Bloom (49,50). They attempted to identify NE neuron recordings by creating microlesions or Prussian Blue spots at recording sites and processing the tissue for catecholamine fluorescence. If a marking lesion or spot was within a cluster of fluorescent neurons, that particular recording was considered to have originated from an NE-containing neuron. They found that only one-fourth of such neurons exhibited their slowest rates during PS, and they also observed similar cells in non-NE recording sites. The remaining three-fourths of this cell population exhibited substantial discharge during PS, with some cells phasically active while others were tonically active. A subset of their neurons yielded greater activity during active waking (i.e., vigilant surveillance of the environment) than during quiet (i.e., non-attentive) waking.

The study in monkeys (41) primarily sought to determine whether LC neurons were antidromically activated from forebrain sites which supported self-stimulation. In this study, LC cells had slow (5 $\pm$ 3 Hz) discharge rates during quiet waking, while subcoeruleus cells exhibited greater activity (15 $\pm$ 2 Hz). Discharge was reported unchanged during operant responding for apple sauce reward, and activity was not studied during sleep. The accuracy of recording site-localizations is difficult to evaluate for this study since in the two monkeys used, 46 and 40 penetrations were made over periods of 6 and 2 months, respectively. Both the large number of penetrations and the long interval between recording

and sacrifice would seem to render accurate histological reconstruction impossible.

In summary, previous studies have demonstrated that there are REM-off cells in the cat LC region whose distribution is roughly the same as that of NE-containing neurons. However, the neurochemical heterogeneity in cat LC (where NE and non-NE neurons are interdigitated) prevents confidently ascribing REM-off properties to NE neurons specifically. In addition, there has been no systemic study of LC discharge during waking as a function of vigilance changes, sensory stimulation, or behavior.

3. Rationale

In this section, the major problem areas in the literature are identified, and the general experimental approach used in the present studies to clarify important issues is described.

Problems and questions raised by the literature. There are at least four basic, unresolved problems in the literature: 1) The type and level of anesthetic, or lack of it, appears to substantially alter fundamental discharge properties of NE-LC neurons. This prevents any useful extrapolations about the activity of these cells in anesthetized animals to physiologically normal contexts. 2) Studies in nondrugged animals have not incorporated convincing verification that any particular recordings were generated by NE neurons. This makes the physiological heterogeneity of cat LC difficult to interpret: It could reflect heterogeneity among NE cells, between NE cells and non-NE cells, or among non-NE cells. 3) There have been no systematic studies of the effect of sensory stimulation or behavior

on the discharge of LC neurons in awake behaviorally responsive animals. 4) NE-LC axon conduction properties have not been studied using patterns of impulse activity similar to those characteristic of unanesthetized behaving animals.

General experimental strategy. To surmount these problems, the present studies incorporated the following features: 1) All recordings were from albino rat, a species whose compact LC is composed entirely of NE neurons. Each recording site was histologically localized. Data from recording sites within LC, therefore, were considered to have been obtained from neurochemically identified NE-LC neurons. 2) Recordings were obtained from unanesthetized behaving, as well as anesthetized, animals. 3) The effects of many different stimuli and behaviors on NE-LC activity were systematically assessed. 4) NE-LC axon conductivity was studied for patterns of impulse activity found in unanesthetized behaving animals.

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Chapter 1 (In press, J. Neurosci.)

Title: Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle.

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INTRODUCTION

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; Siggins et al., 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 Bloom, 1974b; Jones and Moore, 1974). The uncertain neurochemical identity of LC neurons recorded in 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 unanaesthetized 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 can confidently 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 unanaesthetized 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 spontaneously occur in the NE-LC, typically 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.

METHODS

Surgery: One hundred seventeen male albino rats (300-400g) 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 5 to 7 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 MΩ impedance (102 rats), or an array of 2 to 4 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^{o} 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 was then anchored to 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 x 30 cm clear plexiglass 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 later rats were connected to the same counterbalanced cable, but were then 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 - 10 KHz bandpass s signals. Recordings were either monopolar (tungsten microelectrodes) or differential (microwires, typically 100-500 μm apart). SU data were accepted from uniformly superimposing spikes whose amplitudes were at least twice 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. 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 (0.2-0.5 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 continuously monitored on a dual-beam storage oscilloscope.

FP traces were 5 Hz-30 Hz bandpass signals recorded simultaneously with unit activity from the same electrodes. Events in these low frequency traces were scored (from polygraph records) as FPs if they met the following criteria: (1) amplitudes at least twice 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 "theta rhythm" (5 Hz-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-theta 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 μm to 200 μm marking lesion (tungsten microelectodes) or iron deposit (stainless-steel microwires). Rats with marking lesions were allowed to survive for 24 to 72 hours and then 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 perfused with a 5% solution of potassium ferrocyanide in 4% formaldehye 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 Fig.1.

The compact portion of LC was histologically divided 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 pedestial 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 1, 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 Fig. 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. 20 MU recordings during 43 PS episodes yielded an average discharge rate of 0.04 $\pm$ 0.01 Hz (mean $\pm$ 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 were usually associated with phasic movements or bursts of EMG activity.

Spontaneous discharge during S-WC transitions: As quantitatively demonstrated in Fig. 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, were typically anticipated by diminished

discharge. Conversely, SWS-to-W transitions were characteristically preceeded by a burst of impulses (see Figs. 2 and 3). Mean SU discharge rate for the sec prior to W (3.35 Hz) was not only higher than for SWS overall (0.54 Hz), but also higher than SI mean rate (2.14 Hz) ($p < .0005$ 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, PS-to-W was not anticipated by altered NE-LC discharge; rather, discharge remained depressed until EEG theta rhythm vanished, and gave rise to a W signal. We also noted that, by the EEG, PS-W transitions often preceeded, but never followed, the return of tonic EMG activity (see Fig. 4A). Accordingly, therefore, when scored by EMG criteria, PS-to-W transitions were generally preceeded (0.5 to 2.0 sec) by SU as well as MU activity (see inserts 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 sec preceeding spindle onsets; (2) discharge substantially increased during spindles; (3) discharge then decreased for the sec 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.

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 Fig. 6, diminished discharge was not characteristic of intense motor activity per se. (2) Decreased discharge was also 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 consistently associated 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 500μV), 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 Fig. 8. In contrast, during PS a marked dissociation between FPs and unit activity was evident, such 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 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 2).

Some topographical specificity was also found for neurons located near an edge of the NE-LC (less than about 50 μm either inside or outside 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 3).

Whereas 35 of 42 (83%) NE-LC SU recordings yielded predominantly slow tonic spontaneous discharge, only 14 of 91 (15%) non-LC pontine cells (50 to 2000 μm distant) exhibited similar activity. As shown in Table 4, the NE-LC also yielded a higher proportion of neurons whose mean discharge rates were greater during W than during SWS, and 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 preceeding 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 were also 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 were typically

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 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 brain and behavioral states.

Previous reports of LC discharge during the S-WC, all in 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 rat disclosed that increased discharge did not preceed 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 rat at least, other brain activity preceeds 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 theta rhythm, probably generated by 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 cat, Jouvet (1972) concluded that, in addition to maintaining tonic behavioral and cortical arousal, NE-LC neurons may be executive elements for PS, such 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 rat and 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 preceeding and accompanying EEG spindles in rat is consistent with this possibility, indicating that the NE-LC system may also 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. Spindles could

thus 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 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 EPSPs 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. Thus, NE-LC

neurons may be influenced by inhibitory afferents similar to those generating strong tonic inhibition of motorneurons 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 further indicated 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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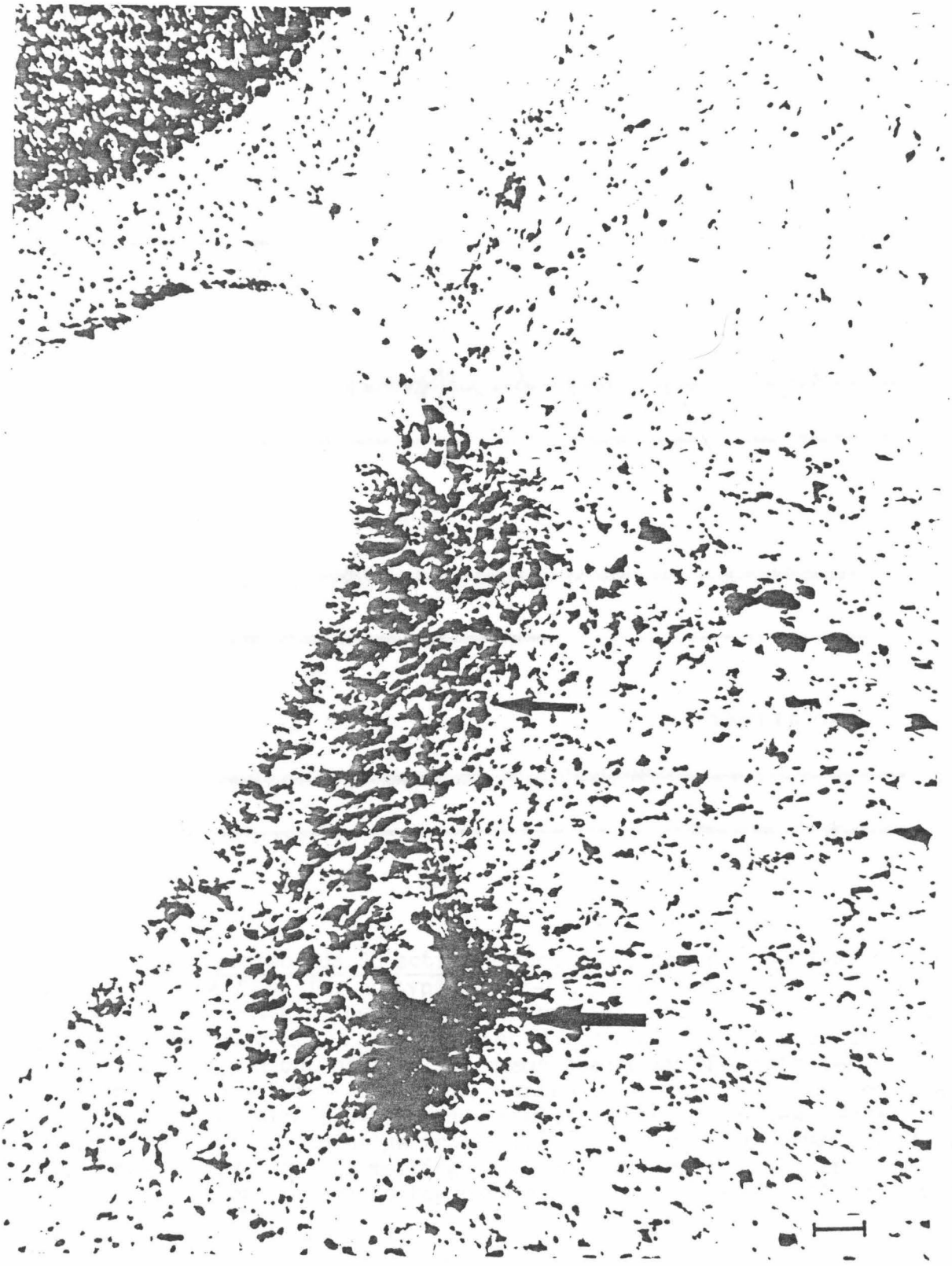

Fig. 1: Histological localization of recording sites. Cresyl Violet-stained 40 µm coronal section through LC (small arrow) in an experimental rat brain. Glia-filled marking-lesion (large arrow) was made by recording microelectrode 100 µm ventral to losing activity characteristic of NE-LC neurons. Calibration bar = 100 µm.

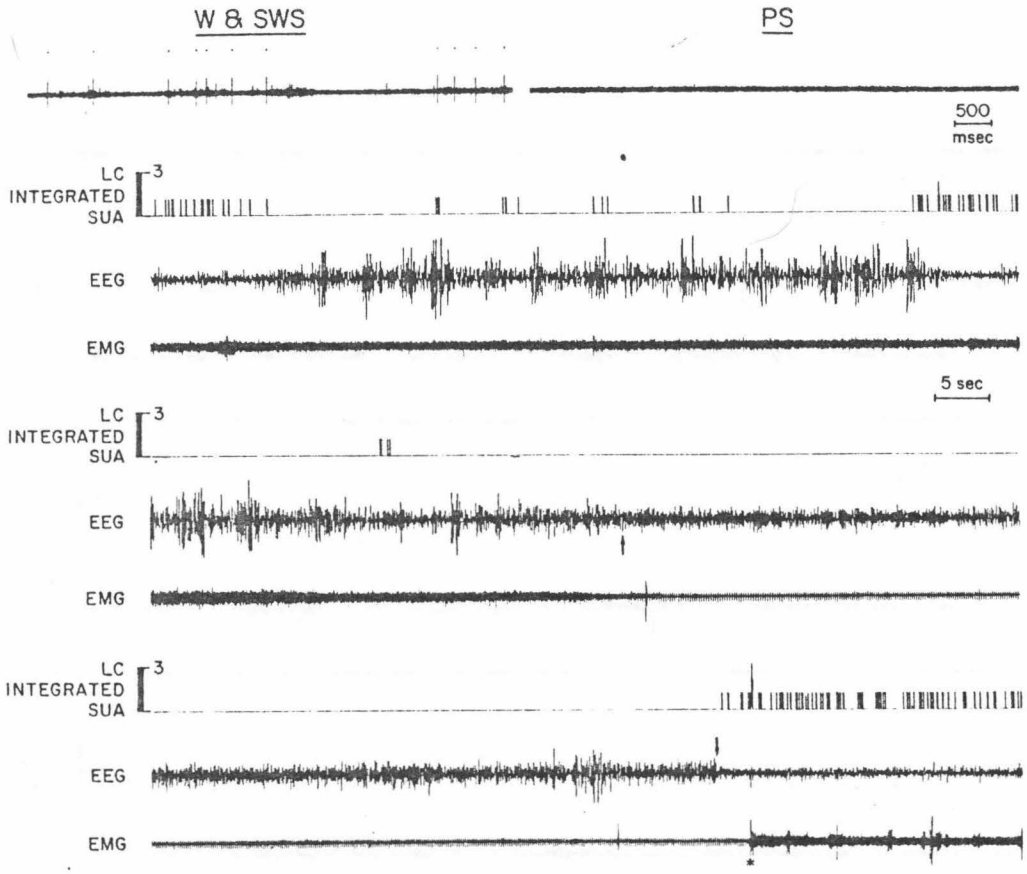

Fig. 2: Spontaneous SU activity during stages of the S-WC. Three epochs from one S-WC for a typical NE-LC single neuron. SWS (high amplitude, low frequency, periodic EEG) contains less discharge than W (low amplitude, aperiodic EEG). Note altered impulse activity anticipating transitions into and out 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 (asterick). 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.

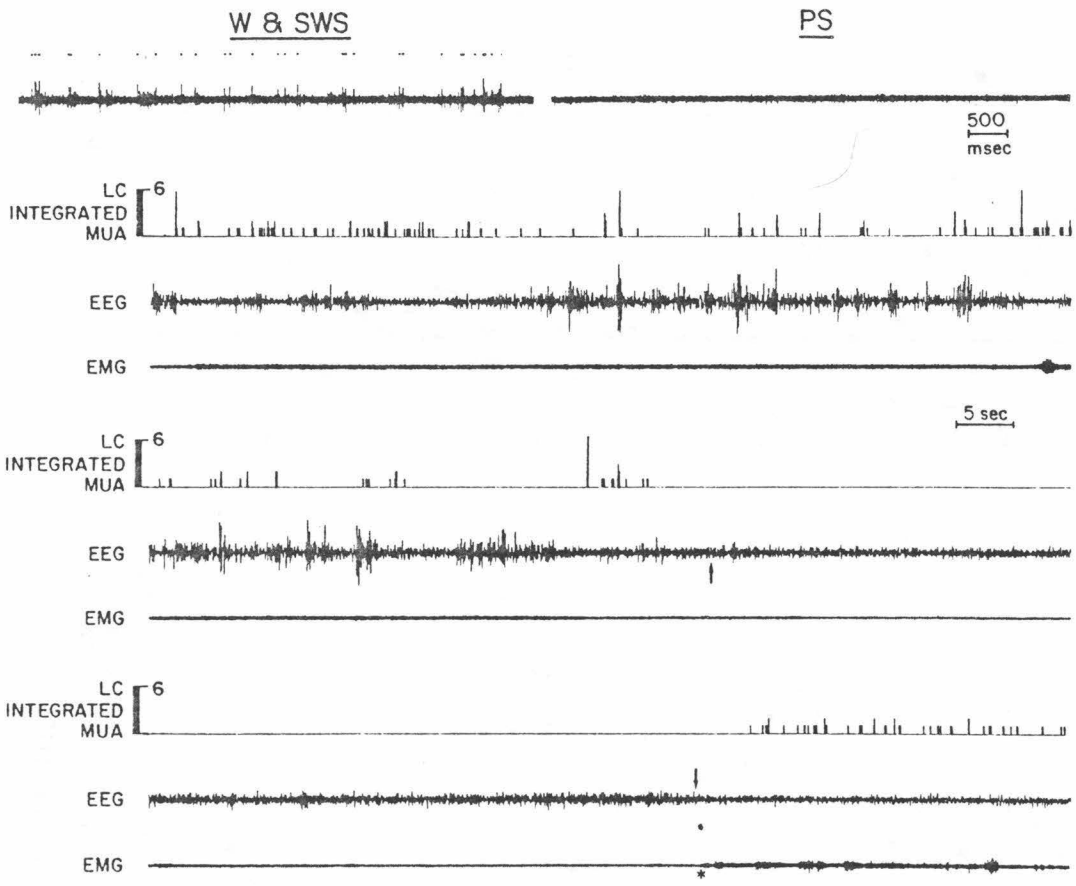

Fig. 3: Spontaneous MU activity during stages of the S-WC. Three epochs from one S-WC for a typical NE-LC MU recording, illustrated as in Fig. 2. Note altered discharge anticipating stage transitions (except PS-to-W), and in association with EEG spindles. Also note that, in this record, PS-to-W transitions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

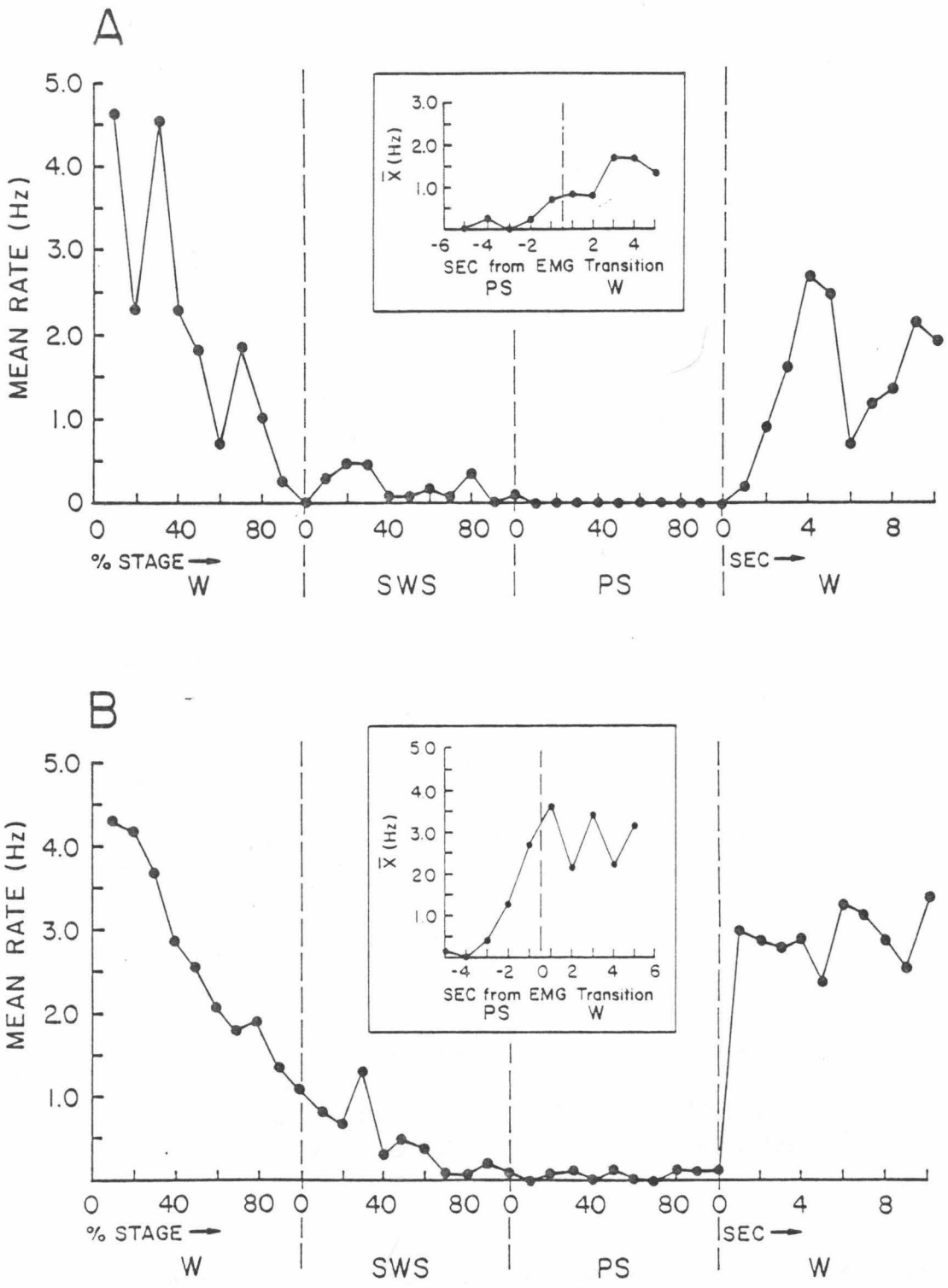

Fig. 4: NE-LC discharge rate during S-WC progression. Mean discharge rates for 6 SU (A) and 9 MU (B) recordings during epochs normalized for percentage of S-WC stage completion, plotted consecutively for complete S-WCs (mean rates for W following PS are plotted in 1 sec epochs, not normalized relative 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 (inserts).

