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19 ABSTRACT (Continue on reverse if necessary and identify by block number) We have developed techniques for recording stable unit activity from individual monkey locus coeruleus (LC) neurons using microwire electrodes (25 μm diameter). A combination of improved electrode design, new microadvancer and methods to accurately localize the LC nucleus now permits stable recordings of high signal/noise (better than 3/1) from single neurons in LC for several hours in the waking monkey performing a vigilance task.

We have found that LC neurons vary activity phasically and tonically during vigilance performance. Phasic responses are selectively evoked by target cues, and follow new targets during acquisition of reversal in this task. Tonically, LC neurons vary activity levels in accordance with attentiveness to the task, as measured by the frequency of foveating a fix spot required to initiate each trial.

Results indicate that the LC functions to regulate the lability of attention. In this view, performance on a task requiring focused attention varies with tonic LC activity in an inverted U relationship. Too little LC activity is associated with poor performance due to non-alertness, while high tonic LC activity corresponds to highly labile attention that prevents focusing attention for long time epochs. Together, these results indicate that optimal vigilance performance (e.g., radar monitoring activity) may require an intermediate level of LC activity and high phasic responsiveness of LC neurons.

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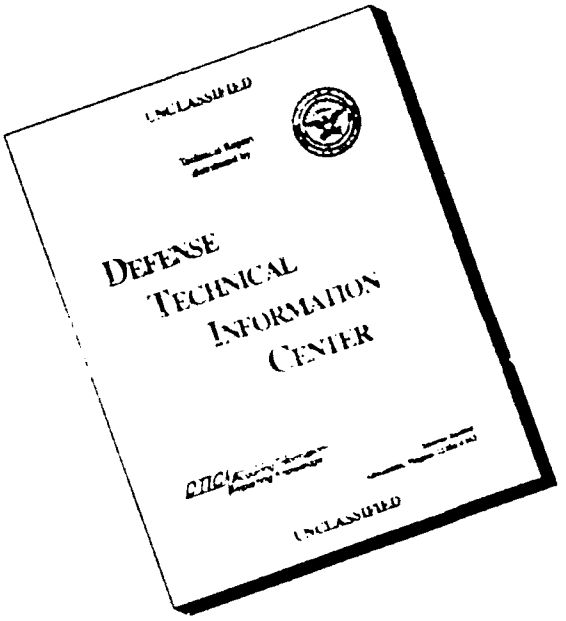

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FINAL TECHNICAL REPORT

AWARD: Grant AFOSR 90-0147

PRINCIPAL INVESTIGATOR: Gary Aston-Jones, Ph.D.

PERIOD COVERED: Dec 15, 1989 through Dec 14, 1992

OBJECTIVES:

(Previous Statement of Work). Our work supported by AFOSR has initiated the study of cellular mechanisms underlying vigilance and selective behavioral responsivity in primates. We have established a behaving primate preparation for recording discharge of locus coeruleus (LC) neurons in brain during performance of a vigilance task that resembles those used in human psychophysical studies. In the present application we propose to continue and extend these studies. (1) We will record monkey LC unit activity during a vigilance task modified to allow a wide range of stimulus presentation and difficulty. Also, eye position and pupillary diameter will be continuously monitored throughout recordings to ascertain (a) trials during which the animal attends to the task and detects sensory cues (gaze directed at task stimuli) vs. those in which attention is directed elsewhere, and (b) concurrent activity in the autonomic nervous system (reflected in pupillary diameter), a measure of stress response during the task and a possibly important concomitant of central systems in mediating adaptive behavioral responsivity. (2) We will monitor cortical electrical events, termed event-related potentials (ERPs), thought to reflect selective processing of meaningful sensory stimuli, and investigate the role of LC in generating these cortical signals in two ways: (a) simultaneous ERP and LC unit recordings will determine the temporal association between these two events; and (b) local microinjections of selective pharmacologic agents will be used to transiently and specifically inactivate or activate NE-LC neurons while recording ERPs. Such specific manipulations of LC will also allow analysis of vigilance behavior while LC is either inactivated or activated. (3) We will challenge animals' performance by varying task parameters and introducing distractors and environmental stressors that are known to influence vigilance in humans. LC and ERP activity will be monitored, and in other experiments LC will be selectively activated or inactivated, to test the role of this system in mediating adaptive behavioral responsivity under adverse conditions.

The proposed studies will examine in detail both the temporal association (via LC recordings) and functional dependency (via LC activation and inactivation) between the brain noradrenergic LC system, higher-order attentional processing (ERPs), and vigilance performance during normative as well as during stressful conditions. Results of these experiments will open the way to examination of afferents to LC in future studies, to understand circuits and mechanisms involved in determination and processing of the specific stimulus attributes (novel, unexpected, or threatening) that activate LC.

STATUS OF RESEARCH EFFORT:

This section reviews our progress on this project during the previous award. As reviewed below, major progress included (i) improvement of techniques to record LC discharge in behaving monkeys, (ii) further characterization of monkey LC activity during naturalistic behaviors, (iii) characterization of LC responsiveness to target cues during a vigilance task in relation to tonic LC activity and behavioral task performance, and (iv) specification of short- and long-term relationships between tonic LC activity and stably focused vs. labile attention.

1. Improvement of techniques to record LC discharge in behaving monkeys. Recording from small, deeply located structures in the brain poses significant technical problems. As the reliability of the technique and quality of data obtained are of the utmost importance in recordings from primate, we devoted considerable time and effort during the last funding period to improving our techniques. Novel and original methods for recording from the LC (including a newly designed microdrive, infrared video eye movement monitoring, alignment frame allowing X-rays in stereotaxic planes, and data acquisition and analysis procedures) were implemented,

tested and further adjusted during all successive recordings. This new technology markedly improved the quality of recordings and heightened the yield of LC neurons recorded. Some of these technical advances are described below

We have developed techniques for recording stable unit activity from primate LC using microwire electrodes (25 μm diameter). In our studies, such electrodes were additionally improved by grinding the tip of the wire to a conical shape. These electrodes yield stable recordings of high signal/noise (better than 3/1) from single neurons in LC for several hours in the waking monkey performing a vigilance task. We find this technique to be much better than conventional etched tungsten microelectrodes for recording LC neurons.

We have also designed and constructed a novel microwire holder and advancer for recording unit activity, and developed improved methods of localizing LC. This recording device is especially suited for penetrations into deep brain structures in behaving animals. In brief, a screw-driven microdrive assembly is attached to a small stereotaxically implanted cylindrical pedestal, with the guide cannula stereotaxically positioned 5 - 8 mm above the LC. This design allows small but accurate repositioning of the guide cannula between recording sessions, permitting rostrocaudal and mediolateral adjustments in the initial stereotaxic position so as to precisely localize LC, and allowing multiple penetrations throughout different areas of the LC nucleus to record from a large number of cells in each hemisphere. Due to the abundance of easily identifiable electrophysiological landmarks in the vicinity of LC (auditory responses in inferior colliculus; cell activity with eye movements in the trochlear and abducens nuclei; cell activity with jaw movements in the Mesencephalic V nucleus; large, fast spikes with distinctive complex spike bursts in the cerebellum), and the readily recognizable discharge characteristics of LC neurons, it typically takes only a few penetrations to locate LC after the surgery. LC neurons are readily identified by their distinctive broad spike waveforms, slow steady discharge, marked decrease in activity with drowsiness, and biphasic (excitatory-inhibitory) responses to novel stimuli. As changing electrode tracks does not entail exposing the dura or brain tissue, the possibility of infection is greatly reduced. In our studies using these techniques, unit recordings have been obtained from individual animals for over 6 months with no signs of infection. Also, as only 1 or 2 microwires (25 μm dia.) pass through the region of LC, damage to this area is slight even with multiple penetrations; no gross damage has been observed in histology from the LC in our previous experiments. The device also allows for replacing damaged electrodes, or switching between single- or multiple-wire electrodes, stimulating electrodes or "chemtrodes" (combined recording/infusion electrodes). *Data have been obtained from more than 200 LC neurons in behaving monkeys with these new techniques.*

We have designed and implemented an alignment frame that permits X-ray images to be made in stereotaxic planes. This device is a simple Plexiglas frame that fits onto the A-P bars of a Kopf stereotaxic frame, over the animal's head. The fixation post is positioned in a post-holder, which is then positioned in the X-ray frame so that the post is properly positioned on the animal' s head. The post is then cemented to the animal's head, and the post-holder is cemented to an opening in the top of the frame. After the cement hardens, the post is released from the post-holder, and the X-ray frame is removed. The post-holder remains cemented to the X-ray frame throughout experiments for the monkey; each monkey has his own X-ray frame (they are recycled after sacrifice). At any later time, the animal can be re-anesthetized with ketamine and placed back into the X-ray frame by inserting the head-mounted post into the post-holder. This places the animal's head in stereotaxic position with respect to the X-ray frame. In making X-ray photographs, the alignment pins and ear bar markers (inserts temporarily placed in the animal's auditory meatus) are used to align the frame with respect to the camera. The ear bar inserts then are used to measure stereotaxic positions in the X-rays. We have found this technique, combined with the distinctive characteristics of LC neurons and the landmarks around LC, to be very useful in guiding our initial adjustments to the LC cannula, and to shortening the time that is required to find LC with recording electrodes after surgery.

2. *Monkey LC discharge during naturalistic behaviors, and in response to unconditioned sensory stimuli.* As previously reported in various species, monkey LC neurons decreased activity with decreased arousal (drowsiness) and during certain other behaviors characterized by

lowered attentiveness to the sensory environment (e.g., grooming and eating). Abrupt transitions in behavioral state to increased arousal were consistently associated with (preceded by) increased discharge of LC neurons. As found for other species, LC activity in monkey was most phasically active during such state transitions to high vigilance, and was highly correlated with orienting behaviors.

LC activity in monkeys was also activated by novel or intense unconditioned sensory stimuli. Similar to results in rat and cat, we found that monkey LC cells responded to such stimuli with a brief excitation following by a more prolonged period of diminished activity. As with spontaneous discharge, sensory-evoked LC activity was most intense for stimuli associated with an orienting behavior indicating increased vigilance; conversely, periods of low vigilance were associated with reduced sensory responsiveness.

These observations for sensory LC responses in monkey are consistent with the overall observation that LC neurons were most active, and responded most intensely to stimuli, in association with orienting behaviors and an apparent increase in vigilance. As conspicuous or complex stimuli most consistently elicited these behavioral responses and associated state changes, the above data for stimuli effective in driving monkey LC fit with our previous behavioral analyses of rat LC activity. Overall, these data indicate that sensory stimuli effective in eliciting LC discharge have specific attributes. It appears that LC is geared to respond to stimuli that are conspicuous to animal: stimuli which by their physical or behavioral properties evoke a change in attention.

3. Discharge of monkey LC neurons during a sustained attention task. If the LC regulates vigilance and attention to salient cues as we have proposed, we reasoned that it should respond not only to physically intense stimuli but also to conditioned, meaningful stimuli that require an immediate behavioral response but that are not conspicuous by virtue of their physical attributes. We tested this prediction in recordings of LC neurons in waking cynomolgus monkeys performing an oddball visual discrimination task. In this task, the animal was required to continuously depress a pedal and attend to visual cues. In most of our recordings, the animal was also required to foveate a small spot in the center of the video display to initiate each trial, thereby ensuring attentiveness to the task. Release of the bar within 500 msec after a target cue (vertical or horizontal bar) was rewarded by juice; incorrect releases or misses were followed by a time out. Target cues were presented randomly on 10% or 20% of trials, and the non-target cue was presented on 90% or 80% of the trials. Thus, this task required the monkey to attend over a long period of time (a single session often lasted one hr), withhold responses to the frequent nontarget cues, and respond selectively to infrequent target stimuli. This task is similar to those used in human studies of vigilance and sustained attention.

Single- and multi-cellular activity were recorded from 161 LC neurons in 4 Cynomolgus monkeys performing this vigilance task. We also recorded cortical event-related potentials (ERPs) in response to the sensory cues, as previous work in humans and in monkeys indicated that such slow-wave activity may signal attentional processing in the brain. Initial results of these studies are found in our recent publications. Impulse activity of LC neurons was stable during performance of this behavioral task; single cells could be routinely tracked for hours. Peri-stimulus time histograms (PSTHs) were generated for target stimuli which were followed by a lever response within the allowable delay (hits), for nontarget stimuli followed by a lever release (false alarms), for target stimuli that did not elicit lever responses (misses), for lever responses regardless of stimuli, and for delivery of juice.

Analyses of these histograms across cells revealed a great deal of specificity in activity of LC neurons during this task. Only one cell had activity specifically related to lever release, and only one cell exhibited no response to any parameter examined. The largest category of cells (81/134) were activated selectively by target stimuli but not by nontarget stimuli, lever release or juice delivery; 9/134 cells were selectively inhibited under the same set of circumstances. Twenty-six of the 134 cells were activated by both target and nontarget stimuli, but for these cells responses to target stimuli were substantially more robust than nontarget-elicited activity. These results indicate that LC neurons are responsive to non-conspicuous stimuli that are meaningful by virtue of conditioning, and that require an immediate response.

Recordings during reversal training further supported these conclusions. Within 15 min of reversing target and non-target cues cells terminated their response to the previous target stimulus and began selectively responding to the new target (previously nontarget) cue. Thus, these responses were specifically related to the meaningfulness of the stimuli, not to their physical attributes. Interestingly, these changes varied closely with behavioral performance, so that responses to the new target cue increased (and responses to the new non-target cue decreased) as the percentage of correct behavioral responses to the new target cue increased (and behavioral responses to the new non-target cue decreased). Together, these results are consistent with the possibility that phasic, sensory responses of primate LC neurons has a role in facilitating responses to significant sensory stimuli.

In addition, cortical ERPs exhibited a similar set of properties. That is, long-latency ERPs (200-300 msec) were selectively elicited by target, but not by non-target, cues. These potentials also reversed with behavior and LC responses during reversal training, so that the long-latency ERPs came to be selectively elicited by the new target cue. These results are consistent with the possibility that LC responses to target stimuli may participate in the generation of the long-latency ERP activity. This would be consistent with other results indicating that LC lesions decrease the amplitude of ERPs in monkeys.

Therefore, there is a close relationship among LC-NE neurons, cortical ERP activity, and behavioral responding to meaningful sensory cues. These results indicate that LC responses can be conditioned to salient stimuli and events in the environment, a potentially important attribute for understanding the role of this system in attentional processing.