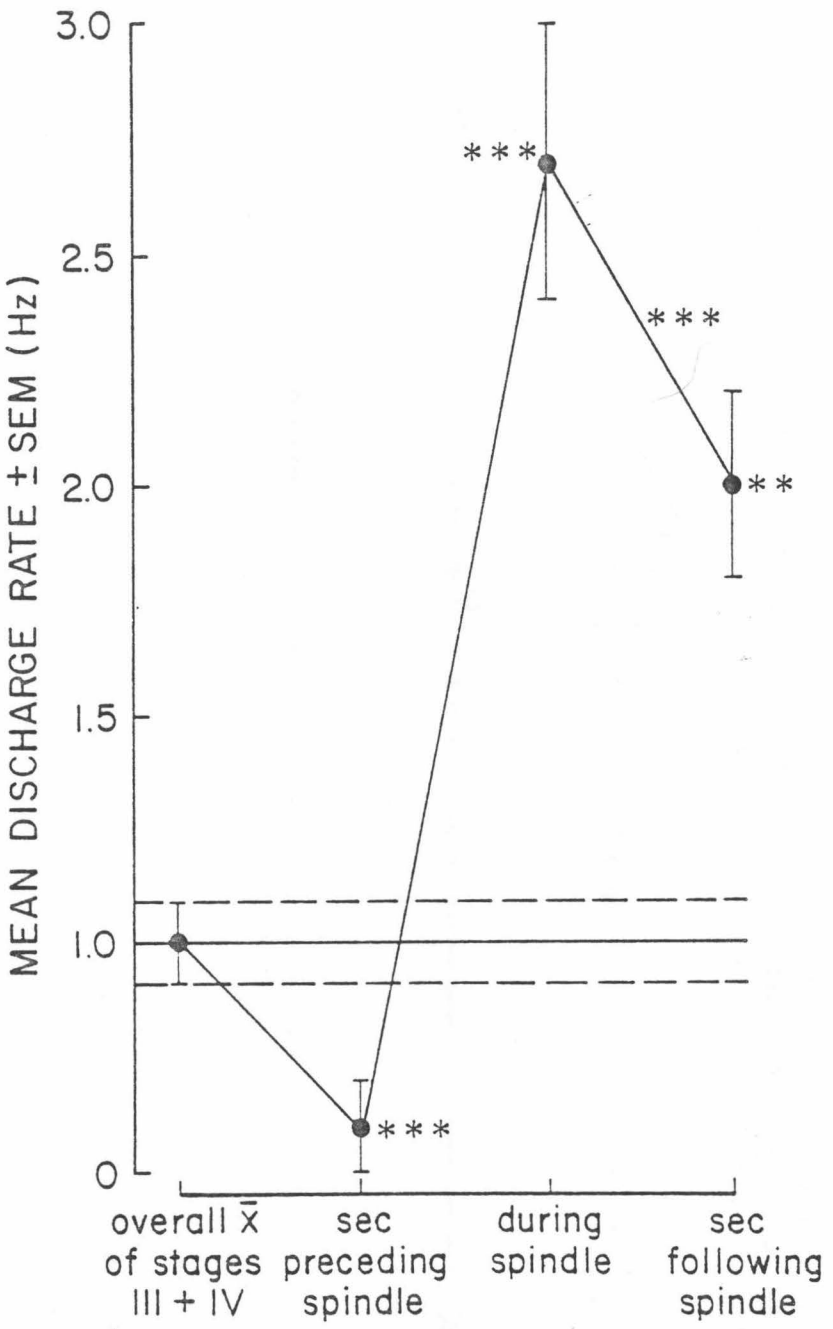

Fig. 5: NE-LC discharge during EEG spindles. Mean SU discharge rate during SWS overall compared to epochs immediately preceeding, during, and immediately following EEG spindles. Corresponding rates differ as follows: one sec epochs preceeding spindles < SWS overall (***p < .005), spindle epochs > SWS overall (***p < .0005), one sec epochs following spindles < spindle epochs (***p < .0005), but > SWS overall (**p < .005); N = 28 cells; paired t-tests.

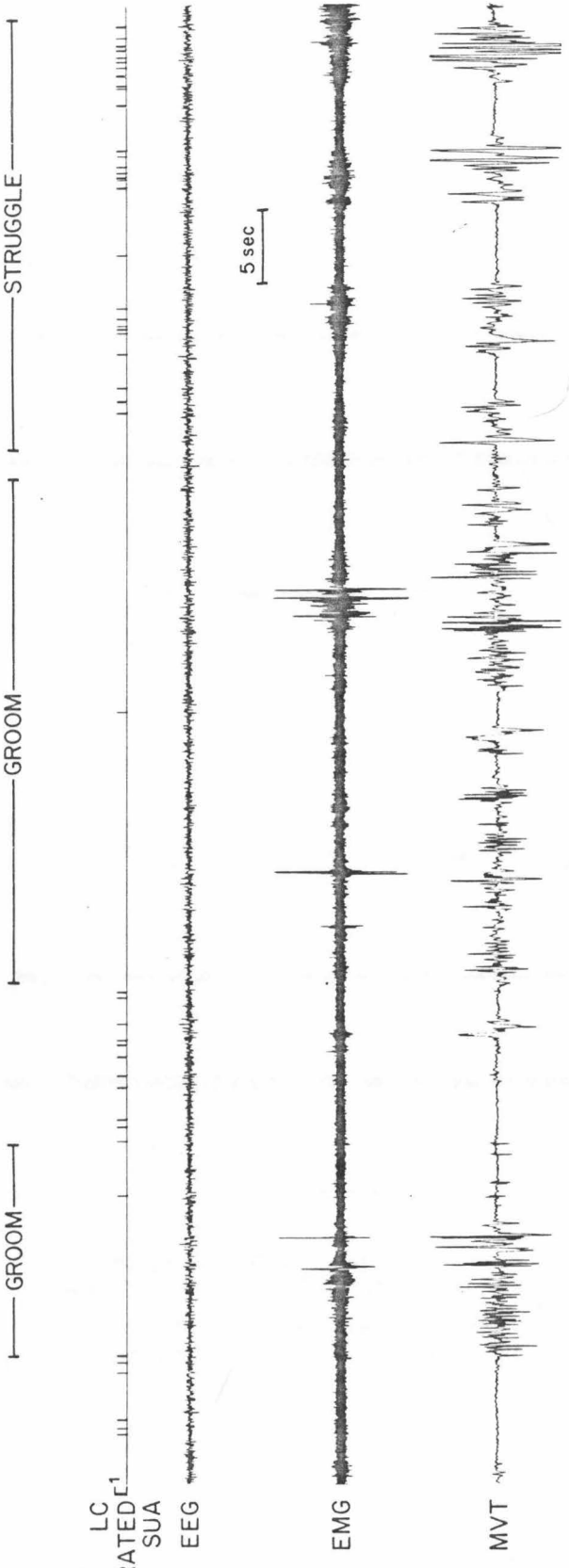

Fig. 6: NE-LC discharge during grooming behavior. Grooming episodes yield less spontaneous discharge than adjacent epochs of active waking, including hand-held struggling.

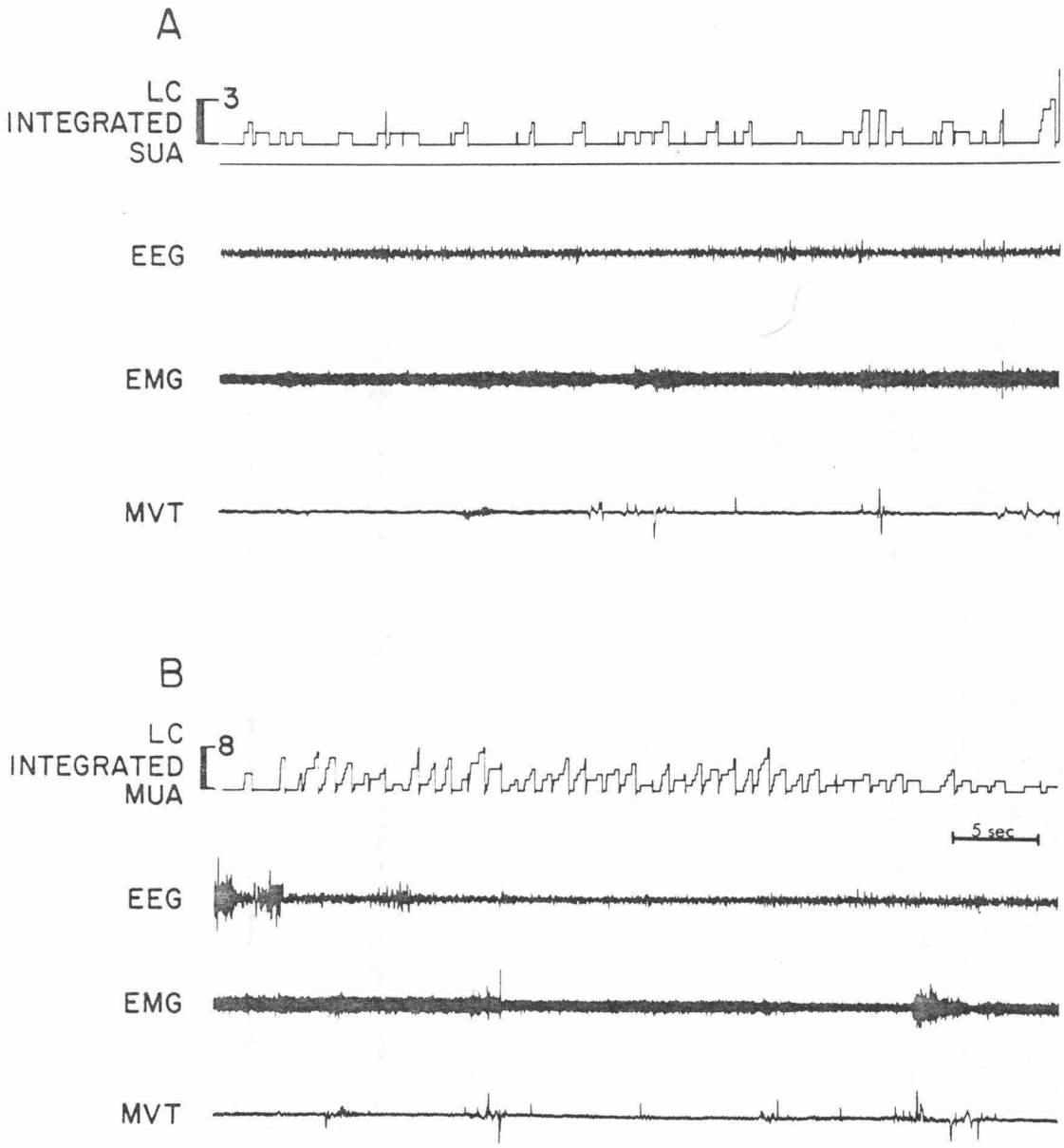

Fig. 7: NE-LC discharge during motor activity. Illustrated in (A) for SU recording, and in (B) for MU recording, discharge does not correspond to intense movement per se (seen in EMG and MVT traces). Note that bursts of impulses sometimes occur before, after, or during movements.

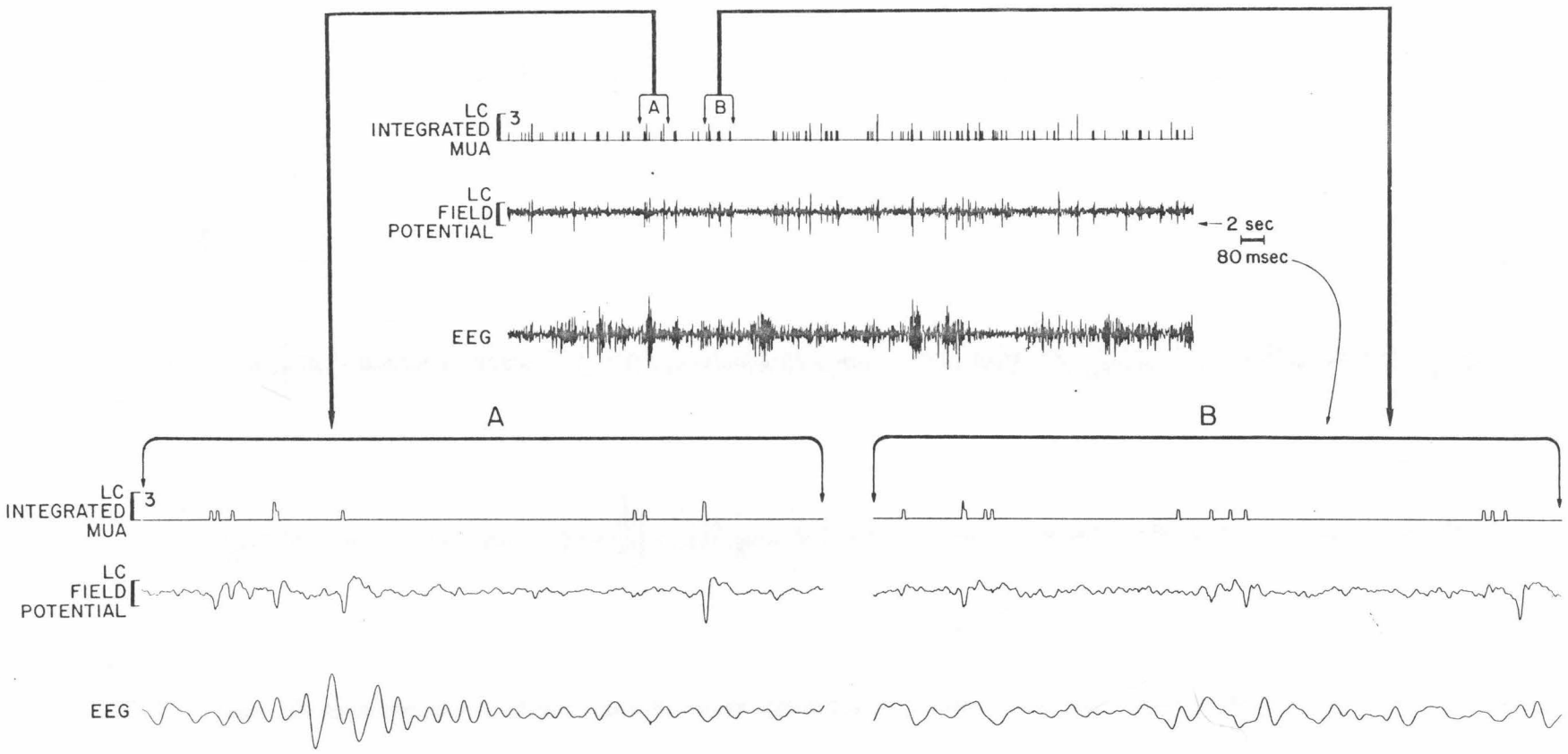

Fig. 8: Spontaneously occurring FPs synchronized with unit activity in the NE-LC. Upper set of traces illustrates tonic spontaneous activity. Bracketed epochs are presented at higher time magnification in lower sets of traces. Note temporal synchrony between FPs and phasically increased unit activity apparent in high resolution records. Differential recordings, separated into FP and unit traces as described in Methods. FP calibration = 100μ V.

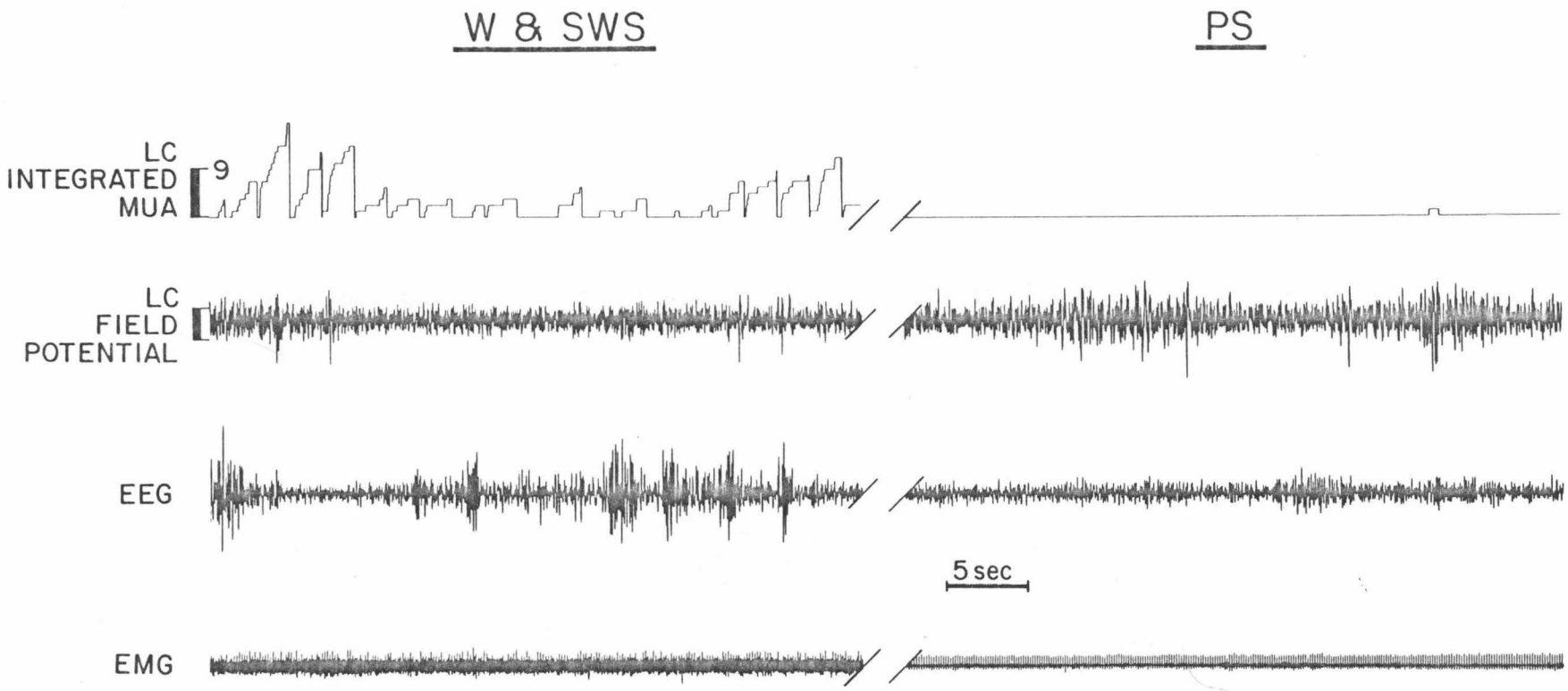

Fig. 9: Spontaneously occurring FPs and unit activity during W and SWS vs. during PS. Two epochs from one S-WC for an NE-LC MU recording, illustrating concurrent FPs and impulses during W and SWS, and their dissociation during PS. Recordings as in Fig. 8. FP calibration = 100 μV.