4. Fluctuations in monkey LC tonic and sensory evoked activities are associated with alterations in focused attentiveness and task performance. LC tonic activity and task performance - In our recent studies we have observed that all 10 LC neurons analyzed from selected long-duration recordings alternated between 2 discrete levels (rates) of spontaneous activity, with each level lasting tens of min. These levels changed in an abrupt "step"-like fashion). In some of our recordings lasting for several hours, LC neurons switched between 2 levels of long-term discharge several times. The difference in discharge rates of these levels was small, in the range of 1 - 2 spikes/sec, but nevertheless quite conspicuous. As described in a preliminary report, these different levels of LC discharge were closely associated with differences in behavioral performance on the vigilance task described above. The periods of elevated LC activity were consistently accompanied by decreased vigilance performance, caused primarily by an increase in the rate of false alarms (lever responses to nontarget stimuli) and increased latencies of bar release following target stimuli, another indication of decreased vigilance performance. In addition, a vigilance decrement is evident for epochs of both low and elevated LC discharge; the major change in vigilance behavior during elevated LC activity is lower *overall* performance than during epochs of lower LC activity. Thus, during the high resting level of LC basal discharge animals responded indiscriminately by eliciting more responses for non-target stimuli, and also exhibited longer latencies in response to target stimuli, perhaps reflecting lower overall vigilance performance. There is also a suggestion in of a more rapid vigilance decrement during epochs of higher LC activity. Additional cases are needed to confirm this possibility. The rate of correct responses ("hits") did not increase with the increased false alarm rate during periods of higher LC activity, as might be expected. In fact, hit rates decreased slightly at these times. Analyses using signal detection theory indicate that during periods of higher LC activity the discriminability of stimuli (d' factor) decreased, while the animal's criterion for responding (ß factor) remained roughly the same as for intermediate levels of activity. One interpretation of these results is that during the higher LC activity the animal was less attentive to the task stimuli (making it more difficult to discriminate target from non-target stimuli), but that his tendency to respond (response criterion) did not change from that of intermediate LC rates. If this analysis is borne out in additional cells and animals, it may mean that LC activity is more involved in the input (sensory) aspects of attention than in the output (motor response) components.

LC fluctuations and changes in focused attentiveness - We also analyzed the frequency with which the animal successfully visually fixated the fix spot, required to initiate each trial. As in

other experiments using this method, foveation of the fix spot in this task is effortful and is a measure of the animal's attentiveness and engagement in the task. As described in a preliminary report, epochs of increased LC activity corresponded to decreased frequencies of fixation; conversely, successful fixation increased during epochs of lower LC discharge. This inverse relationship between LC activity and visual fixation was found to be highly statistically significant for every cell tested using correlation analysis. Note, however, that very low LC discharge was consistently associated with drowsiness as previously reported (described above), and corresponded to low (or no) foveation and little overall task performance. Thus, tonic LC activity in these experiments was related to attentiveness to the task by a curvilinear (inverted U) relationship, with best task performance corresponding to an intermediate level of tonic LC discharge. These results were not expected, and imply that *focused attention corresponds to an intermediate level of LC activity*.

It is possible that these changes in LC activity cause the changes in attention. However, it is also possible that the altered LC activity is not causative of, but rather results from, the changes in attention. Experiments are underway to discern these functional relationships by locally activating or inactivating LC neurons during attentional tasks and measuring the effect on task performance. Our working hypothesis is that very low activity (during drowsiness) provides too little vigilance or alertness for task performance, while high activity results in a level or type of vigilance that is not conducive to focused attention (required for task performance). Specifically, we propose that elevated LC activity may promote a mode of scanning or labile attentiveness, in which the attention span is short and easily altered by exogenous stimuli. This relationship resembles previous arousal models of vigilance function, and may offer a neural substrate for the curvilinear Yerkes-Dodson relationship between arousal and performance.

In addition to the long-term changes in resting discharge described above, LC neurons also exhibited short-lasting fluctuations in activity (epochs 30-60 sec long). These transient discharge levels were too brief to allow ready analysis of different false alarm rates or bar lease latencies as found for the long-term discharge levels described above. However, we found that these short-term changes in LC tonic discharge were often correlated with differences in short-term attentiveness during the task as reflected in different frequencies of visual fixation, similar to the relationship seen with longer-term changes in LC activity described above. Thus, even brief elevations in LC discharge were typically associated with decreased fixation of the fix spot. We have also examined LC activity as a function of simple eye position or movement, and have found no consistent relationship. We have further analyzed short-term changes in LC activity and foveation frequency to ascertain whether changes in LC activity anticipate, and may therefore cause, changes in attentiveness as reflected in successful foveation. To this end, we have analyzed LC spike patterns for the occurrences of bursts. Our preliminary results indicate that a brief pause in LC activity is associated with an increase in foveation frequency, while a burst of LC activity is followed within a few hundred msec by decreased foveation frequency. These results indicate that altered LC activity precedes the associated change in foveation, and are consistent with (but do not prove) the possibility that the LC may cause the change in attentiveness. As noted above, experiments proposed in Aim 1 will directly address this issue further using direct manipulations of LC activity.

Fluctuations in tonic LC activity and changes in LC sensory responsiveness - Analysis of LC responses to target stimuli during the longer epochs of different tonic activity revealed another surprising but marked relationship. Periods of elevated resting activity in a typical LC neuron were consistently associated with decreased responsiveness of that neuron to target stimuli in the vigilance task; the phasic activation of LC neurons typically seen for target stimuli (described above) was observed predominantly during epochs of intermediate tonic LC discharge and best behavioral performance in all 10 cells examined to date. Thus, elevated basal LC discharge corresponds to both decreased behavioral performance (due to indiscriminate responding, long response latencies, and labile or unfocused attention) and decreased phasic activation of LC neurons by target stimuli. Additional analyses are underway to fully evaluate these findings, but they suggest that both phasic evoked responses as well as tonic discharge levels of monkey LC neurons may affect attentional performance. As noted above, manipulations of phasic LC

activity are proposed to determine the causal role of these target responses in vigilance performance.

5. White noise transiently activates LC neurons and disrupts attentiveness. In preliminary studies, LC neurons in one animal were recorded while the animal performed the above vigilance task and white noise was briefly presented (100 db, 5-15 min). There was a transient activation of LC neurons during the noise stimulus, but this quickly subsided even though the noise was still present. In parallel with the increase in LC activity, the frequency with which the animal foveated the fix spot decreased, reflecting decreased attentiveness to the task perhaps in response to distraction by the onset of the noise. Additional presentations of the noise on the same day yielded less or no response in LC activity or behavior, suggesting that habituation to the noise in both cellular and behavioral measures was rapid during task performance. The effects of white noise will be examined in additional LC cells in the proposed studies. In addition to examining effects of brief noise presentation, we will also test whether prolonged exposure (>30 min) exerts additional effects on LC activity or behavior not seen in the brief presentations. We will also examine effects of noise on a task that measures attentional lability, the attentional disengagement task.

6. Acute morphine decreases tonic activity and induces pronounced oscillation of LC discharge in the waking monkey. LC neurons were recorded from waking, chair-restrained cynomolgus monkeys before, and for 0.5 - 4 h after, i.m. injections of morphine sulfate (0.3 to 10 mg/kg). As shown in the attached publication [22], tonic discharge of each LC neuron tested (n=11) decreased after morphine injection; this effect appeared to be dose-dependent for the range of 0.3-3.0 mg/kg. Unexpectedly, these same doses of morphine also induced a pronounced burst-pause discharge pattern in all LC neurons recorded. The bursts in activity corresponded to (and anticipated) orienting behaviors and increased arousal, whereas pauses were associated with apparent sedation. This was demonstrated using a burst analysis of LC activity, where bursts of LC activity after morphine were closely associated with pupillary dilation. Closer analysis revealed that the burst-pause pattern in LC activity was regular, with a period of about 15-35 sec. This observation was confirmed by autocorrelogram analysis. These results indicate that acute opiates may exert a dual effect on LC neurons in waking animals: inhibition of discharge by direct effects on LC cells, and phasic activation mediated by excitatory afferents to the LC. These short-term changes in LC discharge after morphine resembled in frequency the short-term changes described above during the vigilance task, except that the amplitudes of these changes were much greater after morphine. It is interesting to compare the behaviors associated with these fluctuations in discharge in the two conditions: LC bursts after morphine gave rise to apparently increased vigilance while elevated activity in non-opiate testing was associated with a decrease in focused attention. These may be similar effects, in that in both cases the elevated activity may be associated with more labile, less focused attention. It should be noted, of course, that these behavioral observations are not strictly comparable; for example, animals consistently stopped performing the vigilance task after even low doses of opiates. The relationship between these two fluctuations in LC activity remains to be established.