TABLE 1

	SI	SII	SIII	SIV	PS
Mean rate ± SEM (Hz)	2.15 ± 0.16	1.45 ± 0.14	0.68 ± 0.12	0.22 ± .05	0.02 ± 0.01
Mean sample time ± SEM (sec)	54.0 ± 5.7	74.0 ± 12.2	111.1± 9.3	46.6 ± 8.0	187.4± 32.6
Number of cells	31	32	33	22	9

Table 1: Spontaneous NE-LC SU discharge rates during S-WC stages. SI > SII ($p < .0005$, $N = 31$); SII > SIII ($p < .0005$, $N = 32$); SIII > PS ($p < .005$, $N = 22$); SIV > PS, ($p < .05$, $N = 9$); paired t-tests.

TABLE 2

LC Quadrant	SI	SII	SIII	SIV	PS
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 2: Spontaneous SU discharge rates in LC quadrants during S-WC stages. Rates (mean ± SEM Hz) for N cells examined in each quadrant. Mean rates for S-WC stages do not differ significantly across quadrants ($p > .1$ by one-way analyses of variance). However, mean W (SI or SII) rate for VP neurons < corresponding rate for the other quadrants ($p < .05$ by t-tests).

TABLE 3

LC Recording

Location	SI	SII	SIII	SIV	PS
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 3: Spontaneous NE-LC discharge rates during S-WC stages for SUs localized near an edge of LC vs. those located centrally. Rates (mean ± SEM Hz) for N cells in each group. Mean W (SI or SII) rate for edge cells > non-edge cells ($p < .005$ by t-tests). Neither mean SWS (SIII or SIV) nor PS rates differ significantly ($.05 < p < .1$ by t-tests).

TABLE 4

Recording Location	W > SWS (NO PS)	W > SWS > PS
NE-LC	24/24	9/9
Pons, 50 - 250um from LC	2/6	1/3
Pons, 300 - 2000um from LC	2/6	0/4

Table 4: 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/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.

Chapter 2

(In press,
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Title: Norepinephrine-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli.

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INTRODUCTION

Most brain norepinephrine (NE)-containing neurons are located in the pontine nucleus locus coeruleus (LC). The extensive projections of these neurons, together with the pronounced behavioral, clinical and postsynaptic effects produced by manipulations of NE activity in brain, have fostered several possible functions for the NE-containing LC (NE-LC) system (Foote et al., 1975; Segal and Bloom, 1976a,b; Freedman et al., 1977). Depending upon the lability of NE-LC activity, previous data could support the view that this system regulates either long-duration brain activity such as sleep and waking, or short-duration events such as phasic attention and distractability. Other studies (Graham and Aghajanian, 1971; Bunney et al., 1975; Cedarbaum and Aghajanian, 1976; Aghajanian et al., 1977; Bird and Kuhar, 1977; Aghajanian, 1978; Aston-Jones et al., 1980) have consistently reported that NE-LC neurons in anesthetized preparations spontaneously discharge in a slow tonic manner and are apparently responsive only to strongly noxious sensory stimuli. Such tonic discharge, insensitive to nearly all environmental stimuli, is consistent with previous hypotheses linking this system with stages of the sleep-waking cycle (Ramm, 1979; Clark, 1979; Amaral and Sinnamon, 1977; Steriad and Hobson, 1976). On the other hand, some investigators (Lader, 1974; Gray et al., 1975; Redmond and Huang, 1979) have proposed that predominant responsivity to noxious stimuli indicates a role for the NE-LC system in mediating pain and anxiety.

We have found, however, that in unanaesthetized behaving animals the discharge of NE-LC neurons is much more labile than that in anesthetized

preparations. In the preceeding paper (Aston-Jones and Bloom 1981), we reported that spontaneous activity varies not only with tonic stages of the sleep-waking cycle (S-WC), but also with phasic EEG events within some stages (spindle activity during slow-wave sleep). Furthermore, we have demonstrated (Jones et al., 1979; Foote et al., 1980) that NE-LC neurons in freely behaving rats are responsive to mild, non-noxious environmental stimuli of many modalities. In addition, other laboratories (Aghajanian et al., 1977; Takigawa and Mogenson, 1977) have reported similar responses to electrical stimulation of certain peripheral nerves. These various data indicate that the NE-LC system may principally function in more phasic processes than sleep and waking, and in more general phenomena than pain and anxiety.

We therefore undertook a detailed analysis of NE-LC sensory responsiveness in unanaesthetised behaving rats, and now report characteristics not previously described: 1) The magnitudes of response elicited by environmental stimuli vary as a function of vigilance levels. 2) Sensory-elicited discharge is synchronously accompanied by evoked field potentials from the same electrodes. 3) Sensory-evoked discharge decreases within active waking during grooming and consumption.

We conclude that the NE-LC system may play a specific role within the general framework of arousal, altering CNS and behavioral responsivity to salient unexpected stimuli in the external environment.

METHODS

Surgery: Rats were prepared for experimental recording sessions as previously described (Aston-Jones and Bloom, 1981). In brief, the LC was localized during stereotaxic surgery with the aid of unit recording. Skull screws were implanted to provide electroencephalogram (EEG) and ground reference signals, and bilateral subcutaneous wires were permanently sutured through dorsal neck muscles to serve as electromyogram (EMG) electrodes.

Experimental Paradigm: After at least 4 days of recovery from surgery, rats were placed in the experimental chamber either as freely moving (N = 82) or as harness-restrained subjects (N = 35) (Aston-Jones and Bloom, 1981). For each rat, the experimental paradigm alternated between recording spontaneous activity during natural sleep and waking (Aston-Jones and Bloom, 1981), and recording activity during systematic presentation of sensory stimuli (reported here). The investigator monitored all data collection.

Recording Techniques: Filtered and unfiltered unit-electrode traces, EEG, EMG, and digital impulse records were obtained as described (Aston-Jones and Bloom, 1981). These signals, along with digital logic pulses synchronized with experimental stimuli (sync pulses), were recorded on magnetic tape, and also displayed on polygraph paper either on or off-line. Sync pulses and digitized unit activity were fed into a PDP 11-10 or 11-03 computer either on- or off-line to generate peri-stimulus time histograms (PSTHs); inter-spike interval histograms (ISHs) were

similarly generated from spontaneous discharge.

Experimental Sensory Stimuli: The following stimuli were presented at regular 4 or 16 sec intervals, in blocks of 25 to 100 trials per modality: 1) tone pips (4KHz, 20 msec duration, about 96 dB on a 57 dB background); 2) light flashes (10 μsec duration, 50 to 100 candela); 3) brief, mild skin contacts (touches, manually applied to dorso-rostral tail surface); 4) single droplets of a 5% aqueous glucose solution (dispensed from a remote, electrically activated solenoid); sync pulses for licks were generated by a high-impedance circuit upon tongue contact with the solution. Digital sync pulses were precisely synchronized with tone pips, flashes and licks; a manual push-button generated digital pulses approximately synchronized with touches.

Localization of recording sites: Histological procedures were previously described (Aston-Jones and Bloom, 1981). All data were obtained from histologically identified recording sites, using gliotic lesion sites or Prussian Blue reacted iron deposits (illustrated in Fig.1) for reference marks.

Data Analysis: Criteria for acceptable single-unit (SU), multiple-unit (MU) and field potential (FP) data, and for scoring the S-WC are described elsewhere (Aston-Jones and Bloom, 1981). Waking (W) consisted of at least 3 sec of uninterrupted SI or SII, and slow-wave sleep (SWS) contained at least 3 sec of uninterrupted SIII or SIV.

For SU recordings, each stimulus trial was categorized as to EEG arousal levels which occurred before and after the stimulus: 1) SWS/W --

SWS preceeded the stimulus, which was followed within 0.5 sec by W. 2) W/W -- continuous W both before and after the stimulus; 3) SWS/SWS -- continuous SWS both before and after the stimulus. Trials that could not be unambiguously assigned to one of these categories were not included in data analysis. The magnitude of response for each stimulus trial was determined (from polygraph records) by subtracting the number of impulses in the 200 msec epoch preceeding the stimulus from the number of impulses in the 200 msec epoch following the same stimulus.

Response latencies were determined by computer from PSTHs which incorporated at least 25 consecutive trials each, as illustrated in Fig. 6. Baseline mean and standard deviation (SD) for PSTH bins (binwidth = 4 or 8 msec) were calculated for a period of 1 sec or more, beginning at least 2 sec after stimulus onsets. Excitatory response onsets and inhibitory response offsets were defined at the midpoint of the first bin which exceeded baseline mean by 2 SDs or more, and which also was the first of 5 consecutive bins whose mean value met the same criterion. Inhibitory response onsets and excitatory response offsets were similarly defined, but using 20 consecutive bins; for inhibitory responses only, if baseline mean was less than 2 SDs, responses were defined using a 1 SD requirement.

RESULTS

Spontaneous discharge: In general, SU recordings in the NE-LC yielded slow, tonic spontaneous discharge; W rate = 1.74 ± 0.15 Hz (mean ± SEM, N = 30 cells). However, activity was dramatically altered by mild sensory stimuli. This effect was most obvious in MU recordings, which typically contained aperiodic impulse bursts, each followed by a prolonged period of suppressed activity. These phasic bursts were consistently associated with background stimuli in the environment. Upon systematic examination, we found that this discharge pattern matched sensory-evoked activity characteristic of both MU and SU recordings (described below).

Sensory-evoked discharge: As illustrated in Figs.2 through 5 and summarized in Table 1, NE-LC neurons homogeneously responded to mild, non-noxious sensory stimuli of many modalities. Auditory, visual and somatosensory stimuli all elicited similar patterns of biphasic response, consisting of an initial burst of impulses followed immediately by a longer period of decreased activity (closely resembling the phasic pattern of "spontaneous" activity in MU recordings described above).

Table 2 summarizes response latencies for tone pip and flash stimuli. MU recordings consistently yielded less variable latencies and fewer response failures than SU data. The response properties of NE-LC neurons in MU populations were also predominantly homogeneous (see Table 1 and below), as reflected in markedly synchronized response activity among neighboring NE-LC neurons recorded simultaneously. MU activity may, therefore, provide more representative latency data than SU recordings,

owing to the correspondingly larger sample of impulses. MU response latencies for tone pip and flash stimuli are graphically compared in Fig. 7. Excitatory responses to flashes had longer onset latencies than did responses to tone pips (for SUs and MUs, $p < .0005$ by paired t-tests, $N = 10$ and 9 respectively). The same relationship existed for excitation offset latencies in these two modalities ($p < .005$), as well as for the subsequent latencies of inhibitory response onset (for SUs and MUs, $p < .05$ by paired t-tests, $n = 4$ and 7 respectively). Excitatory response durations were longer for flashes in SU data ($p < .005$ by paired t-test), but did not differ significantly in MU recordings ($p > .1$ by paired t-test); the difference here between SU and MU data may be a consequence of the relatively high variability in SU data due to the intrinsically small sample of impulse activity, as noted above. Inhibitory response durations for tone pips were longer than for flashes (for SUs, $p < .01$, $N = 4$; for MUs, $p < .025$, $N = 7$; paired t-tests), but the latencies of inhibitory response offset were similar for the two modalities in both SU and MU recordings ($p > .1$ by paired t-tests).

Six SU and 3 MU touch PSTHs were quantitatively analyzed; although sync pulses were not precisely synchronized with somatosensory stimuli, consistent response patterns were approximated: SU excitatory response duration = 206.6 $\pm$ 40.9 msec (mean $\pm$ SEM) and subsequent inhibitory response duration = 532.0 $\pm$ 58.0 msec; MU excitatory response duration = 240.0 $\pm$ 24.0 msec and subsequent inhibitory response duration = 605.3 $\pm$ 127.2 msec. Touches apparently elicited more pronounced responses than tone pips or flashes. Similarly, auditory responses were generally more

robust than responses to visual stimuli, consistent with the comparatively large number of visual response failures (seen in Tables 1 and 2) and flash responses insufficient in magnitude to permit single-trial analysis (see below). It was also apparent that touches elicited greater orienting response than tone pips which, in turn, were more effective than flashes. Thus, stimulus modalities yielded the same order of efficacy for NE-LC unit response and for behavioral orienting responses. This indicates that NE-LC responsiveness may vary with evoked vigilance increase more directly than with stimulus modalities per se.

Several NE-LC recordings were examined with olfactory stimuli (acetic acid-soaked swab placed under the nose) and painful tail-pinches (manually applied). Each of the four recordings tested with olfactory stimulation exhibited an excitatory response, but a subsequent inhibitory response was difficult to identify (perhaps due to less abrupt and more prolonged stimulus administration). Tail-pinches elicited a strong response in all 4 recordings tested, yielding a biphasic (excitatory-inhibitory) pattern of discharge similar to that characteristic of responses to non-noxious auditory, visual and somatosensory stimuli.

In contrast to the biphasic response pattern typically evoked by sensory stimuli in the above studies, gustatory stimulation (during voluntary consumption of a preferred 5% aqueous glucose solution) elicited only a decrease in NE-LC discharge. These responses were most apparent in cumulative PSTHs, as seen in Figs. 3 and 5. Quantitative PSTH analysis yielded the following results: for SUs, the latency of inhibitory response onset = 59.0 $\pm$ 22.4 msec (mean $\pm$ SEM), and offset latency = 357.0 $\pm$ 32.4

msec (N = 4); for MU recordings, the latency of inhibition onset = 44.7 ± 16.9 msec, and offset latency = 420.0 ± 75.4 msec (N = 6). Gustatory responses were unrelated to the motor activity involved in licking, since there was no consistent pattern of NE-LC activity in PSTHs which were triggered at the onset of every lick in an episode (typically 6 to 7 licks/sec for about 2 to 3 sec); consistent NE-LC response occurred only in PSTHs which were synchronized with the first lick in each trial, i.e., the first lick only for each drop of solution.

Sensory response magnitudes: In our initial studies, NE-LC responses appeared to habituate and dishabituate in rapid succession. Typically, the first 5-10 stimuli elicited robust responses while the following set of 5-10 stimuli elicited very little or no response, followed by pronounced responses to the next few stimuli, etc. (see Figs. 2, 4 and 8). Upon closer examination, however, we found that these fluctuations in response corresponded to changes in the animal's level of vigilance. As illustrated in Fig. 8, the magnitudes of response to tone pips were directly correlated with EEG arousal. For quantitative analysis, each stimulus trial was placed in one of three categories according to the S-WC stages immediately preceeding and following stimulus presentation, and response magnitude was calculated for each trial (as described in Methods). As shown in Fig. 9, response magnitudes for tone pips presented during uninterrupted waking (W/W) were significantly greater than for those presented during uninterrupted SWS (SWS/SWS) ($p < .0005$ by paired t-test, N = 16 cells). Furthermore, the largest response magnitudes occurred for tone pips in the SWS/W category, i.e., for stimuli that awakened the animal

(SWS/W > W/W, $p < .005$ by paired t-test, N = 13 cells). The mean difference (D) between the absolute discharge rates immediately following stimuli in the SWS/W vs. W/W category (D = 1.21; SWS/W rate > W/W rate, $p < .05$ by paired t-tests) was not as pronounced or confident as the corresponding difference between response magnitudes (D = 3.05). Conversely, the difference between response magnitudes in the W/W vs. SWS/SWS categories (D = 3.05) was smaller than the difference between corresponding absolute discharge rates (D = 4.50). Responses to flash stimuli were generally not sufficient in magnitude to permit quantitative single-trial analysis, but similar general results were observed. Mild auditory, visual or somatosensory stimuli presented during uninterrupted PS elicited no response in the 5 NE-LC recordings examined. No habituation of NE-LC response was observed (over 25-100 trials) independent of such changes in the level of vigilance.

Sensory-evoked NE-LC responsiveness, as well as spontaneous discharge (Aston-Jones and Bloom, 1981), was observed to decrease during grooming and sweet-water consumption, similar to results obtained for sleep (above). Furthermore, these three behavioral states were also correlated with reduced orienting behavior in response to sensory stimuli. However, any sensory stimulus that successfully disrupted such ongoing behavior elicited a robust response in NE-LC activity (qualitative observations). Thus, tonically reduced NE-LC activity accompanied behavioral states characterized by low levels of vigilance, whereas phasic, intense NE-LC discharge corresponded to abrupt increases in vigilance.

Sensory-evoked field potentials: FPs were evoked in the NE-LC by the same sensory stimuli used in the above unit studies, yielding waveforms similar to those occurring spontaneously. As illustrated in Figs. 10 through 13, sensory-evoked FPs were temporally synchronized with unit responses simultaneously recorded from the same electrodes (see Methods). A negative FP wave accompanied the onset of excitatory unit responses, closely followed by a positive FP deflection. Sensory-evoked FP magnitudes ranged from 100 μV to 300 μV in the negative component, and from 50 μV to 150 μV in the positive component.

As found for unit responses (above), the magnitudes of sensory-evoked FPs fluctuated with changes in vigilance. FPs in the SWS/W category were apparently larger than those in the W/W category, which, in turn, were generally larger than FPs in the SWS/SWS category (illustrated in Figs. 8 and 14). In contrast to unit activity, however, FPs continued to be evoked in the NE-LC during PS (Fig. 15), although with smaller amplitudes than those typically elicited during other S-WC stages.

Topographical specificity: The NE-LC was histologically divided into quadrants (Aston-Jones and Bloom, 1981) to test for possible topographical segregation of unit response properties. There were no differences in Table 1 properties for SU or MU recordings in different quadrants. Similarly, one-way analyses of variance for response latencies across quadrants yielded no significant differences (for SUs, $p > .05$, N = 5,4,4 and 4 for tones, and 3,3,3 and 2 for flashes in the dorsoanterior (DA), ventroanterior (VA) dorsoposterior (DP) and ventroposterior (VP) quadrants, respectively; for MUs, $p > .05$, N = 7,3,3 and 2 for tone pips, and 5,0,3

and 1 for flashes in the DA, VA, DP and VP quadrants, respectively).

However, when comparing quadrants for response magnitudes, some significant differences emerged. Only DP neurons exhibited SWS/W magnitudes that were significantly greater than those in the W/W category ($p < .05$ by paired t-test, $N = 3$ cells; $p > .1$ by paired t-tests in the DA and VA quadrants, $N = 7$ and 2 cells, respectively; the VP quadrant was not analyzed as there was only 1 cell common to these categories). Also, response magnitudes for DP neurons were significantly greater in the W/W than in the SWS/SWS category at the $p < .01$ level (by paired t-test, $N = 4$ cells), while cells in the VA quadrant yielded a similar difference at the $p < .05$ level only (by paired t-test, $N = 4$ cells), and DA cells exhibited no significant difference ($p > .1$ by paired t-test, $N = 7$ cells); the VP quadrant was not analyzed (as $N = 1$ cell common to these categories). One-way analyses of variance revealed a significant effect of quadrant for response magnitudes in the W/W category only ($p < .02$, $N = 16$ cells). Subsequent t-tests revealed that DP response magnitudes in the W/W category were significantly greater than those for cells in the VA quadrant at the $p < .005$ level ($N = 4$ cells in each quadrant), while neurons in the DA quadrant ($N = 7$ cells) yielded significantly higher W/W magnitudes than those in the VA quadrant at the $p < .05$ level only; the VP quadrant was not analyzed (as $N = 1$ cell for this category). Thus, cells in the DP quadrant tended to exhibit larger responses to tone pips during W than other NE-LC neurons. This also appeared to be true for flash responses, but quantitative comparison across quadrants was precluded by an insufficient number of cells amenable to single-trial analysis; however,

of the 3 cells with response magnitudes permitting that analysis, 2 were in the DP quadrant.

During the course of these studies, we noted that anomalous activity was often exhibited by NE-LC neurons situated near an edge of the nucleus. We compared our results for neurons located within about 50 m on either side of NE-LC boundaries (edge cells) with results for neurons located more centrally. Thirteen of the fourteen exceptions in Table 1 SU properties were from edge cells, as were all 3 exceptions in MU properties. However, PSTHs for SU and MU edge recordings yielded response latencies similar to those for non-edge recordings ($p > .1$ by t-tests; $N = 5$ edge and 12 non-edge SUs for tone pips, $N = 2$ edge and 8 non-edge SUs for flashes; $N = 2$ edge and 13 non-edge MUs for tone pips; there were unsufficient cases to compare inhibitory flash response latencies or MU excitatory flash response latencies).

Qualitative data collected on 91 non-LC pontine SUs during the course of these studies are summarized in Table 3. Comparing Table 1 with Table 3 reveals that a relatively small percentage of non-LC pontine neurons exhibited discharge properties that were characteristic of NE-LC neurons (percentages differ between corresponding properties in Table 1 and Table 3 at $p < .001$ by Chi-square tests for 2 independent samples). There were too few non-LC neurons quantitatively examined to confidently compare their latencies or durations with NE-LC neurons.

DISCUSSION

The present results expand the set of characteristic properties for NE-LC activity in unanesthetized behaving rats (Aston-Jones and Bloom, 1981). These neurons exhibit prompt, biphasic responses to non-noxious auditory, visual and somatosensory stimuli. Such responses, consisting of a short burst of impulses followed by a prolonged silence, are typically exhibited for each stimulus modality in individual NE-LC recordings. In contrast, these neurons exhibit only inhibitory responses to voluntarily consumed, preferred gustatory stimuli. Thus, NE-LC discharge is characterized by polysensory responsiveness, yielding two modality-specific patterns of evoked activity.

Tone pip response latencies were 30 to 50 msec shorter than for flash stimuli; this difference may be attributable to retinal transmission time, estimated at 30 msec in cat (Creutzfeldt, 1970). These auditory response latencies approximate the latencies reported for similar responses to electrical stimulation of certain peripheral nerves (Aghajanian et al., 1977; Takigawa and Mogenson, 1977).

Excitatory response magnitudes fluctuated during trains of stimuli in the present study, which might be interpreted as habituation and dishabituation in the NE-LC. However, these fluctuations were systematically associated with simultaneous changes in the level of vigilance, such that the largest responses occurred for stimuli that awakened rats, and the smallest were elicited by identical stimuli during uninterrupted sleep. Although habituation of NE-LC responsiveness may

eventually occur dissociated from vigilance changes, none was observed here with up to 100 consecutive stimuli. Sensory response magnitudes were also apparently reduced for certain behaviors within active waking, i.e. during grooming or consumption of sweet water, times when behavioral orienting responses were suppressed. However, stimuli that successfully interrupted either of these behaviors elicited robust responses in NE-LC neurons. Thus, sensory-evoked activity in the NE-LC, like spontaneous discharge (Aston-Jones and Bloom, 1981), is tonically suppressed during sleep, grooming and consumption, but is phasically enhanced when such ongoing behavior is disrupted, corresponding to a change in behavioral state.

Mild sensory stimuli also evoked FPs in the NE-LC, eliciting waveforms closely resembling those occurring spontaneously. Similar events have been recently observed in the LC area by Kaufman and Morrison (1981) in unanesthetized behaving rats, but their use of large-diameter electrodes makes it difficult to specify sites which generate recorded signals. The present study, however, provides more definitive evidence that FPs do arise from NE-LC activity: (1) FPs were recorded from sites discretely localized to the NE-LC. (2) FPs were obtained with monopolar etched tungsten microelectrodes, as well as with differential pairs of adjacent microwire electrodes. Both of these recording techniques probably monitor activity from a relatively small volume of tissue only. (3) FPs were temporally synchronized with unit activity simultaneously recorded from the same electrodes. In particular, the negative FP component was typically accompanied by a burst of unit impulses, as expected for neurons that generate the corresponding FP current sink (Steriade and Hobson, 1976).

(4) NE-LC neurons were generally homogeneous in their discharge properties, and neurons in MU populations were markedly synchronized during bursts of impulses. Such concerted activity in a tightly packed group of cells fulfills theoretical requirements (Steriade and Hobson, 1976) for sites generating FPs, and implies that NE-LC neurons may function as an homogeneous ensemble.

The relationship between sensory response magnitudes in the NE-LC and vigilance levels implies that there are two significant, distinct influences on NE-LC activity: (1) excitatory inputs mediating sensory-evoked discharge, and (2) inhibitory systems that modulate NE-LC excitability according to vigilance or behavioral state. The present FP data offer additional insight as to the factors controlling NE-LC activity. Spontaneous and sensory-evoked FPs without unit activity during PS may reflect concerted EPSPs in the presence of strong, tonic inhibition which prevents discharge. Phasic, excitatory inputs concurrent with tonic, inhibitory inputs resemble factors known to be operating on motoneurons during PS (Chase, 1980), where impulse generation is prevented despite intense excitatory barrages. This would be consistent with previous proposals (McCarley and Hobson, 1975) that NE-LC discharge is incompatible with PS, and would suggest that suppression of these neurons plays a critical role in PS episodes.

Future experiments are planned to determine if varying intensities of excitatory and inhibitory inputs to NE-LC neurons similarly underlie certain present results for W and SWS as well. Increased inhibition during SWS, grooming and consumption could produce the corresponding decreases

observed in spontaneous and sensory-evoked impulse activities. Thus, the NE-LC may integrate CNS signals that reflect external sensory events with those that convey internal vegetative requirements. The relative intensities of activity in these two afferent systems could determine NE-LC discharge level which may then influence the global orientation of brain and behavioral activities.

Although some sensory response characteristics of NE-LC neurons in unanesthetized behaving rats resemble results reported for anesthetized rats, many fundamental properties differ markedly. Previous studies (Graham and Aghajanian, 1971; Bunney et al., 1975; Cedarbaum and Aghajanian, 1976; Aghajanian et al., 1977; Bird and Kuhar, 1977; Aghajanian, 1978), as well as our own observations, indicate that these cells in anesthetized animals respond only to strong, noxious environmental stimuli. In contrast, we found that mild, non-noxious sensory stimuli of many modalities elicit pronounced NE-LC responses in unanesthetized behaving preparations. In our unanesthetized preparation, pronounced lability was observed in spontaneous NE-LC discharge with MU recordings, apparently resulting from responses to background environmental stimuli. (Such bursty discharge was not so apparent in spontaneous SU activity, probably due to the smaller sampling of impulse activity). In fact, increased sensory responsivity is the most prominent difference between behaving and anesthetized rats' NE-LC discharge. Reduced responsivity under anesthesia may correspond to the relationship between response amplitude and vigilance found in the present study. By maintaining a low vigilance level, anesthesia may decrease NE-LC sensory responsiveness much

like sleep does in unanesthetized rats. The ineffectiveness of most sensory stimuli in anesthetized rats has led some investigators to propose that the NE-LC system is primarily involved in nociception, fear or anxiety (Lader, 1974; Gray et al., 1975; Redmond and Huang, 1979). The present results indicate a much broader range of environmental influences on NE-LC discharge, and therefore a more general role for this system in brain and behavioral activity.

Summary and Hypothesis

The preceeding (Aston-Jones and Bloom, 1981) and present studies have demonstrated the following set of characteristic properties for NE-LC neurons: (1) Spontaneous discharge covaries with stages of the S-WC, exhibiting rates directly related to the level of vigilance. (2) Spontaneous discharge fluctuates with and anticipates phasic cortical epochs (spindle activity during SWS) as well as tonic cortical periods (S-WC stages, except W after PS). (3) Discharge is not apparently linked to specific movements, but does correspond to orienting, grooming and consumption behaviors. (4) FPs occur spontaneously in the NE-LC, temporally synchronized with unit activity (during W and SWS) simultaneously recorded from the same electrodes. (5) Biphasic FPs and synchronous unit responses are evoked by mild, non-noxious environmental stimuli of many modalities. (6) Spontaneous and sensory-evoked FPs persisted during PS, in the virtual absence of unit activity. (7) Spontaneous as well as sensory-evoked activity is tonically reduced during sleep, grooming and consumption, but is phasically enhanced when such behaviors are interrupted. (8) SU and MU recordings throughout the NE-LC yield homogeneous discharge properties.

Our present results, together with previous data on the postsynaptic effects of NE (Foote et al., 1975; Segal and Bloom, 1976a,b; Freedman et al., 1977), lead us to re-evaluate proposals of NE-LC function, and to offer a new working hypothesis for the role of this system in brain and behavioral activity. A global release of NE (e.g., following environmental stimulation that elicits robust NE-LC discharge) may enhance signals in

brain systems engaged by exogenous sensory stimuli, and simultaneously suppress CNS activity associated with tonic vegetative functions which are low in priority for phasic adaptive behavior. Sleep, grooming and consumption, as endogenously generated repetitive behaviors, may critically depend upon low-levels of NE-LC impulse activity: Vigorous discharge may disrupt or disengage such internally oriented behavioral patterns by enhancing signal-to-noise characteristics (and, therefore, transmission flow) in CNS pathways important for appropriate response to unexpected external events. We propose, therefore, that the NE-LC system may function to facilitate transitions between behavioral states. By selectively augmenting CNS activity engaged by rapidly changing exogenous stimuli, robust NE-LC discharge may bias the global orientation of behavior towards coping with phasically imperative events in the external environment.

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Fig. 1: Histological localization of recording sites. Sagittal, 40 µm-thick section from experimental rat brain stained with Neutral Red, through LC (small arrow). Prussian-Blue spot (large arrow) marks iron deposited by recording electrode 100 µm below typical LC activity. Calibration bar = 100 µm.

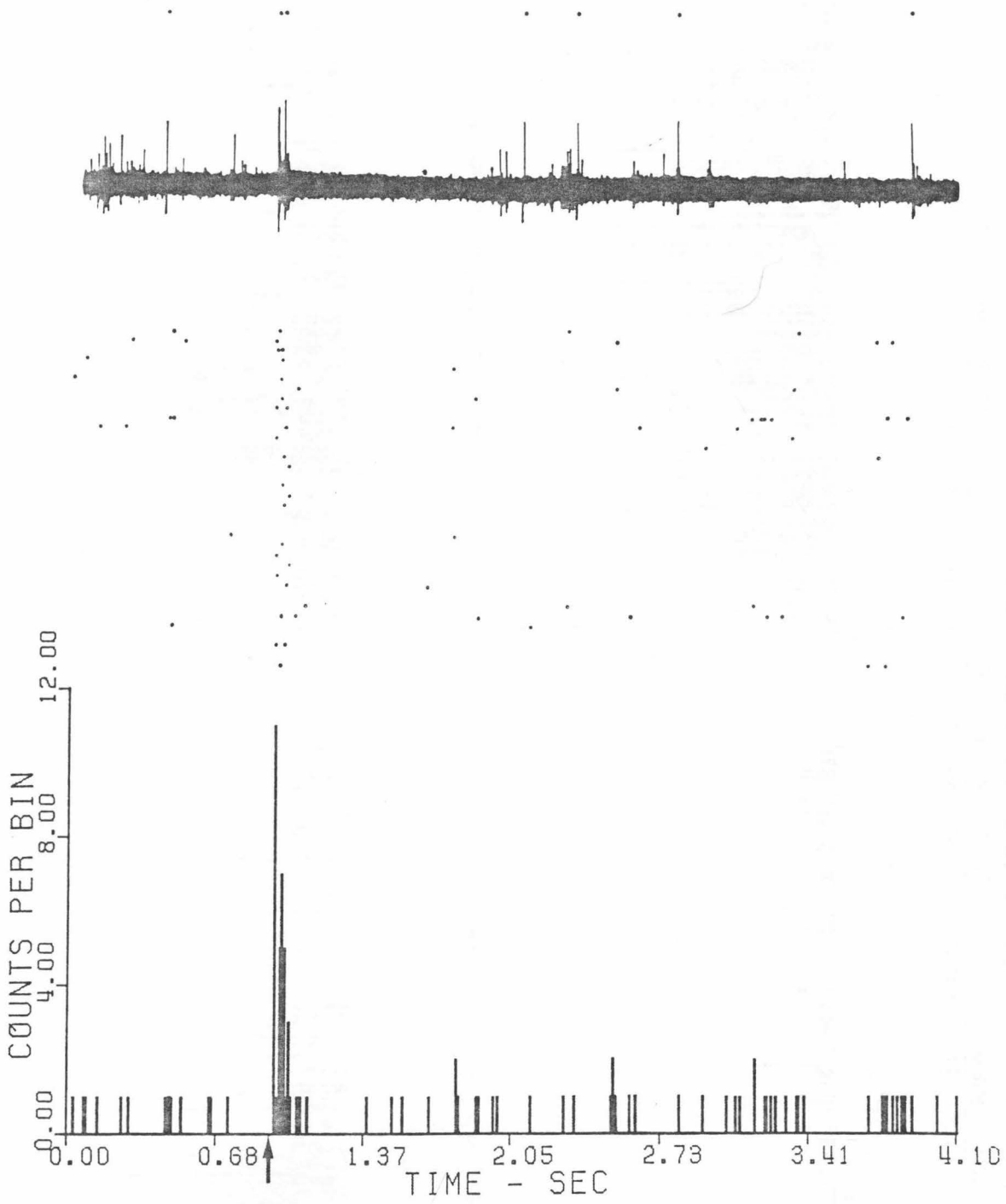
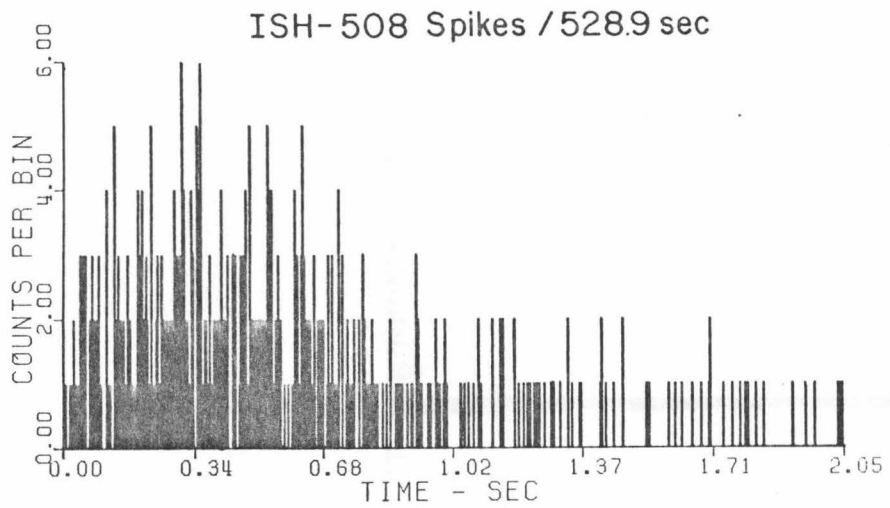
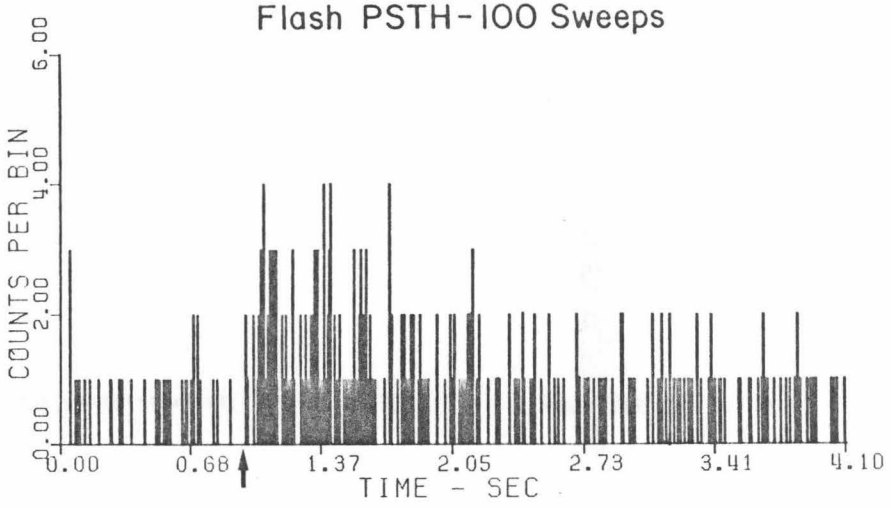
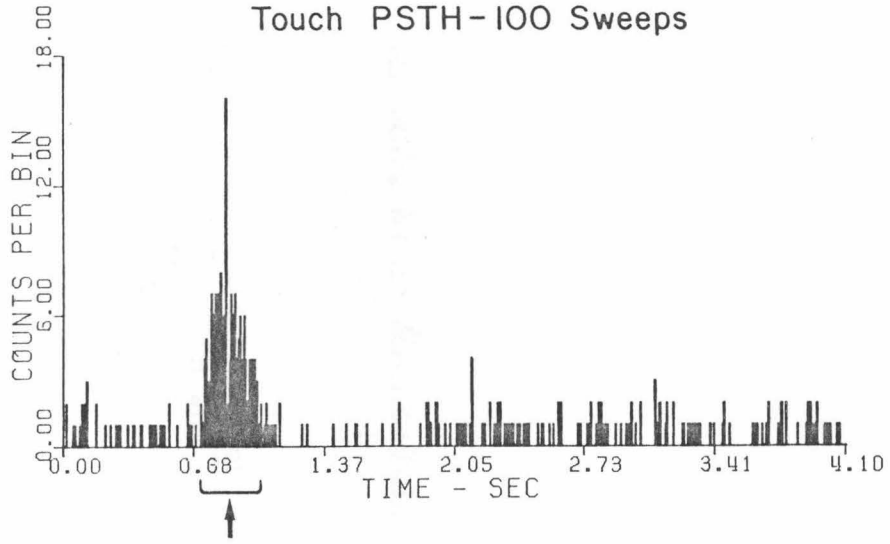


Fig. 2: Tone pip - evoked discharge in a single NE-LC neuron. Upper panel - single oscilloscope sweep of analogue discharge trace for one trial. Dots were generated for spikes meeting waveform discriminator criteria. Middle panel - raster display of impulse activity for 40 consecutive trials, in sequence from top to bottom. Lower panel - PSTH accumulated for 50 consecutive trials (bin width = 8 msec). Time axis and tone pip onsets (arrow) apply to all panels. ISH and other sensory modality PISHs for this neuron are given in Fig. 3.