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PROFESSIONAL PERSONNEL:

Gary Aston-Jones, Ph.D., Professor (Principal Investigator)	20%, 3 years
Tatiana Alexinsky, Ph.D. Research Associate	100%, 0.5 year
Janusz Rajkowski, Ph.D., Research Assistant Professor	100%, 3 years
Piotr Kubiak, Ph.D., Postdoctoral Fellow	100%, 2 years

INTERACTIONS:

Invited presentations at national and international meetings:

European Winter Conference on Brain Research, Les Arcs, France, March, 1990.

International Symposium on the Neurobiology of the Locus Coeruleus, Post Falls, Idaho, May, 1990.

XIIIth Congress of the International Primatological Society, Kyoto, Japan, July, 1990.

European Brain and Behavior Society Symposium, "Functions of the forebrain cholinergic and noradrenergic systems", Stockholm, Sweden, September, 1990.

McDonnell Foundation Workshop on Emotion, Montauk, New York, September, 1990.

Chairman and speaker, "The Ventrolateral Medulla: A Site for Integration of Pain, Sympathetic Activity and Arousal", Special Panel, Winter Conference on Brain Research, Vail, Colorado, January, 1991.

Chairman and speaker, "The Ventrolateral Medulla: A Site for Integration of Pain, Sympathetic Activity, Respiration and Arousal", Workshop, IBRO 3rd World Congress of Neuroscience, Montreal, Canada, August, 1991.

Chairman and speaker, "The Locus Coeruleus-Norepinephrine System: New Basic and Clinical Perspectives", Panel, American College of Neuropsychopharmacology (ACNP), San Juan, Puerto Rico, December, 1991.

Symposia presentations (2), Seventh International Catecholamine Symposium, Amsterdam, June, 1992.

International Research Conference of the American Academy of Osteopathy, on *Nociception and the Neuroendocrine Immune Connection*, Cincinnati, Ohio, June, 1992.

American College of Neuropsychopharmacology (ACNP) Panel, "Neural Mechanisms of Learning and Memory: Relevance to the Consequences of Severe Psychological Trauma", San Juan, Puerto Rico, December, 1992.

Chairman and speaker, "Catecholamines and Attention: New Basic, Clinical and Modeling Approaches", Workshop, Winter Conference on Brain Research, Whistler, British Columbia, January, 1993.

The above does not include more than 15 invited seminars at other universities.

Title

FROM THE MEDULLA TO ATTENTION THROUGH THE LOCUS COERULEUS: CELLULAR PHYSIOLOGIC AND ANATOMIC STUDIES.

Authors and addresses

G. Aston-Jones, J. Rajkowski, P. Kubiak, T. Alexinsky[1], M.T. Shipley[2], M. Ennis[2], H. Akaoka[3] and B. Astier[4]. Div Behav Neurobiol, Dept. Mental Health Sci, Hahnemann Univ., M.S. 403, Philadelphia, PA 19102, USA; [1]U Rene Descartes, Dept. Psychophysiol, Paris, 75006, France; [2]Dept Cell Biol Anat, U Cincinnati Coll Med, Cincinnati, OH 45267, USA; [3]INSERM U171, Hospitalier Lyon-Sud, 69310 Pierre Benite, France; [4]U. Claude Bernard Fac.Pharm., 69008 Lyon, France.

Noradrenergic neurons of the locus coeruleus (LC) in both rat and monkey decrease their tonic discharge with sleep but also with aroused, non-vigilant behaviors (grooming and consumptive behaviors). These cells respond to sensory stimuli of many modalities, both internal and external, with greatest responses occurring for stimuli that cause orienting behavioral responses. Together with the broad efferent projections of LC axons, and the modulation of target cell activity by NE, these results suggest that the LC functions to regulate vigilance, defined as surveillance of the environment and readiness to respond to salient stimuli. New studies recording LC neurons in monkeys performing an oddball discrimiation task reveal that these neurons are selectively phasically activated by meaningful stimuli. Additionally, tonic activity fluctuates with minute-to-minute fluctuations in attention (measured by frequency of visual fixation), such that optimal focused attention and performance occurs with intermediate levels of LC activity. These results suggest that phasic activation of LC denotes urgent meaningful stimuli, while fluctuations in tonic LC activity may regulate whether animals are drowsy (activity too low), able to focus attention (intermediate activity), or are too highly aroused to focus attention and respond selectively ("scanning" mode; activity too high). Anatomic studies of the sources of afferents to LC that may be responsible for such discharge properties have revealed that major inputs originate in 2 rostral medullary cell groups, the nuclei paragigantocellularis (PGi) and prepositus hypoglossi (PrH). The PGi provides potent excitatory amino acid (EAA) and inhibitory adrenergic inputs, while the PrH inhibits LC via GABA-A receptors. We and others have recently found that the PGi-EAA input is responsible for several sensory-evoked responses of LC neurons, and also for the bulk of the hyperactivity that occurs in LC during opiate withdrawal. Agents that interact with this EAA input modulate LC activity during these and other stimuli, and may have a variety of clinical applications. Prominent circuit and functional features of the PGi and PrH extend our understanding of the LC as a vigilance system. The PrH is linked to orienting behaviors, while the PGi is a key sympathoexcitatory region. These findings suggest that the LC is in a critical position to regulate vigilance and attention in parallel with orientation to salient sensory events and sympathetic activation of peripheral systems for adaptive responses to salient urgent stimuli.

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TONIC AND SENSORY-EVOKED ACTIVITIES OF NORADRENERGIC LOCUS COERULEUS (LC) NEURONS IN PRIMATE VARY WITH DISCRIMINATION PERFORMANCE IN A VIGILANCE TASK. P. Kubiak, J. Rajkowski, G. Luthin* and G. Aston-Jones, Div. Behavioral Neurobiol., Dept. Mental Health Sci., Hahnemann University, Philadelphia, PA 19102.

Previous studies indicate that the LC regulates vigilance, or attentiveness to sensory stimuli. Consistent with this idea, we have recently reported that monkey LC neurons typically respond preferentially to target stimuli in a vigilance task (Aston-Jones et al., Prog. Brain Res. 88: 501, 1991). We extended this analysis to include changes in discrimination performance and basal discharge rates of LC neurons.

Individual LC neurons were recorded in 2 cynomolgus monkeys performing a vigilance task which required bar release within 700 msec of a target stimulus (10 % of trials) but no response to non-target stimuli (90% of trials). Stimuli were horizontal or vertical bars presented on a video screen, one of which occurred per trial immediately after foveation of a central fix spot. The mean baseline discharge rates of LC neurons were typically between 1 and 4 spikes/sec. During prolonged task performance (more than 30 min), each of 15 LC neurons analyzed to date alternated between two levels of tonic activity which differed by 0.5 - 1.5 spike/sec; animals were continuously alert throughout the task. These episodic changes in LC activity corresponded to altered task performance such that epochs of elevated discharge were accompanied by decreased discrimination, reflecting lowered attention to task stimuli. In addition, LC neurons appeared to be unresponsive to both target and non-target task stimuli during such periods of elevated discharge. In contrast, when LC activity resumed the lower level of discharge, discrimination performance markedly improved and neurons exhibited the typical phasic activation by target stimuli. Thus, a strong relationship exists among tonic LC discharge rate, sensory responsiveness of LC neurons and vigilance performance. These and other results (see Rajkowski et al., this volume) support a role for the LC in attention and vigilance. Additional work is underway to determine how these changes in LC activity contribute to the accompanying changes in attention. *Supported by AFOSR grant 90-0147.*

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ACTIVITY OF LOCUS COERULEUS (LC) NEURONS IN BEHAVING MONKEYS VARIES WITH CHANGES IN FOCUSED ATTENTION. J. Rajkowski*, P. Kubiak, & G. Aston-Jones. Div. Behavioral Neurobiol., Dept. Mental Health Sci., Hahnemann University, Philadelphia, PA 19102.