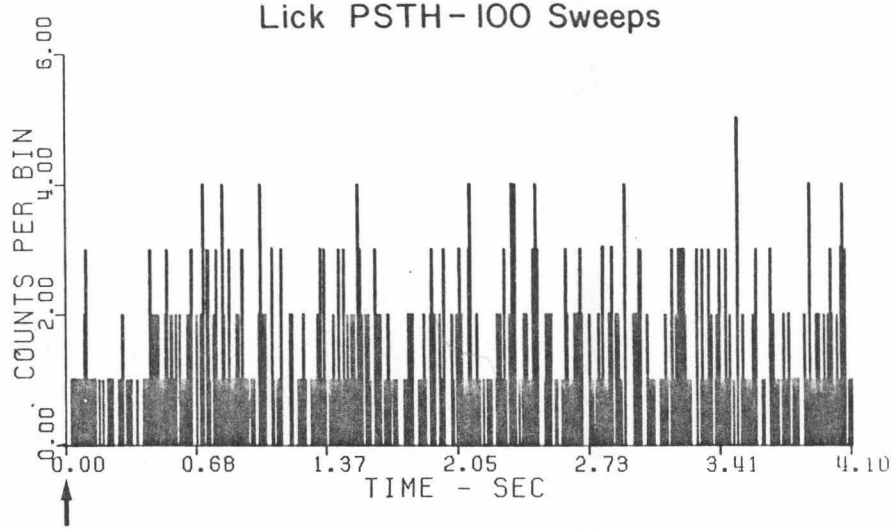


Fig. 3: Spontaneous and sensory-evoked discharge in a single NE-LC neuron. Same neuron in A,B,C and D. Tone pip data for this neuron are given in Fig. 2. Arrows indicate stimulus onsets.

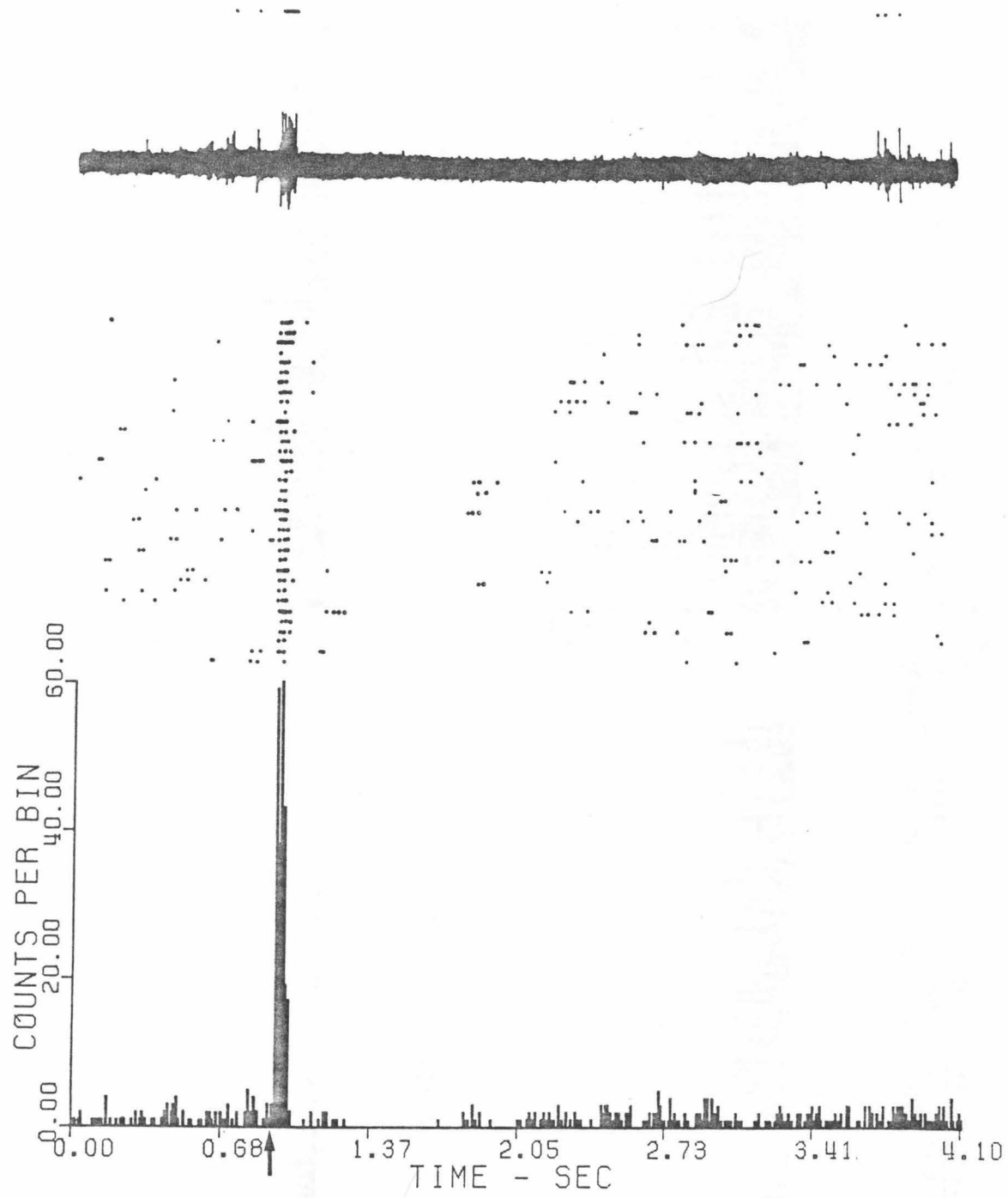


Fig. 4: Tone pip-evoked discharge in an NE-LC MU recording. Panel format, time scale and stimuli are as in Fig. 2. ISH and other sensory modality PISHs for this recording are given in Fig. 5.

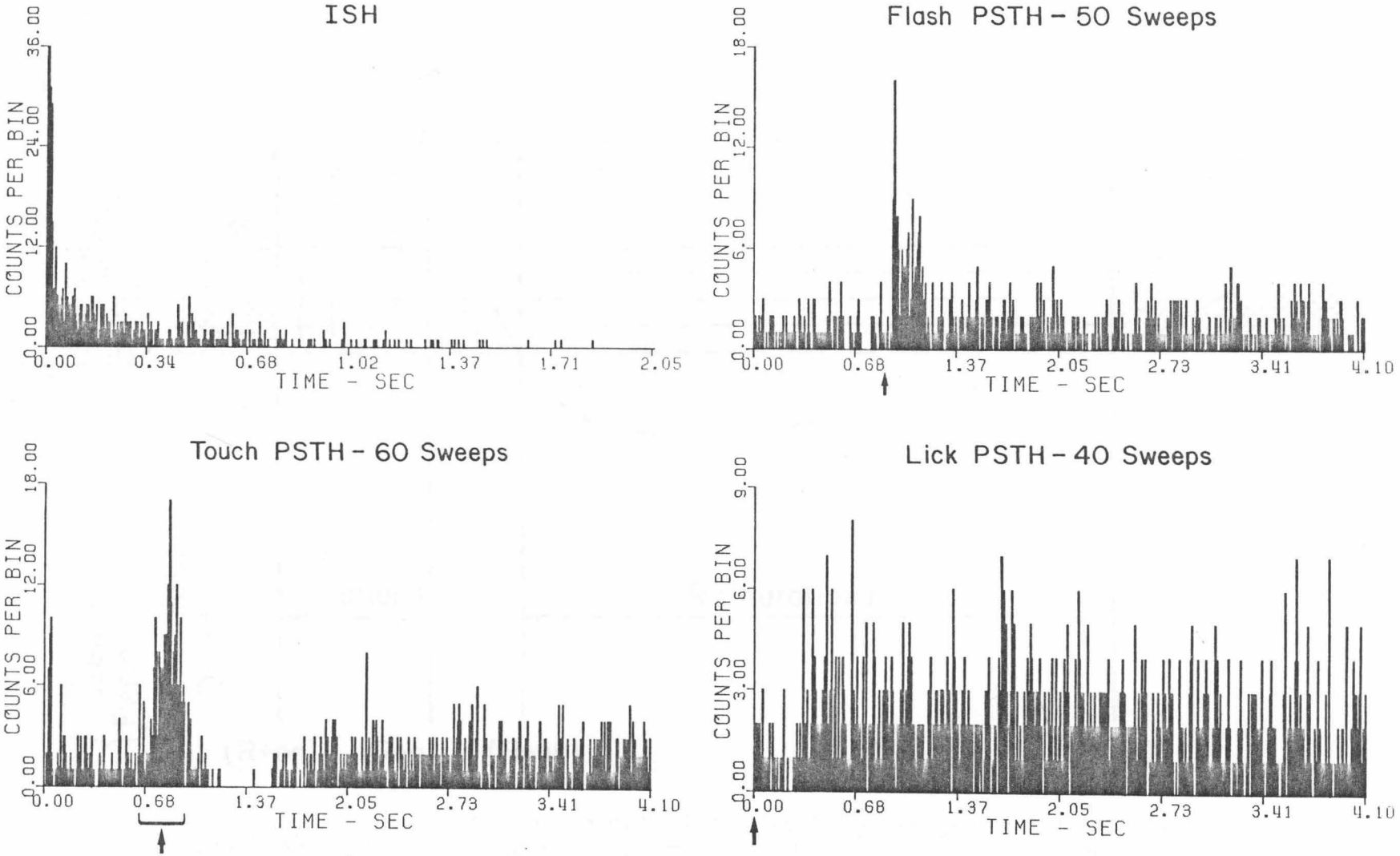


Fig. 5: Spontaneous and sensory-evoked discharge in an NE-LC MU recording. Same MU recording in A,B, C and D. Tone pip data for this recording are given in Fig. 4. Arrows indicate stimulus onsets.

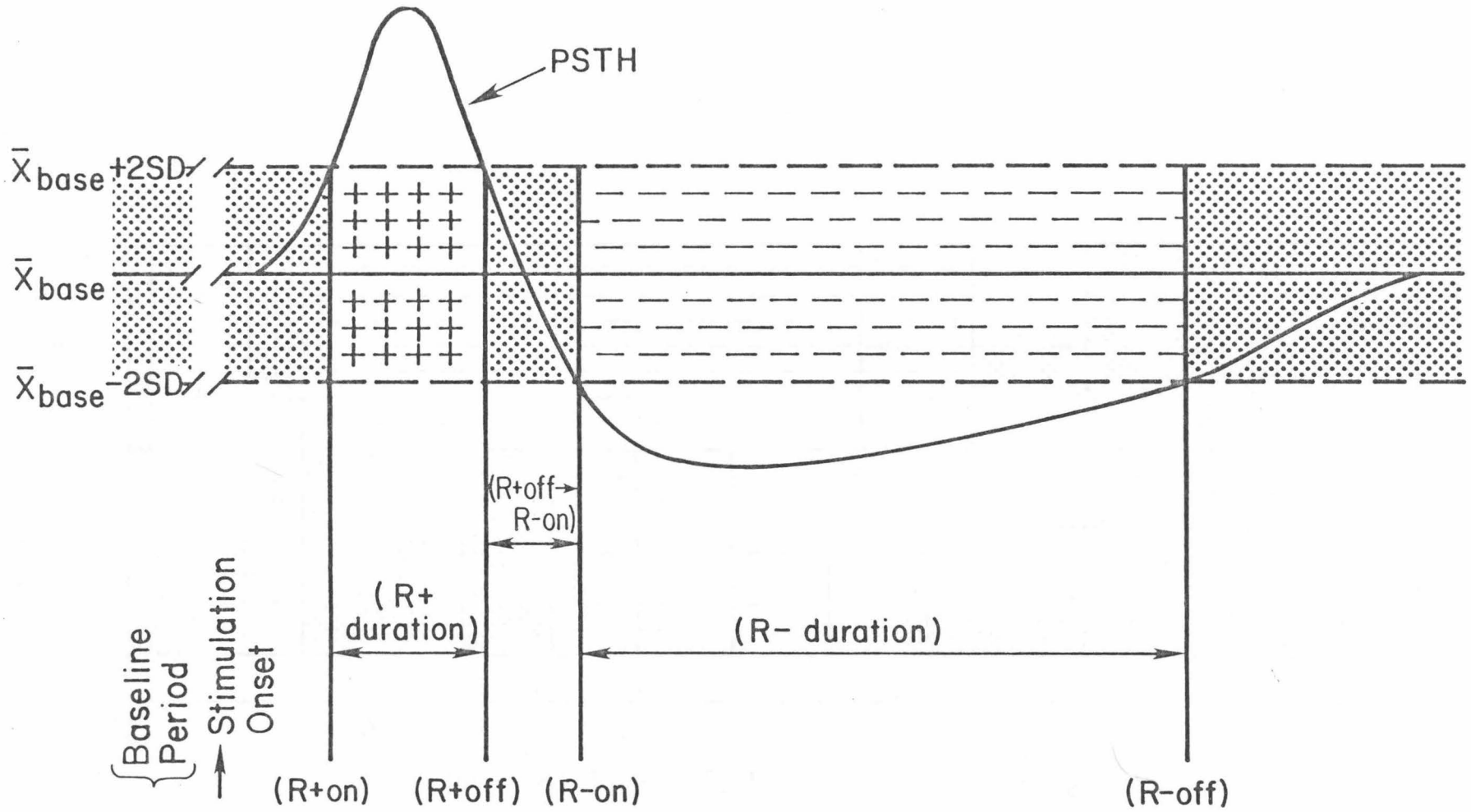


Fig. 6: Method used to calculate discharge response latencies. Latencies of excitatory response (R+on and R+off) and inhibitory response (R-on and R-off) were taken at PSTH bins differing from baseline mean (X_{base}) by predetermined standard deviation (SD) criteria, as described in Methods. Areas filled with dots, plus (+) signs and minus (-) signs indicate non-significant, excitatory and inhibitory response periods, respectively. Response durations (dur) were taken between R on and R off.

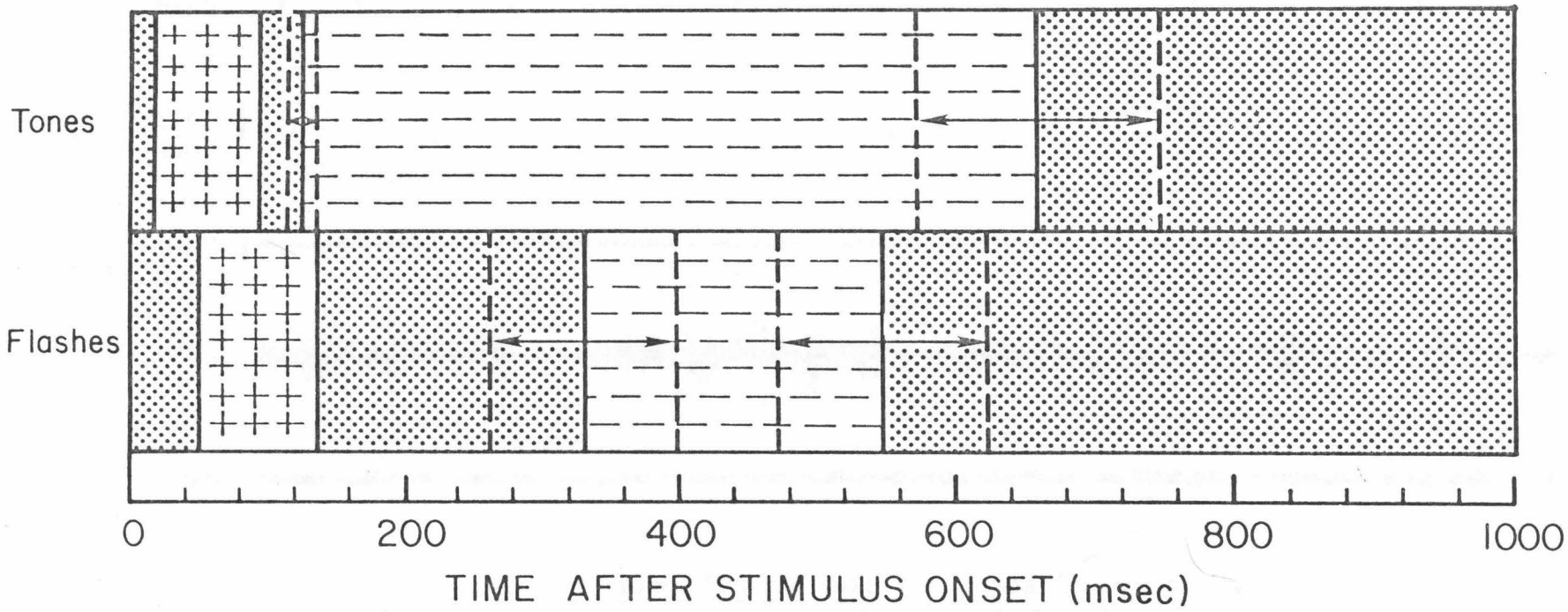


Fig. 7: latencies of evoked response in NE-LC discharge for tone pip vs. flash stimuli. Data averaged for one group of 7 MU recordings tested with both tone pip (T) and flash (F) stimuli. Solid, and adjacent dashed, vertical lines indicate mean and SEM latencies, respectively. Other symbols as in Fig. 6. (T+on) < (F+on)***; (T+off) < (F+off)**; (T+dur) $\simeq$ (F+dur)^{o}; (T-on) < (F-on)*; (T-off) $\simeq$ (F-off)^{o}; (T-dur) > (F-dur)*; (T-on minus T+off) < (F-on minus F+off)*; ***p < .0005; **p < .005; *p < .05; ^{o}p > .1; paired t-tests.

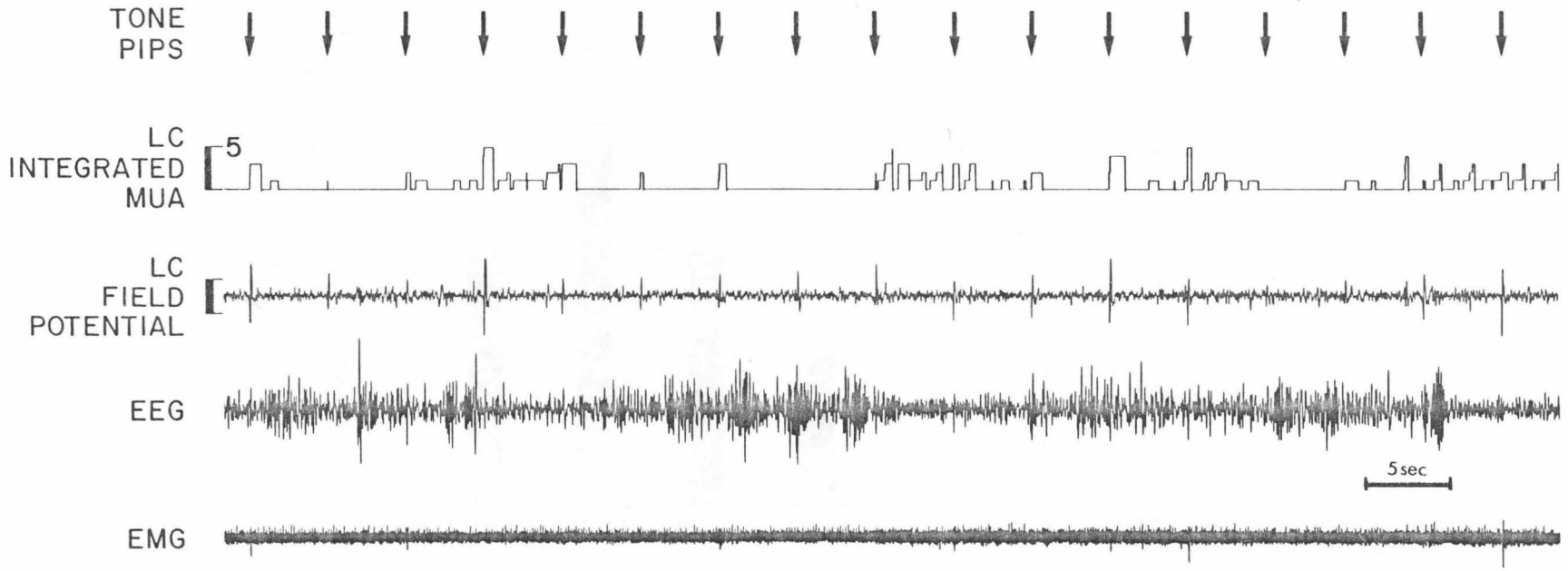


Fig. 8: Sensory-evoked FPs and unit responses in the NE-LC during fluctuating cortical arousal. Tone pips (at arrows) evoke smaller magnitudes of MU response during SWS (high amplitude, low-frequency, periodic EEG) than during W (low-amplitude, aperiodic EEG). Largest amplitudes occur for stimuli that evoke abrupt W from SWS. Differential recordings, separated into FP and discharge traces as described in Methods.