Our previous results revealed that sensory responses of LC neurons in behaving monkeys are selectively elicited for attended stimuli in a discrimination task (Aston-Jones et al., Prog. Brain Res. 8: 501, 1991). Here we report that tonic LC discharge also varies in close correspondence with attentiveness.

Discharge of individual LC neurons was recorded from 2 cynomolgus monkeys performing an attentional task (oddball visual discrimination). The results of activity during this task are presented in an accompanying abstract (Kubiak et al., this volume). This task required that the animal foveate a central fix spot to initiate each trial of stimulus presentation; proper response to target stimuli resulted in juice reward. Such foveation is effortful and reflects attentiveness to the task. During drowsiness there was typically no task performance and LC activity was very low (< 0.5 spikes/sec). We observed that during continuous alertness and task performance the frequencies of both LC discharge and foveation fluctuated over short (10-30 sec) and long time intervals (10-30 min). The long-term changes in LC discharge were consistently inversely correlated with task behavior, such that slightly elevated LC activity (by 0.5 to 1 spike/sec) was accompanied by decreased foveation frequency and poorer task performance. Correlation analyses revealed that this relationship was highly significant (of the 6 cells quantitatively analyzed to date, typically ~~r = -0.5,~~ $p < 0.001$). In addition, even short-term increases in LC tonic activity often corresponded to marked, short-lasting reductions in foveation frequency. These results suggest that focused attentiveness varies with tonic LC discharge in an inverted U relationship. Very low LC activity is associated with drowsiness and inattentiveness, while high tonic LC discharge corresponds with labile attention and restlessness; optimal focusing of attention occurs with intermediate levels of tonic LC activity. Additional studies are underway to test whether fluctuations in tonic LC activity cause or reflect changes in attentiveness. *Supported by AFOSR grant 90-0147.*

KEY WORDS: (see instructions p. 4)

1. norepinephrine
2. vigilance
3. primate behavior
4. foveation

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ACUTE MORPHINE DECREASES DISCHARGE AND INDUCES PERIODIC BURSTING OF LOCUS COERULEUS NEURONS IN THE WAKING MONKEY. J. Rajkowski, H. Akaoka and G. Aston-Jones. Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann Univ., Philadelphia, PA 19102.
The discharge activity of six neurons tentatively identified as noradrenergic locus coeruleus (LC) units (>2 ms spike, low frequency, burst-pause response to salient stimuli) was recorded before, and up to 4 hours after i.m. injections of morphine (2 cells each for 1 mg/kg, 3 mg/kg, or 10 mg/kg) in a chair restrained Cynomolgus monkey with fixed head. Throughout sessions the animal's eyes were open and he visually explored the environment, occassionally shivered or scratched himself, but did not respond to most environmental stimuli. By 1 hr or more after morphine, the eyes exhibited slow drifts and subsequent saccades, the pupil diameter oscillated widely, and there were occasionally short periods of drowsiness.

LC activity pre-drug was characteristically tonic and regular, with small amplitude periodicity (0.04 Hz). Three to 7 min after morphine, each of the 6 LC cells exhibited clear periodic bursting activity which continued for the entire recording session. These larger bursts also occurred with a period of 0.04 Hz for each cell, and were highest in amplitude 20 min after injection. Both interburst pauses and interspike intervals increased with time after injection, and resulted in an overall decrease of impulse activity. By 2 hr post morphine, LC activity remained at a very low level even when the animal had his eyes fully opened, and bursts of unit discharge that accompanied awakening predrug were reduced to a few spikes. The above effects were observed for all 6 cells, even with the lowest dose tested (1 mg/kg). Supported by AFOSR grant 90-0147 and PHS grant DA 06214.

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DECREASED TONIC DISCHARGE AND INDUCTION OF PERIODIC BURSTING OF LOCUS COERULEUS (LC) NEURONS AFTER ACUTE MORPHINE IN WAKING MONKEYS

J.Rajkowski, H. Akaoka, C.J. Kovelowski, II and G. Aston-Jones Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann Univ. Philadelphia, PA 19102.

Substantial evidence indicates that the LC may be an important site of action for exogenous opiates. However, the effects of opiates on the electrical activity of LC neurons in conscious animals remain controversial, and never have been reported in primates. Here, the discharge activity of 11 neurons located in the LC region and tentatively identified as noradrenergic cells (>2 ms spike, low frequency, burst-pause response to salient stimuli) was recorded before, and up to 4 hours after i.m. injections of morphine sulphate in a chair restrained Cynomolgus monkey (3 cells each with 0.3 mg/kg, 1 mg/kg or 3 mg/kg, and 2 cells with 10 mg/kg). After injection, the animal sat quietly appearing sedated with his eyes open. One-half to 1 hour following morphine administration, the animal's eyes exhibited episodic slow shifts, the pupil diameter oscillated widely, and there were occasional short periods of drowsiness.

LC activity prior to drug administration was characteristically tonic and regular; closer analysis revealed that there were small oscillations in discharge rate occuring at a frequency of about 0.04 Hz. At 3 to 7 min after morphine injection, LC neurons showed pronounced periodic bursting activity which continued for the duration of the recording session. Such bursting also occurred with a frequency of 0.04 -0.05 Hz, as detected by autocorrelation of unit discharge, and was most pronounced about 20 min after injection. Interspike intervals increased with time after injection, resulting in an overall decrease of impulse activity. By 2 hrs following a high dose of morphine, LC neurons were nearly silent, even though the animal's eyes were fully open. Although most pronounced for higher doses of morphine, the above effects were observed for all cells tested.

Supported by AFOSR grant 90-0147 and PHS grant DA 06214.

KEY WORDS: (see instructions pg. 4)

1. Opiates
2. Norepinephrine
3. Unit activity
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PHYSIOLOGICAL CORRELATES OF ADAPTIVE BEHAVIOR IN A VISUAL DISCRIMINATION TASK IN MONKEYS. T. Alexinsky[1], G. Aston-Jones, J. Rajkowski and R.S. Revay, Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann Univ., Philadelphia, PA, USA, and [1]U. René Descartes and LPN2 CNRS, 91198, Gif-sur-Yvette, France.

Previous studies have implicated the rostral pontine nucleus locus coeruleus (LC) in vigilance and adaptive sensory-behavioral responding. Here we have examined this framework by recording neurons in the LC area of cynomolgus monkeys performing an "oddball" visual discrimination-vigilance task. Monkeys were trained with colored lights serving as S+ or S-. Four bundles of 6 micro-wires (25-μm) were implanted bilaterally in the LC area for recording neuronal impulse activity. Event-related potentials (ERPs) were recorded from skull screws. Stimulus duration, time to respond, interstimulus interval, S+/S- ratio, and session duration were systematically varied to alter task difficulty and attentiveness. Monkeys were also subjected to reversal training.