FP calibration = 100 μ V.

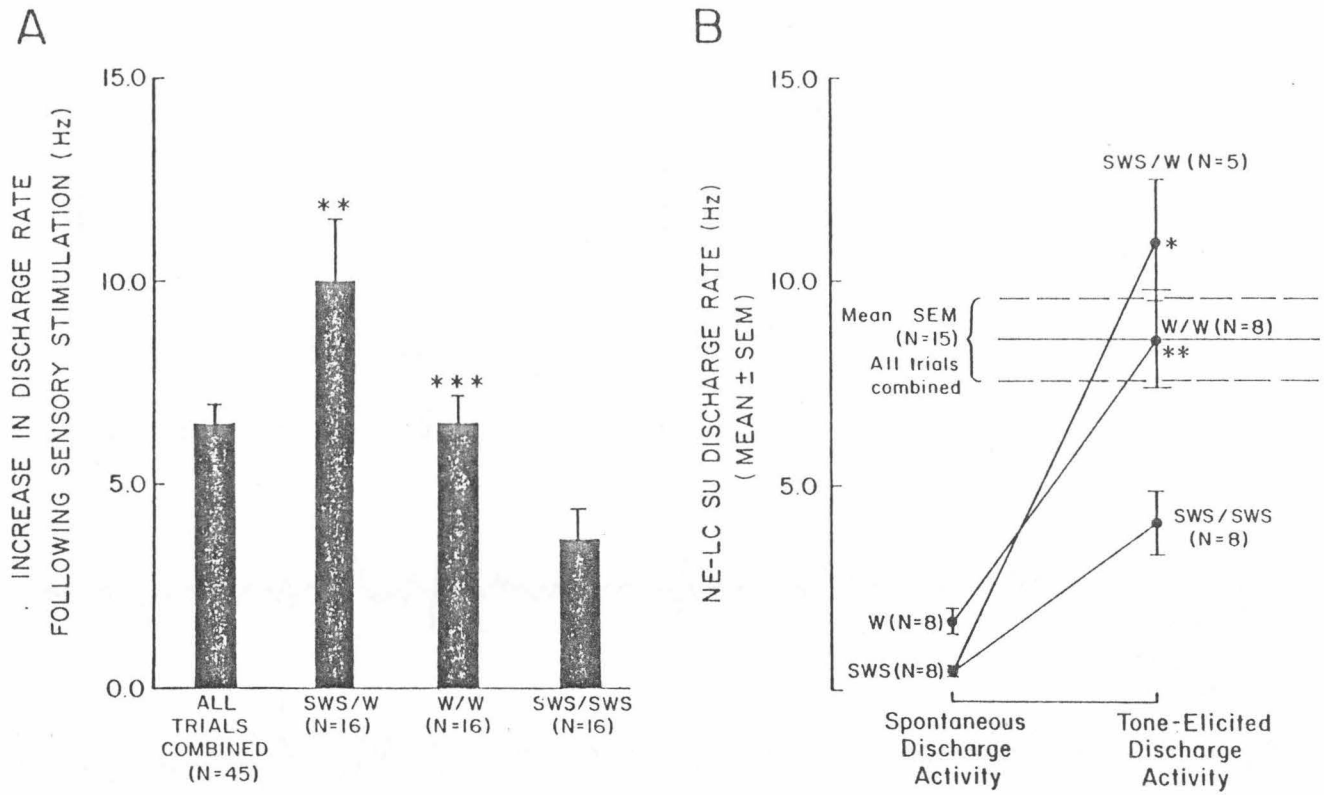


Fig. 9: Sensory-evoked response magnitudes and discharge rates in NE-LC SU's as functions of arousal. A) Mean response magnitude for SWS/W trials > W/W trials, **p < .005; mean W/W magnitude > mean SWS/SWS magnitude, ***p < .0005; mean SWS/W magnitude > mean magnitude for all trials combined > mean SWS/SWS magnitude, p < .0005 for each; paired t-tests. B) Mean absolute discharge rates for 200 msec epochs immediately following stimuli grouped in arousal categories, and mean spontaneous discharge rates for these neurons during tonic W and SWS. Lines connecting dots illustrate relative increases in discharge rates. SWS/W > W/W, *p < .05; W/W > SWS/SWS, **p < .0005; SWS/W > all trials combined > SWS/SWS, p < .005 for each; W > SWS, p < .005; SWS/W > SWS or W, p < .005 for each; W/W > W, p < .0005; SWS/SWS > SWS, p < .0005; paired t-tests.

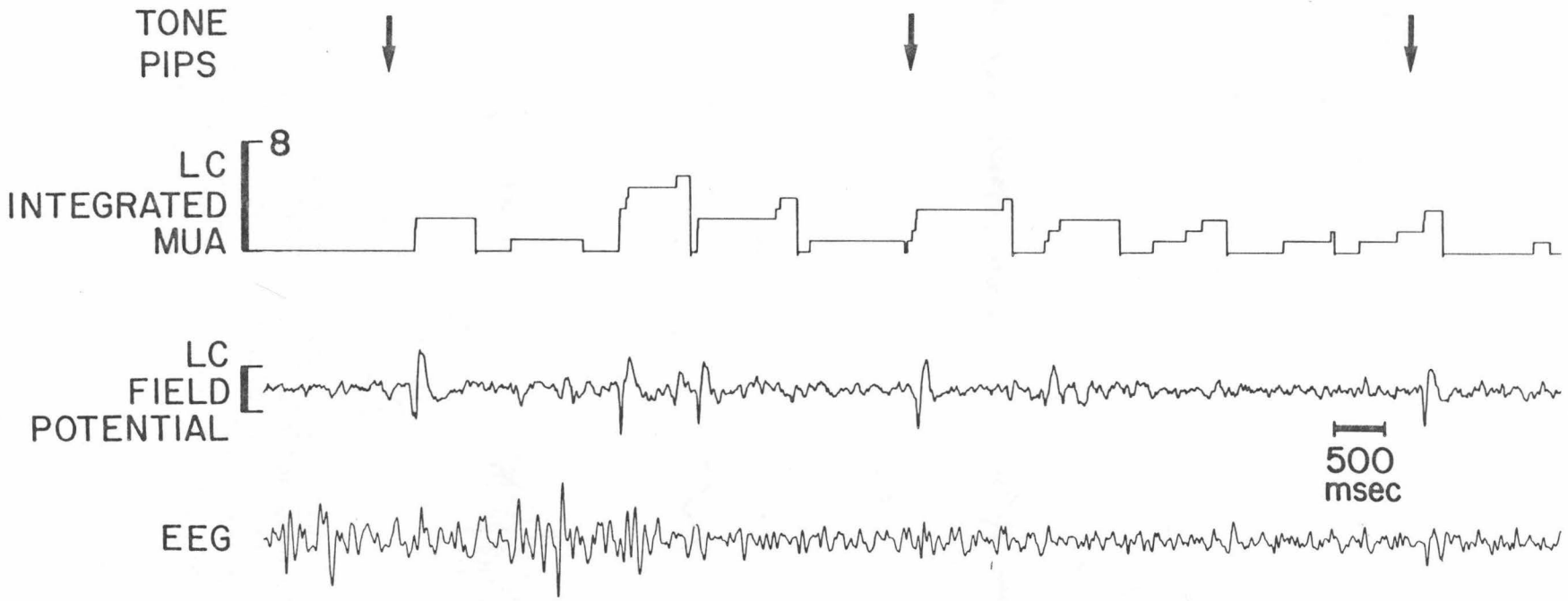


Fig. 10: Sensory-evoked FPs and synchronous unit responses in the NE-LC. Tone pips (at arrows) evoke MU and FP responses synchronously. Similar FP waveforms and synchronous MU bursts spontaneously occur in ancitipation of W from SWS (between first and second stimuli). Differential recordings, separated into FP and discharge traces from the same electrodes as described in Methods. FP calibration = 100μ V.

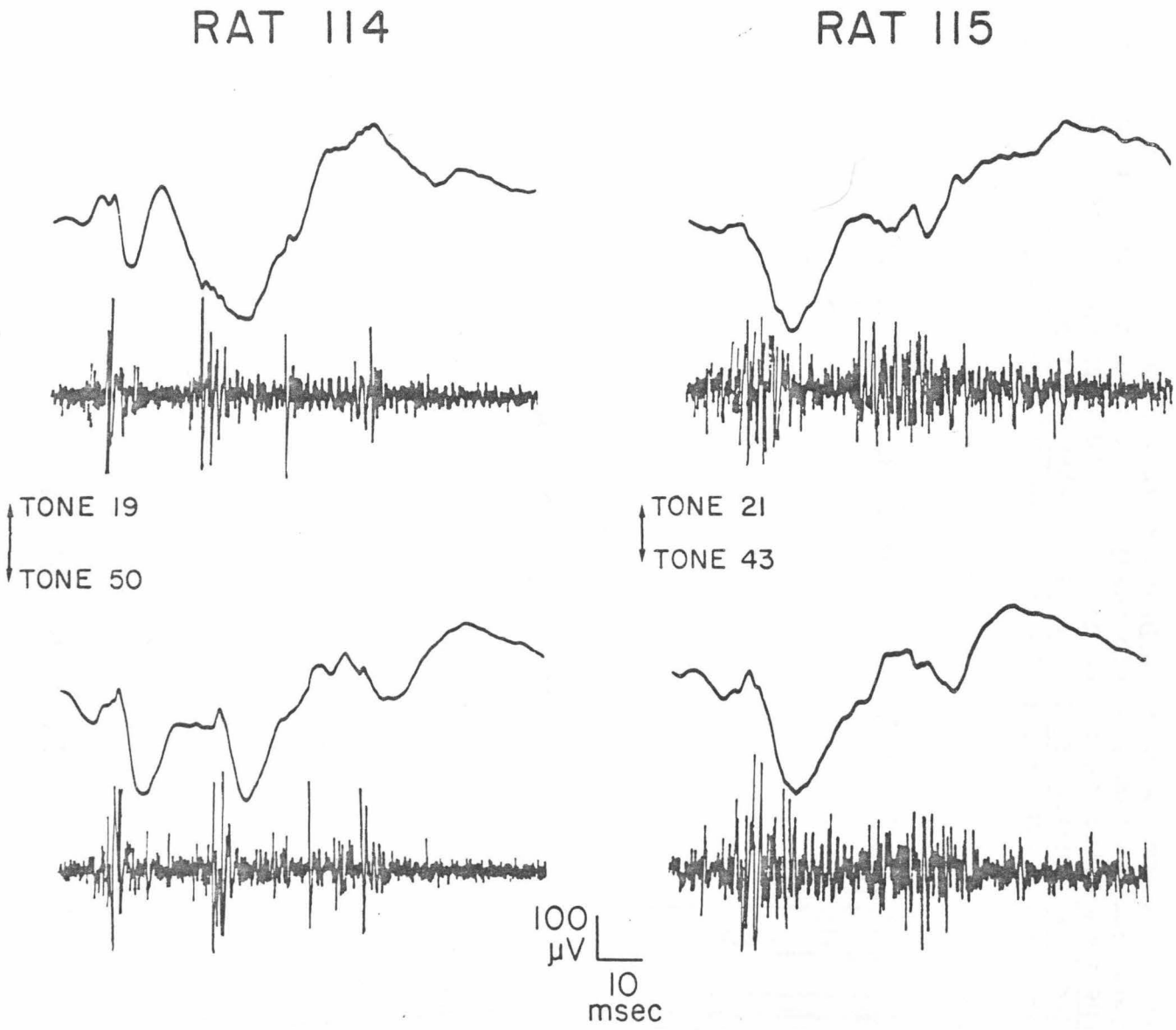


Fig. 11: Analog sensory-evoked NE-LC unit responses and FPs. Single oscilloscope sweeps for 2 tone pip trials for each of 2 NE-LC recordings. Differential recordings, separated into FP and unit traces from the same electrodes as described in Methods. Tone pip onsets are indicated by arrows. Apparent lag in FP vs. unit response partially reflects phase-shifts in FP signal produced by filtering.

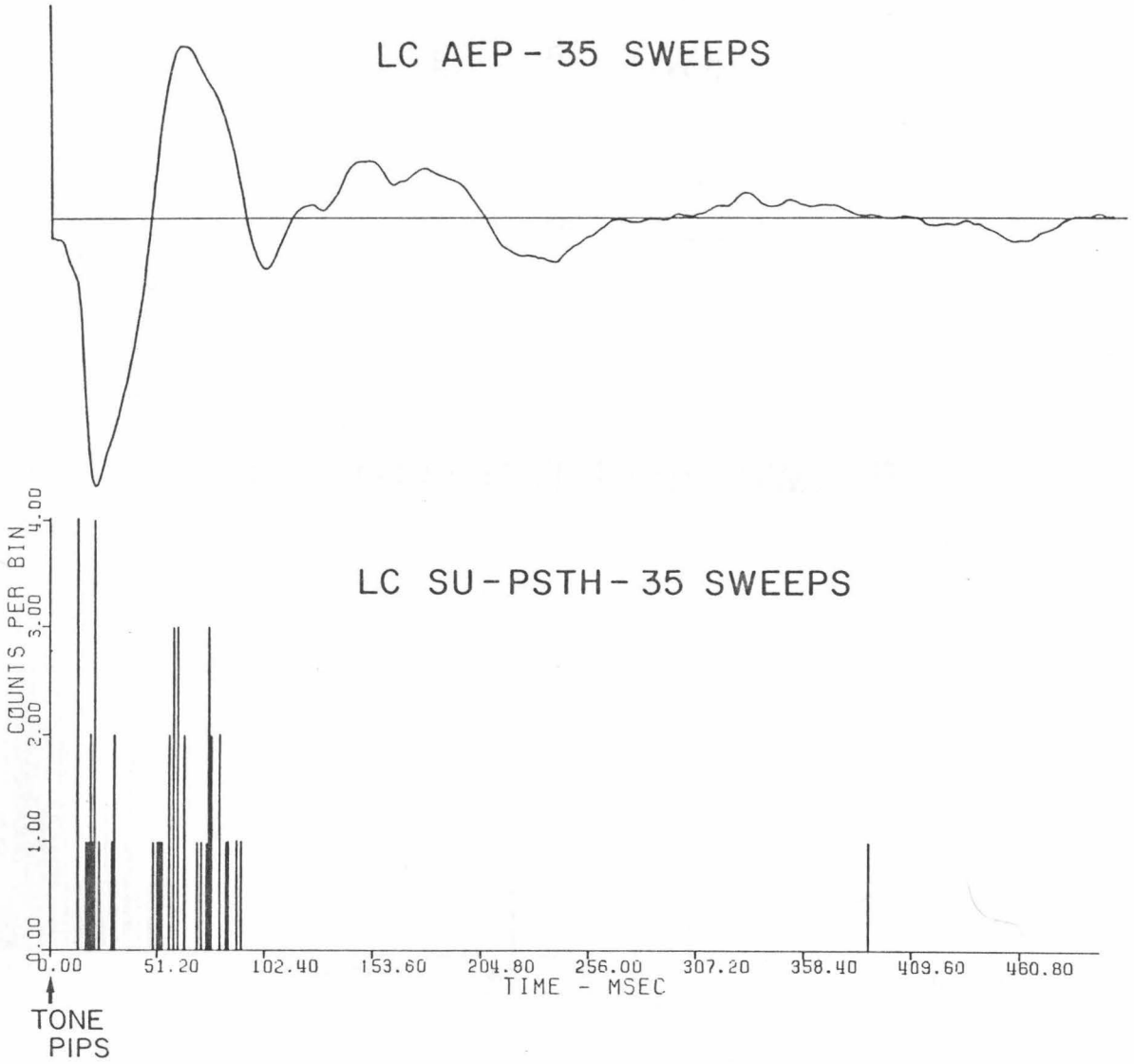


Fig. 12: Sensory-AEP and corresponding SU-PSTH for one NE-LC recording site. Differential recordings, separated into FP and unit traces from the same electrodes, as described in Methods. Both records accumulated for the same tone pip trials (onsets at arrows). PSTH time axis serves for both records. PSTH bin width = 1 msec.

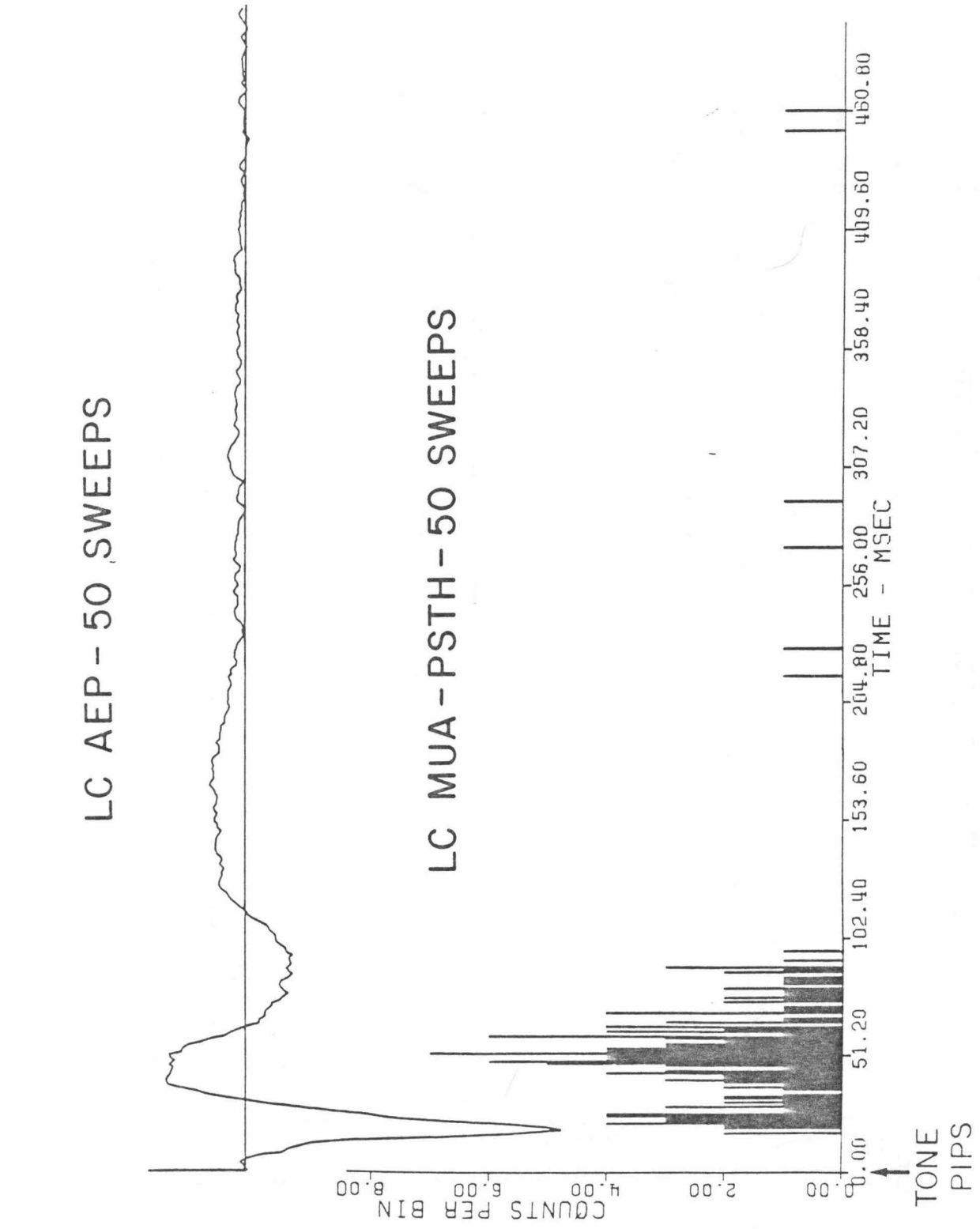


Fig. 13: Sensory-AEP and corresponding MU-PSTH for one NE-LC recording site. Recordings obtained and data illustrated as described for Fig. 12.