Histologic reconstruction of all recording sites is not completed, but certain classes of neuronal responses in the rostral pons are apparent. Apart from cells that could not be driven by any aspect of the task (25%), many neurons were classified as sensory (23%), motor (14%) or reward cells (2%). However, a large population of cells in the LC area (36%) exhibited activity that was specifically related to meaningful stimuli (i.e., driven by S+ but not S-). These cells altered their responsiveness to be activated by the new S+ during reversal training in close correlation with behavioral performance. ERPs were also specifically evoked by the S+, whether overlearned or during stimulus reversal (correlation between ERP amplitudes and behavioral performance during reversal = 0.89). Thus, strong relationships exist among activity of certain cells in the LC area, cortical ERPs and adaptive behavior in a task requiring sustained attention. These relationships are being examined in more detail by monitoring attention via eye position and autonomic activity via pupillary diameter in a more sophisticated discrimination task.

Supported by AFOSR grant 90-0147, and ONR contract N00014-86-K-0493.

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AUTHORS and Address: T.Alexinsky & G. Johnson, Université René Descartes and LPN2 CNRS, 91198 Gif/Yvette FRANCE

Abstract:

In a reversal paradigm, a previously reinforced target or S+, is no longer reinforced and undergoes the process of extinction, wherein the former S- becomes the new S+. This task required detection and selective response to an infrequent colored stimulus (10%) embedded in a sequence of nontarget stimuli (90%). When the monkey (macaca fascicularis) depressed a lever, a randomized series of colored lights were presented. At the onset of the infrequent target light, a rapid release of the lever (in less than 500ms) was rewarded by juice. Performance during the reversal process was studied. We examined the physiological correlates of this behavior at a global level using averaged EEG activity triggered by S+ and S- and at neuronal level, unit and multi-unit activity of cells located in the rostral pontine area.

Results showed that:

- 1°monkeys were able to learn the signification of a new S+ after a few presentations.
- 2°S+ was followed by an event related potential (ERP) (latency :250ms +/-15, amplitude 19.5 +/- 4.5 uV). The correlation between the amplitude of the ERPs and the performance in the task was .89.
- 3° cells located in the LC region showed also the specific response for the target. During reversal, this response was shown to be reduced progressively for the former S+ and conversely increased for the new S+. These data show clearly that both at the global EEG and at the cellular brainstem levels, there is a significant correlation between physiological measures and behavior attested by the performance in an attention task.

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Progress in Brain Research, Vol. 88
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CHAPTER 35

Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance

G. Aston-Jones [1], C. Chiang [1] and T. Alexinsky [2]

[1] *Division of Behavioral Neurobiology, Department of Mental Health Sciences, Hahnemann University, Broad and Vine, Philadelphia, PA, U.S.A. and* [2] *Université René Descartes, Department de Psychophysiologie, Laboratoire de Physiologie Nerveuse 2 CNRS, Gif-sur-Yvette, Cedex, France*

Recordings from noradrenergic locus coeruleus (LC) neurons in behaving rats and monkeys revealed that these cells decrease tonic discharge during sleep and also during certain high arousal behaviors (grooming and consumption) when attention (vigilance) was low. Sensory stimuli of many modalities phasically activated LC neurons. Response magnitudes varied with vigilance, similar to results for tonic activity. The most effective and reliable stimuli for eliciting LC responses were those that disrupted behavior and evoked orienting responses. Similar results were observed in behaving monkeys except that more intense stimuli were required for LC responses.

Our more recent studies have examined LC activity in monkeys performing an "oddball" visual discrimination task. Monkeys were trained to release a lever after a target cue light that occurred randomly on 10% of trials; animals had to withhold responding during non-target cues. LC neurons selectively responded to the target cues during this task. During reversal training, LC neurons lost their response to the previous target cue and began responding to the new target light in parallel with behavioral reversal. Cortical event-related potentials were elicited in this task selectively by the same stimuli that evoked LC responses.

Injections of lidocaine, GABA, or a synaptic decoupling solution into the nucleus paragigantocellularis in the rostral ventrolateral medulla, the major afferent to LC, eliminated responses of LC neurons to sciatic nerve stimulation or foot- or tail-pinch. This indicates that certain sensory information is relayed to LC through the excitatory amino acid (EAA) input from the ventrolateral medulla.

The effect of prefrontal cortex (PFC) activation on LC neurons was examined in anesthetized rats. Single pulse PFC stimulation had no pronounced effect on LC neurons, consistent with our findings that this area does not innervate the LC nucleus. However, trains of PFC stimulation substantially activated most LC neurons. Thus, projections from the PFC may activate LC indirectly or through distal dendrites, suggesting a circuit whereby complex stimuli may influence LC neurons.

The above results, in view of previous findings for postsynaptic effects of norepinephrine, are interpreted to reveal a role for the LC system in regulating attentional state or vigilance. The roles of major inputs to LC from the ventrolateral and dorsomedial medulla in sympathetic control and behavioral orienting responses, respectively, are integrated into this view of the LC system. It is proposed that the LC provides the cognitive complement to sympathetic function.

Key words: locus coeruleus, vigilance, monkey, rat, behaving, cortex

Introduction

The noradrenergic locus coeruleus (LC) system has been proposed to be involved in almost as many brain and behavioral phenomena as there are investigators who study this structure. These neurons have been implicated in fundamental brain processes ranging from vegetative activities

such as sleep and cerebral blood flow, to more complex, cognitive phenomena such as selective attention and memory (reviewed in Aston-Jones *et al.*, 1984). Correspondingly, this system has been a favorite suspect in the "who done it" of clinical etiology, being implicated in disorders ranging from anxiety and panic to dementia and schizophrenia. How can so few cells (estimated to be 15,000 per hemisphere in humans) (Foote *et al.*, 1983) do so many things? While some may think that investigators have waxed overly enthusiastic in some claims about this enigmatic little nucleus buried in the pontine brainstem, it is possible that the LC system serves a fundamental, general function in brain activity and thereby is involved in a number of brain and behavioral processes. The thesis of this paper is that, indeed, the LC provides a very general function, which is to regulate attention to the broad range of environmental stimuli and the degree to which behavior is engaged by the ever-changing sensory surround. The hypothesis to be developed in this report is that the LC system functions to control vigilance, defined as the surveillance of the environment, or readiness to respond to unexpected environmental events. We will develop this model using a host of cellular attributes of the LC system, particularly anatomic, physiological and pharmacological properties of these neurons. We will extend this hypothesis by incorporating recent findings pertaining to the neural systems that are afferent to LC neurons, and by using known functions of these major inputs to expand our thinking about the LC's role in brain and behavior. We will also describe recent results of LC impulse activity in waking monkeys performing an "oddball" visual discrimination task designed to manipulate and measure vigilance. Finally, we will define a very general perspective from which to examine LC function, in which this system is viewed as a "random search generator" whose activation serves to disrupt stable activity in neural loops and associated behaviors and generate a search for a new activity that is more consistent with the most recently sampled sensory information. This view may be useful to general nervous system models and neural network analyses.

The ultimate goal of this functional analysis is to derive an algorithm for LC function, so that given a certain input, one could predict a functional outcome of LC system activation. We believe that the cellular physiological and anatomic properties of the LC system are key elements in such an analysis.

Background

Efferent organization

The LC system has been the subject of intense study for more than two decades. The interest in this nucleus began in earnest when Swedish researchers (Dahlström and Fuxe, 1964; Ungerstedt, 1971) discovered that these cells give rise to an enormously divergent set of efferent projections; it is noteworthy that this small nucleus innervates more different brain areas than any other single nucleus yet described. This, and the fact that these neurons appeared to use the then recently discovered neurotransmitter norepinephrine (NE) to communicate with their target cells, generated great enthusiasm for understanding their possible function(s).