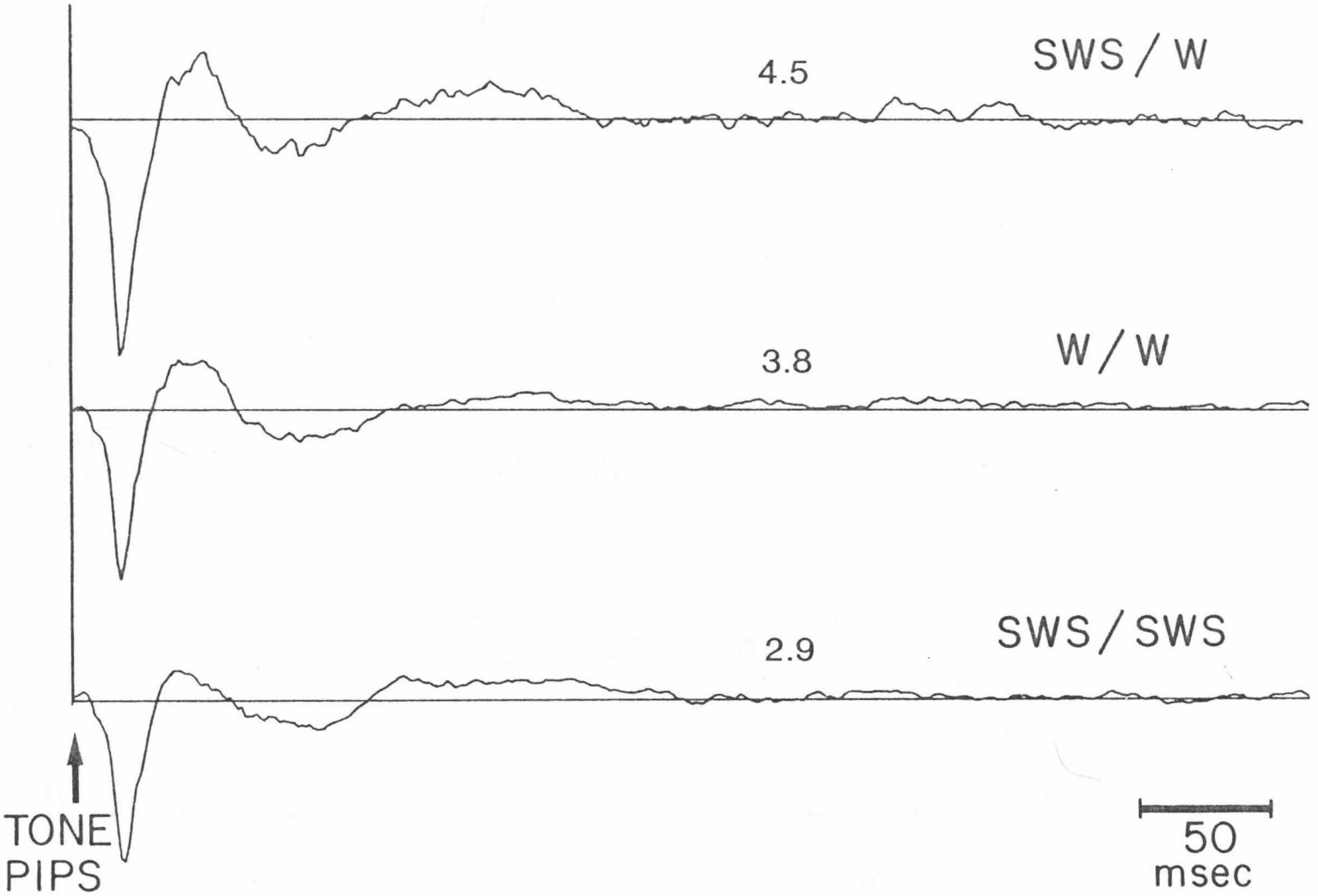


Fig. 14: Sensory-AEP magnitudes in NE-LC as a function of cortical arousal. Three AEPs generated from a single series of consecutive tone pip trials, segregated by cortical arousal categories. Magnitudes were normalized for number of trials. Above each AEP is the corresponding mean number of MU impulses (from the same electrodes) per trial. Differential recordings; 0.1 Hz-150 Hz bandpass filtering for FP trace.

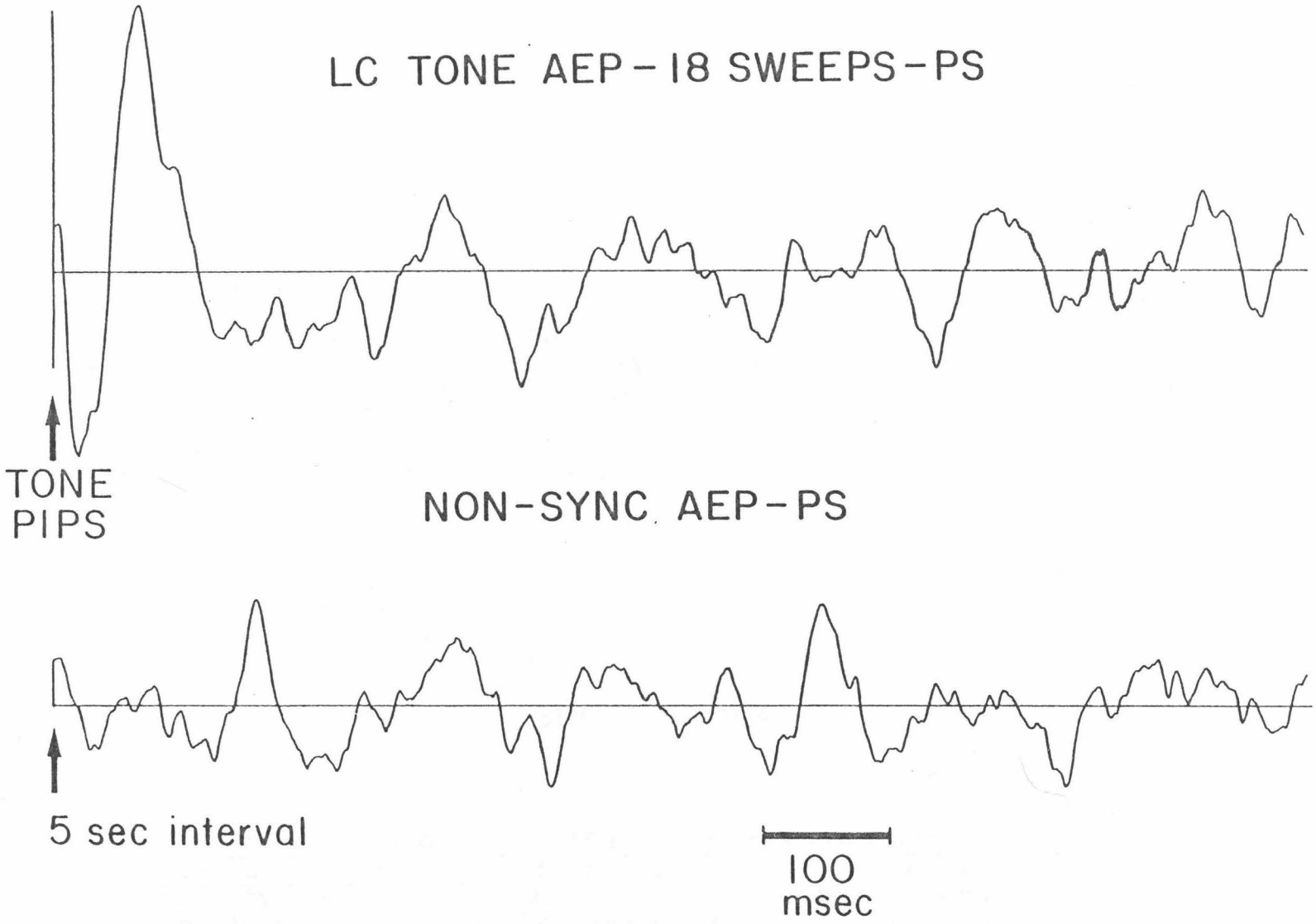


Fig. 15: Sensory-AEP in LC during PS. Sweeps incorporated in the upper trace were triggered at tone pip onsets (arrows; 4 sec intervals). Lower trace was generated from the same PS recording-epoch (on tape), but sweeps were triggered at regular 5 sec intervals, independent of stimulus presentation. Differential recordings, 0.1 Hz- 150 Hz bandpass.

TABLE 1

LC Recording	SLOW, TONIC SPONTANEOUS DISCHARGE	PHASIC, BURSTY SPONTANEOUS DISCHARGE	INITIAL + AUDITORY RESPONSE	INITIAL + VISUAL RESPONSE	INITIAL + TOUCH RESPONSE	BIPHASIC (+ -) RESPONSES IN AT LEAST 2 MODALITIES
SU	35/42		37/38	22/27	24/24	29/30
MU		52/53	51/51	42/43	42/42	46/47

Table 1: Proportions of NE-LC recordings exhibiting characteristic discharge properties. Number of recordings exhibiting column property/number of recordings examined. Properties in columns apply to SU and MU recordings, except for spontaneous discharge, as listed. Plus (+) and minus (-) signs indicate excitatory and inhibitory, respectively.

TABLE 2

STIMULATION	RECORDING	EXCITATION Onset	EXCITATION Offset	INHIBITION Onset	INHIBITION Offset
Tone Pip	SU	18.0 ± 1.0	96.6 ± 5.4 (N=17**)	232.8 ± 54.2	610.1 ± 66.5 (N=17=9** + 8*)
	MU	16.9 ± 0.7	97.6 ± 5.5 (N=15**)	122.1 ± 10.5	760.9 ± 74.9 (N=15=12** + 3*)
Flash	SU	70.8 ± 9.0	324.1 ± 52.5 (N=10**; N=1NS)	961.5 ± 143.6	1141.5 ± 130.2 (N=4=3** + 1*; N=7NS)
	MU	52.0 ± 3.7	136.0 ± 9.9 (N=7**)	329.4 ± 68.4	548.6 ± 75.6 (N=7=6** + 1*; N=5NS)

Table 2: Latencies for sensory-evoked responses in NE-LC discharge. Latencies in msec (mean ± SEM), determined from PSTH analysis using 2 SD criteria (**) or 1 SD criteria (*) (see Methods); note that some flash PSTHs did not meet response criteria (NS = no significant response).

TABLE 3

RECORDING LOCATION	SLOW, TONIC SPONTANEOUS RATE	INITIAL + AUDITORY RESPONSE	INITIAL + VISUAL RESPONSE	INITIAL + TOUCH RESPONSE	BIPHASIC (+ -) RESPONSES IN AT LEAST 2 MODALITIES
	PONTINE SU 50 to 250um from LC				
Parabrachial Nuclei	1/6	1/5	1/2	0/2	1/2
Mesencephalic RF	0/2	-	-	-	-
Pontine RF	3/10	3/5	1/3	1/4	1/3
Mesencephalic Nuc. V	0/4	2/3	1/1	3/3	0/3
Central Gray	1/8	3/7	2/4	1/4	1/3
	PONTINE SU 300 to 3000um from LC				
Parabrachial Nuclei	1/2	0/1	0/1	0/2	-
Mesencephalic RF	3/19	2/9	2/7	4/12	1/3
Pontine RF	2/28	7/15	2/12	2/12	0/6
Mesencephalic Nuc. V	2/5	1/3	0/2	1/2	1/1
Central Gray	1/7	5/5	1/1	2/2	1/5

Table 3: Proportions of non-LC pontine SU recordings with discharge properties characteristic of NE-LC neurons. Same format as in Table 1. Properties in columns apply to all pontine recording locations. RF = reticular formation; Nuc. V = nucleus of the fifth cranial nerve.

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Brain aminergic axons exhibit marked variability in conduction velocity

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Impulses in rat locus coeruleus neurons exhibit pronounced conduction latency decreases, followed by even larger latency increases (of over 20 msec in some cases) during a single train of antidromic activation. The magnitude of latency fluctuation varies as a function of basal antidromic latency, frequency of stimulation, and number of stimuli in a train. These and additional data indicate that this variability in latency is a consequence of altered impulse conduction velocity along the axons, perhaps reflecting reduced ion concentration gradients resulting from impulse propagation. These latency changes may allow thin unmyelinated axons to influence target cells most effectively with short bursts of activity, and suggest that myelination and large axon diameter provide for high fidelity as well as for high velocity of impulse flow in nervous tissue.

It is generally conceived that axons transmit impulses with constant velocity and act as simple transmission lines between soma and synapse. Recent studies, however, have suggested that certain axons can modulate their own impulse flow[8,18,24,25]. We now report that noradrenergic neurons of the rat locus coeruleus (LC) exhibit marked alterations in axonal conduction velocity following spontaneous or evoked impulse activity. This property may serve to modulate the effectiveness of LC activity on its target cells, and suggests that fine, unmyelinated fiber systems may be unable to transmit information with the temporal fidelity of large diameter axons.

Nineteen male Sprague–Dawley rats (300–400 g) were anesthetized with chloral hydrate (400 mg/kg i.p.) and a twisted pair of 250 μm wires was placed in the anterior cingulate cortex (CC) and also, in 9 of these rats, in the olfactory bulb (OB) for stimulation of LC axons (Fig. 1A). Stimulations ranged from 0.5 to 2 mA amplitude and from 0.2 to 0.5 msec duration (about 1.5–2 times threshold). Glass micropipettes filled with a Pontamine sky blue (PSB) dye solution recorded isolated impulses extracellularly from single neurons. Verification of each LC-recording site was accomplished by iontophoresis of PSB from the recording electrode at the end of each penetration, followed by careful histological examination (Fig. 1C).

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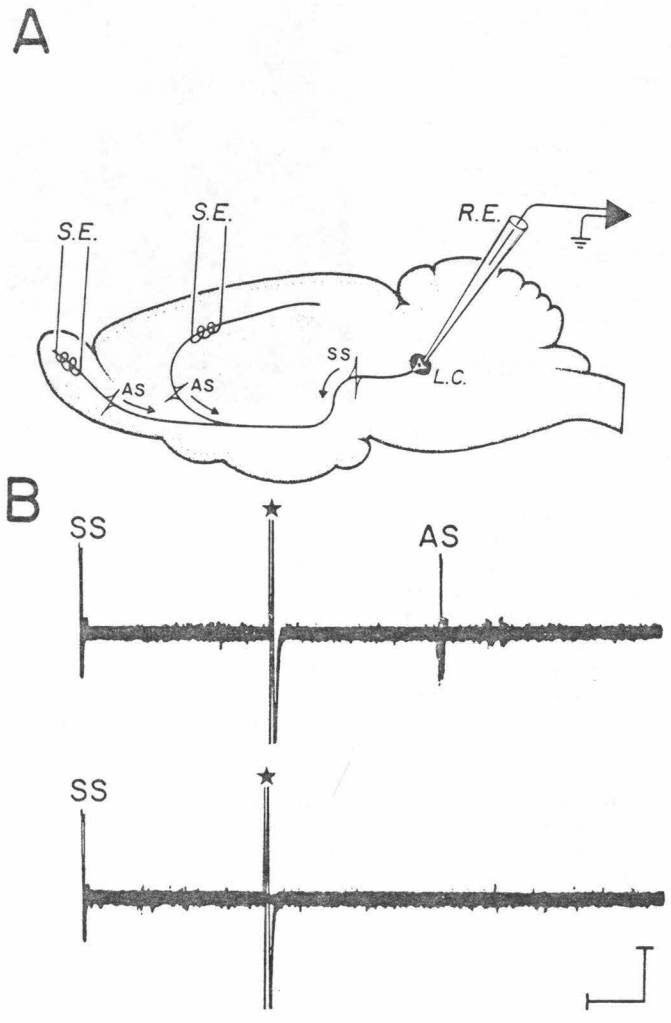
Driven spikes were considered antidromic (AD) if they met collision technique criteria[7], being occluded when stimuli were triggered within a critical interval after spontaneous impulses (Fig. 1A, B). AD impulses typically exhibited a pronounced initial segment (IS) spike followed by a markedly variable delay before soma activation (see Fig. 2D). AD latencies were therefore measured from stimulation onset to the IS spike component in order to determine axon conduction latency specifically.

In the course of these tests, we observed striking alterations in AD latency not reported in previous studies of LC neurons[1,4,20,27,28]. During constant frequency stimulation as low as 2–5 Hz, the AD latency for each of 15 neurons increased in a gradual additive manner, reaching an apparent asymptote after 50–400 stimuli; the value at which the AD latency asymptotically stabilized was greater with higher frequencies of stimulation (Fig. 2A). Furthermore, the magnitude of increase in AD latency was closely correlated with each neuron's basal AD latency ($r = 0.97$; Fig. 2B). However, the *percentage* of increase over basal latency was more constant: many