Although some investigators have argued that NE may be released from LC fibers in a non-synaptic manner, providing a hormone-like, paracrine influence on many neurons within a diffusion-limited area (Beaudet and Descarries, 1978), more recent studies have shown that LC terminals in several brain structures make conventional synapse-like appositions with postsynaptic specializations on target neurons (Koda *et al.*, 1978; Olschowka *et al.*, 1981; Papadopoulos *et al.*, 1989; Papadopoulos and Parnavelas, 1990). There is, in fact, a great deal of both regional and laminar specificity in the innervation of target structures by LC axons (*e.g.*, Morrison *et al.*, 1982). Finally, although there have been reports of NE fiber apposition to blood vessels (Edvinsson *et al.*, 1973; Hartman, 1973; Swanson *et al.*, 1977), more re-

cent studies in LC terminal areas do not find a preference for apposition of dopamine-β-hydroxylase fibers on capillaries (Olschowka *et al.*, 1981; Papadopoulos *et al.*, 1989; Papadopoulos and Parnavelas, 1990). While such findings cannot rule out a possible involvement of LC projections in blood flow and metabolism in target areas, they indicate that this system is most prominently structured to provide conventional synaptic input to brain neurons.

Postsynaptic effects

Using microiontophoresis, early studies (Hoffer *et al.*, 1973; Segal and Bloom, 1974) found that NE inhibited basal discharge of cerebellar or hippocampal neurons in anesthetized rats, effects which appeared to be mediated by β-adrenergic receptor activation of a cyclic AMP second messenger system (Siggins *et al.*, 1971; Foote *et al.*, 1983). However, subsequent experiments by Foote, Segal and colleagues (Foote *et al.*, 1975; Segal and Bloom, 1976) found that, in addition to decreasing basal discharge, NE may also enhance the selectivity of target cell discharge, so that in the presence of this neurotransmitter neurons respond with increased preference to their most strongly determined inputs. In these and studies by others that extended these findings (see contributions by Waterhouse *et al.*, and by Woodward *et al.*, this volume), NE acting at presumed β receptors decreased spontaneous impulse activity to a greater extent than activity evoked by afferent or sensory stimulation. This effect has recently been described for motor-related activity in primate cortex as well (Sawaguchi *et al.*, 1990), indicating that it is not limited to sensory areas. It is noteworthy that in many cases NE has been found to augment evoked activity (either excitatory or inhibitory) while decreasing spontaneous discharge of the same neuron (Waterhouse and Woodward, 1980; Waterhouse *et al.*, 1980, 1984). Such selective enhancement of responses to strong inputs relative to low-level or basal activity has been likened to an increase in the "signal-to-noise" ratio of target neurons by NE. Although other effects of NE have been described for various target areas, such biasing of target cells to respond preferentially to their strongest inputs is most significant for the present analysis.

LC discharge in unanesthetized rats and monkeys

Before considering data concerning when LC neurons are active in behaving animals, it is pertinent to point out some important technical issues. Our recordings of LC discharge have utilized species (rat and monkey) whose LC is composed entirely of noradrenergic neurons. Thus, in these species one can record from known NE-containing LC neurons by using simple histological verification of recording sites. This is an important consideration, since the wide interest in LC stems from its noradrenergic cell population. Similar experiments in other species (*e.g.*, cat) in which the nucleus LC is composed of interdigitated NE and non-NE neurons could only positively ascribe discharge to NE-containing neurons if intracellular staining and double-labeling is carried out, a procedure not reported for any such study to date.

Spontaneous LC discharge and the sleep-waking cycle

One predominant hypothesis of LC function is that these neurons control various stages of the sleep-waking cycle (Jouvet, 1969; Hobson *et al.*, 1975; McCarley and Hobson, 1975). We found that spontaneous LC discharge covaries consistently with stages of the sleep-waking cycle, firing fastest during waking, more slowly during slow-wave sleep, and becoming virtually silent during paradoxical sleep (PS) (Aston-Jones and Bloom, 1981a). In rat, the nearly total lack of activity in this nucleus during PS is evident not only from the consistent quiescence of single neurons, but especially when several neurons in the densely packed noradrenergic cell group are recorded simultaneously. In such cases, the entire population typically becomes silent, with a prominent

decrease in "background noise" as well. These observations, the first of their kind for known NE-containing neurons, support previous proposals that a similar subpopulation of unidentified cat LC neurons may be noradrenergic (Hobson *et al.*, 1975; Rasmussen *et al.*, 1986). However, other activity profiles of purported noradrenergic neurons have been reported in cat LC (Chu and Bloom, 1973, 1974).

We also recorded spontaneously occurring field potentials from rat LC during sleep and waking. These slow potentials are synchronous with bursts of unit activity during waking and slow-wave sleep, but occur at their highest rates during PS in the absence of unit activity (Aston-Jones and Bloom, 1981a). These observations indicate that the absence of LC discharge during PS is due to active inhibition of these neurons, not simply disfacilitation. This is consistent with results demonstrating that LC cells in brain slices are auto-active, in the absence of synaptic inputs (Aghajanian *et al.*, 1983; Williams *et al.*, 1984).

Further analysis revealed that LC impulse activity also changes within stages of the sleep-waking cycle, in anticipation of the subsequent stage (Fig. 1). Thus, during waking, LC neurons progressively decrease in activity as slow-wave sleep approaches, and likewise during slow-wave sleep before the onset of PS (Hobson *et al.*, 1975; Aston-Jones and Bloom, 1981a). If waking rather than PS follows slow-wave sleep, LC neurons abruptly emit phasically robust activity 100–500 msec prior to waking. As indicated in Figure 1, the one exception to such stage-anticipation in LC discharge occurs for the PS-to-waking transition. Rat LC neurons return to waking activity either coincident with or slightly after the cessation of PS as measured by the EEG (theta activity). Thus, although anticipatory LC activity during most stage transitions is consistent with a role in generating the subsequent stage, this nucleus cannot be responsible for the termination of PS (Aston-Jones and Bloom, 1981a; Aston-Jones *et al.*, 1984; however, see also Hobson *et al.*, 1975).

We have also monitored discharge of LC neurons in unanesthetized, chair-restrained primates (Foote *et al.*, 1980; Aston-Jones *et al.*, 1988; Grant *et al.*, 1988). Although these animals do not exhibit normal sleep and waking under our experimental conditions, we have observed LC activity during alertness and drowsiness as measured by EEG. As described above for rat LC, monkey LC neurons vary their activity closely with the state of arousal, even during unambiguous waking. Thus, periods of drowsiness are accompanied by decreased LC discharge, while alertness is consistently associated with elevated LC activity. Also as in rat, such changes in LC activity preceded the corresponding changes in EEG state by a few hundred msec. PS has not been observed in our chair-restrained monkeys.

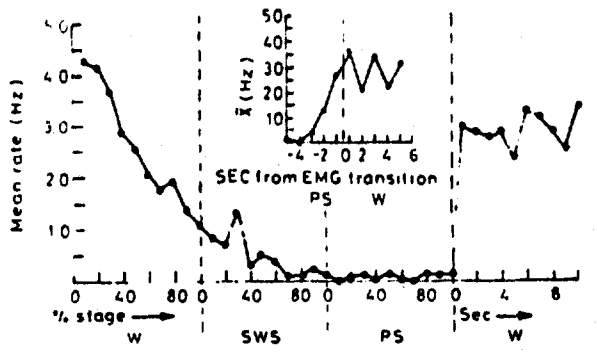


Fig. 1. Locus coeruleus (LC) discharge rate during sleep-waking cycle (SWC) progression. Mean discharge rates for LC neurons in behaving rats during epochs normalized for the percentage of SWC stage completion are plotted consecutively for complete SWCs. Note that when paradoxical sleep (PS)-to-waking transitions are judged by the EEG (main plot), cellular activity does not anticipate the transition, and that LC cannot be the primary agent terminating PS. However, discharge is enhanced in anticipation of these same transitions scored by EMG criteria (inset). (From Aston-Jones and Bloom, 1981a.)