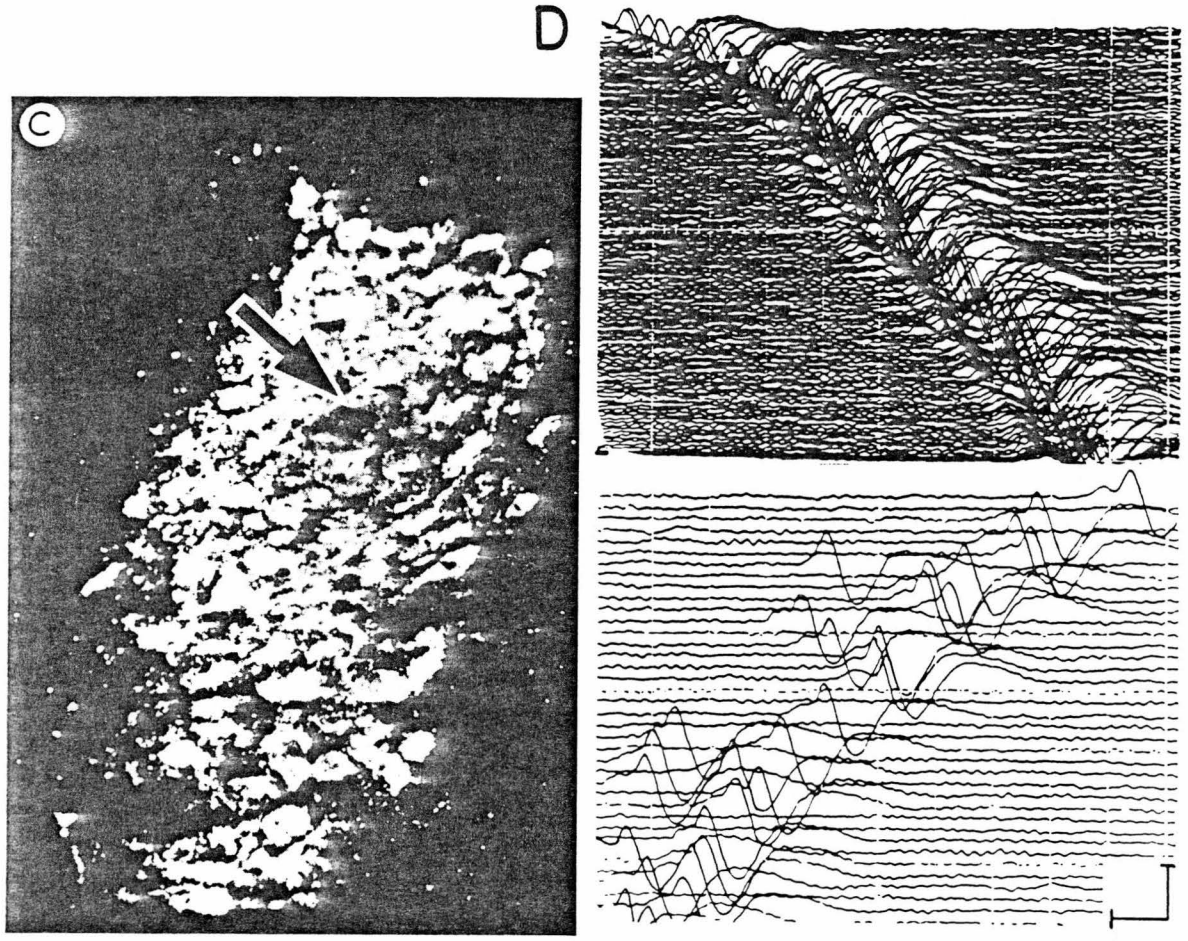
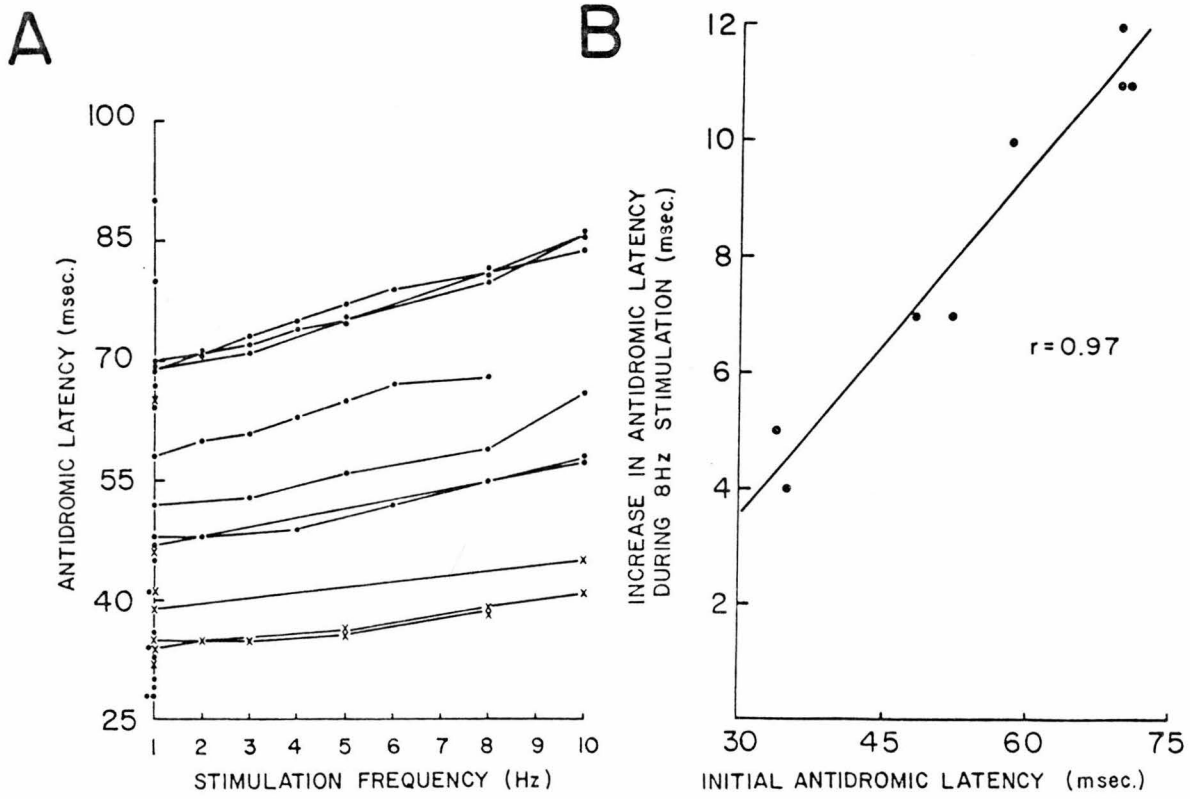


Fig. 1. A: sagittal view of rat brain, illustrating experimental paradigm. Stimulating electrodes (S.E.) in olfactory bulb and cingulate cortex initiate antidromic spikes (AS) in fiber of LC neuron which also carries spontaneously occurring orthodromic spikes (SS); this activity is monitored with recording electrode (R.E.) in LC. B: collision test confirms antidromic (AD) nature of driven spike[7]. In upper trace, spontaneous discharges (SS) are followed by stimuli (marked by star) 65 msec later, which in turn elicit constant latency spikes (denoted AS) 60 msec later. As seen in lower trace, when the stimulation follows SS by 63 msec no driven spikes are observed, demonstrating collision between SS and AS along the same axon. Ten oscilloscope sweeps each trace. Calibrations: 20 msec, 300 μV. (Filtered recordings.) C: red fluorescent PSB spot (arrow; as revealed by recently developed techniques[17]) among green fluorescent LC neurons confirms recording site. D: upper sweeps, latency of typical AD spike increases during a 10 Hz stimulation train. Lower set of sweeps demonstrates recovery of AD latency when stimulation frequency lowered to 1 Hz. Oscilloscope time base was triggered 48 msec (the basal AD latency for this neuron) after each stimulation. Sweeps are displayed in consecutive order from top to bottom. Note that during the 1 Hz stimulation, spontaneous spikes sometimes occluded driven spikes. The larger jumps in latency apparent in some of the 1 Hz sweeps may reflect reduced AD latency typically observed when spontaneous discharges closely precede AD stimuli (see text and Fig. 2D). Calibrations: horizontal bar = 1 msec for upper and lower sweeps: vertical bar = 1.25 mV, containing about 15 sweeps (upper); 1.0 mV, containing about 5 sweeps (lower). (Filtered recordings.)

cells differing widely in basal AD latency and magnitude of latency increase yielded similar percentage increases in latency. In addition, each increased latency gradually recovered to basal value (over 1–2 min) when stimulation frequency was reduced to 1 Hz. In contrast to the number of stimuli, stimulation frequency and basal AD latency, increased stimulus amplitude (up to 3 times threshold) produced no AD latency increase in any of 4 neurons tested (2 of these cells did exhibit a 2 msec latency decrease, probably reflecting AD spike initiation further from the stimulating electrode).

Closer examination also revealed that early stimuli in 10 Hz trains yielded slightly *decreased* AD latencies, followed by increased AD latencies (described above) during subsequent stimuli in the same train (Fig. 2C). In each of 3 LC neurons stimulated with pulse pairs (of 10–100 msec inter-pulse intervals), a 0.5–3.5 msec decrease in the AD latency of the second driven spike was consistently observed. As with AD latency increase, the magnitude of AD latency decrease was positively correlated with basal AD latency magnitude. Furthermore, in each of 16 LC neurons a similar decrease in AD latency resulted when spontaneous impulses triggered stimuli after a short fixed delay (ranging from 1 to about 20 msec greater than the collision interval for each cell); these AD spikes also yielded more constant latencies than those obtained with 1 Hz, non-contingent stimuli (Fig. 2D). Thus, variations in spontaneous activity preceding AD impulses may result in markedly variable AD latencies (of over 3 msec in the present experiments).



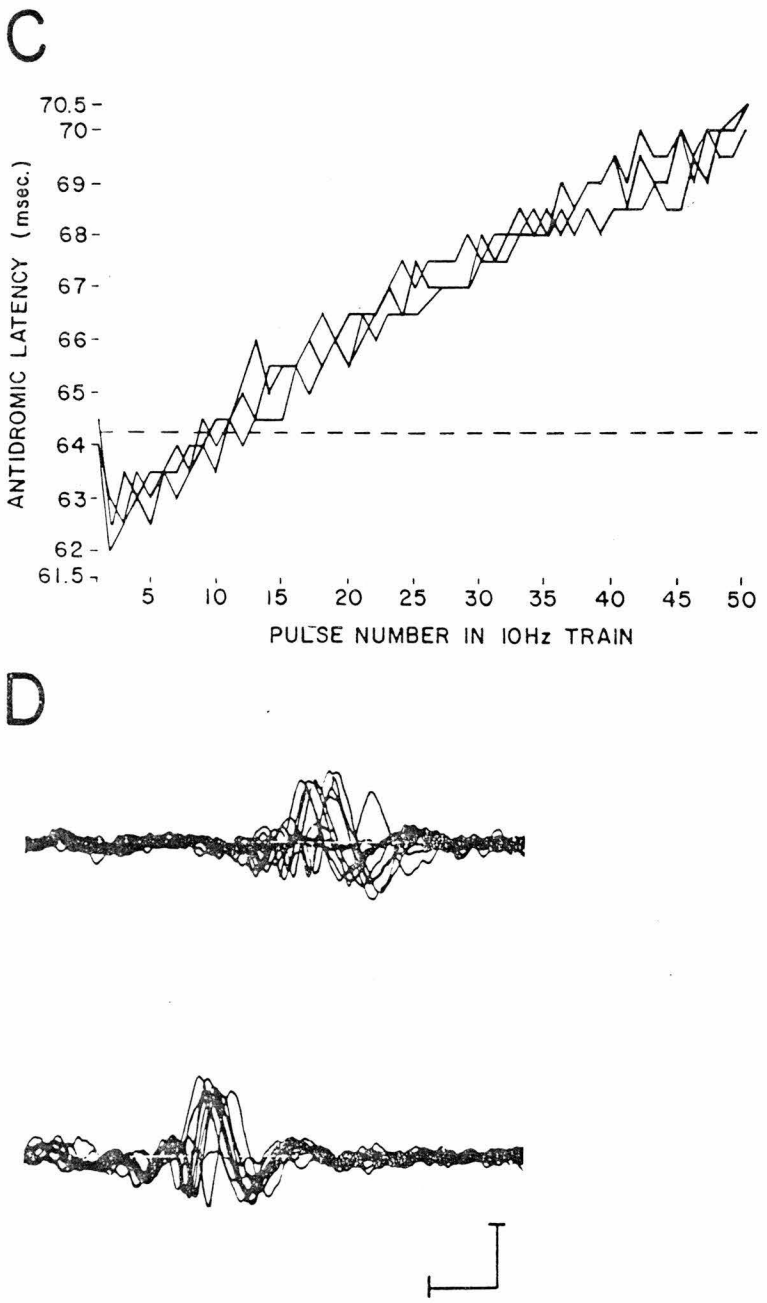


Fig. 2. A: maximum observed antidromic (AD) latency of each LC neuron plotted as a function of stimulation frequency. Each line connects the data points obtained for a single neuron. Stimulation at each frequency was presented until an apparent asymptote in AD latency had been achieved. Filled circles denote CC stimulation and Xs denote OB stimulation. B: maximum observed increase in AD latency during 8 Hz stimulation plotted for each neuron as a function of each corresponding basal AD latency during 1 Hz stimulation. C: latency of each AD spike plotted as a function of stimulus pulse number; results from 3 successive, identical trains of 10 Hz stimulation for the same neuron are superimposed. Dotted line denotes basal AD latency of this neuron, obtained with 1 Hz stimulation. Note the period of latency decrease followed by latency increase during each train. D: upper trace is 10 superimposed oscilloscope sweeps showing AD spikes (latency about 69 msec) generated with 1 Hz stimulation in CC, irrespective of the cell's spontaneous discharges. Lower trace is 10 superimposed sweeps recorded from the same LC neuron when stimulation was triggered 74 msec after spontaneously occurring spikes; note shorter and less variable latency, especially for the IS component of AD spikes. This reduced variability may arise from the less variable history of impulse activity preceding AD stimuli. Oscilloscope time base for all traces was triggered 65 msec after stimuli. Calibrations: 1 msec; 200 μV (Filtered Recordings.)

Four lines of evidence suggest that altered impulse conduction velocity along LC axons underlies these AD latency fluctuations: (1) increasing the stimulation voltage produced no increase in latency; (2) the magnitude of decrease and of increase in AD latency was positively correlated with each neuron's basal latency value; (3) spontaneous spikes closely preceding AD stimuli led to decreased AD latencies. Also, our pilot studies revealed that tonically increased spontaneous discharge activity (induced by piperoxane i.p.[3], from about 1.8 Hz before to 3.5 Hz) resulted in an increased basal AD latency (from 69 msec before to 73 msec). Thus, conduction time variability results from spontaneously occurring orthodromic, as well as antidromically driven, impulse activity; and (4) in preliminary studies of AD activation from both CC and OB in the same neuron, 10 Hz stimulation of either site resulted in increased AD latencies not only for later impulses in that train, but also for initial AD impulses when the stimulation was switched to the other, previously non-stimulated, site. This rules out the stimulation site as the locus mediating AD latency increases.

Whereas most axons studied have sufficient internal volume to buffer the ion fluxes produced by many thousands of action potentials, impulses in very fine diameter axons (estimated at 0.1 μm[12] for LC), with correspondingly large surface area-to-internal volume ratios, may significantly decrease the axon K^+ concentration gradient and thereby decrease its resting membrane potential. Indeed, our calculations (using values obtained from rabbit vagus C fibers[16]) indicate that 100 impulses at 10 Hz in a 0.1 μm diameter unmyelinated axon could reduce the internal $[K^+]$ enough to depolarize the resting axon by about 7 mV. Such tonic depolarization could, by increasing the membrane's resting Na^+ inactivation level and G_K, significantly increase the threshold for impulse production and thereby slow impulse conduction velocity and increase AD latency. Our calculations also indicate that the first 5 impulses in such a train would only depolarize the resting membrane by about 0.3 mV, perhaps enough to speed impulse conduction (thereby decreasing AD latency) by moving the resting membrane potential closer to impulse threshold, but not enough to significantly alter the threshold level. The myelination and large axon diameters in many other fiber systems may, therefore, provide not only high impulse conduction velocity but also high temporal fidelity among impulses.

Our results resemble data previously reported for other systems, in demonstrating: (1) a conduction latency decrease at the onset of stimulation[8,18,25]; (2) a latency increase after a longer period of stimulation[25]; (3) a similar range of percentage change in AD latency (2–25%); and (4) variability in AD latency as a function of variability in spontaneous impulse activity immediately preceding stimuli[26]. However, a number of novel results in the present report should also be noted: (1) significant latency increases occurred with stimulation frequencies as low as 2 Hz; (2) AD latencies increased and recovered gradually, exhibiting no sharp threshold but rather a continuously additive, asymptotic change during the period of activation; (3) decreases in AD latency preceded AD latency increases during the same train of constant frequency stimulation; and (4) the magnitudes of fluctuations in AD latency (over 20 msec in some cases) were much larger than those reported for other axons. These novel results were fundamental in suggesting (and strongly support) the

above proposed mechanism for LC impulse latency changes; the similarities between the present results and previous data from other labs suggest further that our proposed mechanism underlies not only AD latency fluctuations in LC neurons, but also those reported for other fiber systems, and may, therefore, reflect general nervous tissue properties[6,13,15,21]. Supporting this possibility, we have also observed AD latency increases in the thin unmyelinated axons of the raphe and substantia nigra systems similar to those reported here for LC neurons.

Our results are consistent with the notion[19] that the function of the locus coeruleus system involves global events of long duration (hundreds of msecs) in which exact temporal fidelity among impulses may not be required. In addition, the phenomenon of activity-dependent latency variability may serve to modulate the post-synaptic efficacy of impulses in thin unmyelinated axons: the effectiveness of early impulses (in a high frequency train) on target cells would be enhanced as a consequence of temporal convergence and synaptic facilitation. In contrast, the temporal summation of later spikes in such a train would be reduced, as would the quantity of transmitter each would release (due to tonic depolarization), thereby decreasing their efficacy. In addition, we anecdotally observed that LC axons often fail to conduct long trains ($>$ 20 sec) of impulses stimulated at frequencies of about 20 Hz or more. Our results predict, therefore, that long high frequency trains of impulses in LC neurons would produce less marked effects on target cells than lower frequency or shorter trains of activity. In fact, many previous studies on the post-synaptic effects of LC stimulation support this prediction[2,11,22,23,29]. Additional support is found in the results of our studies using freely behaving rats[5,14]: in this preparation, LC neurons spontaneously fire at a low rate (about 2 Hz) and typically exhibit a higher frequency (about 10–20 Hz) burst of 2–6 impulses in response to sensory stimulation or arousal. Thus, decreased conduction latency (and consequent increased post-synaptic efficacy) may occur for impulses in LC neurons during physiologically normal brain activity.

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CONCLUSION

Chapters 1 and 2 described studies of impulse and FP activities in the NE-LC of unanaesthetized behaving rats with the following general results: (1) Spontaneous discharge co-varied with stages of the S-WC, yielding tonic rates that were directly proportional to vigilance. (2) Spontaneous discharge consistently changed in anticipation of tonic cortical epochs (S-WC stages, except W after PS) as well as phasic cortical events (spindle activity during SWS). (3) FPs occurred spontaneously in NE-LC recordings, temporally synchronized with unit activity from the same electrodes during W and SWS, but at highest rates during PS, when discharge was virtually nil. (4) Responses in discharge and FP activity were synchronously evoked by mild, non-noxious environmental stimuli. (5) The magnitudes of sensory-evoked responses varied as a function of vigilance. (6) Spontaneous and sensory-evoked discharge decreased during grooming and consumption, similar to results obtained for sleep. (7) Spontaneous or sensory-evoked interruptions of ongoing behavior (such as sleep, grooming or consumption) were typically accompanied by phasic robust discharge. (8) Discharge was not apparently linked to movement *per se*. (9) SU and MU recordings throughout the nucleus yielded remarkably homogeneous results. (10) Phasic robust discharge was typically synchronized among neurons in MU populations.

Chapter 3 described studies on the impulse conduction properties of NE-LC axons. General results were: (1) Impulse conduction velocity fluctuated as a function of basal conduction latency, impulse rate, and number of impulses in a train of activity. (2) Impulse conduction velocity

initially increased, then gradually decreased markedly for later impulses in the same train of activity. (3) Large magnitude increases in conduction latency occurred during low-frequency trains of impulses. (4) Calculations indicated that these axons may modulate their own impulse flow as a result of ion fluxes during spike propagation.

Previous studies have demonstrated that NE released from LC neurons can increase signal-to-noise ratios in target cell impulse activity (see Introduction, Chapters 1 and 2). These results in combination with present data indicate that, in physiologically normal contexts, salient environmental stimulation or spontaneous alertness is accompanied by a global release of NE, which may then enhance CNS activity linked to strong stimuli and simultaneously dampen activity associated with weak inputs. Behaviors which do not have significant value in coping with phasic, unexpected external events (such as sleep, grooming or consumption) are propagated by relatively weak or low-priority CNS activity, rendering them easily disruptable by robust NE-LC discharge. This would be consistent with the present proposal that such tonic, endogenously generated vegetative behaviors may be enabled by tonically reduced NE-LC discharge.

The previous data on the postsynaptic effects of NE are crucial in generating an hypothesis of NE-LC function. Other previous results are also important in this regard: (1) The ubiquitous NE-LC efferent fiber system indicates that these neurons serve a very global function, one that is generalizable over widely distant and disparate CNS processes. (2) The pronounced clinical and behavioral effects produced by pharmacological manipulations of NE activity in brain indicate a substantial and pervasive

suggests that robust NE-LC activity has a role in generating the associated change in behavioral state.

Thus, NE-LC discharge may be controlled by phasic, exogenously determined inputs as well as by tonic, endogenously controlled afferents. Such powerful gating suggests specific behavioral consequences of NE-LC activity. A working hypothesis generated in the present studies states that robust NE-LC output may suppress CNS processes which have minimal value in phasically coping with unexpected external events, and simultaneously enhance driven activity within systems primarily concerned with such immediate behavioral responses. Conversely, tonically reduced NE-LC discharge may enable endogenously generated brain programs which mediate tonic vegetative behaviors. In this way, the NE-LC system may bias the global orientation of behavior between the external and internal environments.