Spontaneous LC discharge and waking behavior

We further observed that LC discharge is altered during certain spontaneous waking behaviors. During both grooming and consumption of a glucose solution, rat LC discharge decreases compared to that in other epochs of similar EEG arousal (Aston-Jones and Bloom, 1981a). Similar results were obtained for LC activity in behaving

primates (Grant *et al.*, 1988). These results indicate that LC discharge is reduced not only for periods of low arousal (drowsiness or sleep), but also during certain behaviors (grooming and consumption) when animals are in an active waking, but inattentive (nonvigilant) state (see below).

LC discharge also varies strongly with orienting behavior. In both rat (Aston-Jones and Bloom, 1981a,b) and monkey (Foote *et al.*, 1980; Aston-Jones *et al.*, 1988; Grant *et al.*, 1988), the highest discharge rates we observed for LC neurons were consistently associated with spontaneous or evoked behavioral orienting responses. LC discharge associated with orienting behavior is phasically most intense when automatic, tonic behaviors (sleep, grooming, or consumption) are suddenly disrupted and the animal orients toward the external environment (Aston-Jones and Bloom, 1981a,b). Thus, as found following sleep, grooming, or consumption, there is close correspondence between spontaneous bursts of discharge and interruption of automatic, preprogrammed behaviors with an increase in attentiveness and vigilance (see below).

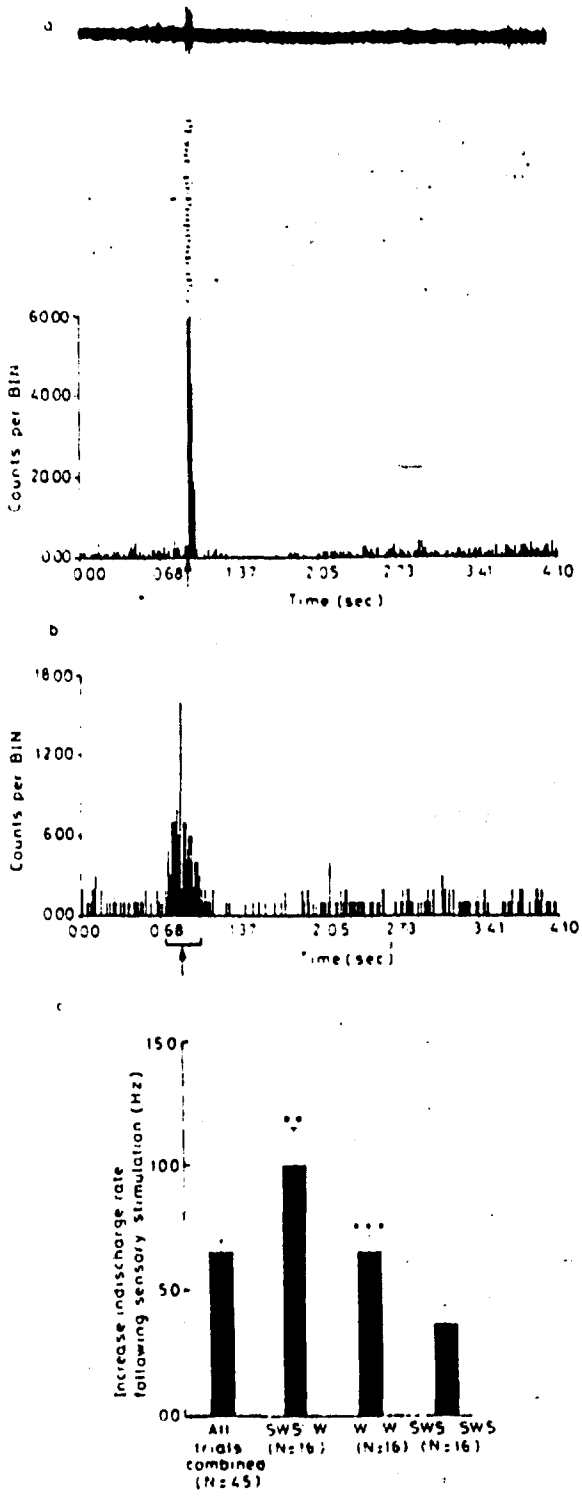
LC sensory responsiveness

In addition to the above fluctuations in LC spontaneous discharge, we found that these neurons in unanesthetized rats and monkeys were responsive to non-noxious environmental stimuli (Fig. 2a,b; Foote *et al.*, 1980; Aston-Jones and Bloom, 1981b). In waking rats, LC activity is markedly phasic, yielding short-latency (15–50 msec) responses to simple stimuli in every modality tested (auditory, visual, somatosensory, and olfactory). Responses were most consistently evoked by intense, conspicuous stimuli, though sporadic responses were also observed for nonconspicuous stimuli as well. These responses were similar for the different sensory modalities, and consisted of a brief excitation followed by diminished activity lasting a few hundred msec (Fig. 2a,b).

While sensory responsiveness was qualitatively similar for LC neurons in rat and monkey, there were important differences as well. In rat, any of a variety of intense stimuli evoked LC responses in a majority of sensory trials. In contrast, monkey LC was less strongly influenced by such stimuli, with responses fading after the first few trials. However, more complex stimuli, such as a new face or a meaningful but unexpected stimulus (see below), was consistently capable of eliciting LC responses in monkey (Aston-Jones *et al.*, 1988; Grant *et al.*, 1988).

More generally, we found that stimuli effective in eliciting LC responses were also those that disrupted ongoing behavior and elicited a behavioral orienting response by the monkey. It appears, then, that the difference in stimulus-responsiveness in rat vs. monkey LC is closely related to the difference in behavioral responses evoked by stimuli in the two species. Compared to rats, monkeys require much more complex stimuli to interrupt behavior and evoke an orienting response; their LC responsiveness follows the same pattern.

We quantified this linkage between behavioral disruption/orientation and LC sensory responses for rat LC neurons (Aston-Jones and Bloom, 1981b). As illustrated in Figure 2c, the largest responses were elicited by stimuli that caused an abrupt transition from sleep to waking, with associated behavioral orientation. Responses evoked during uninterrupted slow-wave sleep were much smaller in magnitude, whereas no response occurred during uninterrupted PS. In addition to these results for sleep, we found that response magnitudes during uninterrupted grooming or consumption of sweet water were reduced, whereas stimuli that disrupted such activity and generated orienting behavior elicited strong responses. Thus, there was a strong correspondence in rat, as in monkey, between sensory-evoked responsiveness in behavior and LC discharge, and a common factor for stimulus-responsivity in the two species is behavioral disruption and re-orientation. In sum, in both rat and monkey, stimuli that disrupt behavior and evoke an orienting response evoke LC response.



Habituation of LC responsiveness independent of habituation of behavioral state did not occur: response magnitudes for stimuli after 100 or more presentations were similar to those for initial stimuli when analyzed for similar behavioral states and orienting responses.

LC neurons in monkeys respond to meaningful stimuli during an "oddball" discrimination / vigilance task

The above results suggested that the essential property of stimuli to elicit LC responses was meaningfulness, so that intense stimuli elicited responses because their intensity made them meaningful but that non-intense, meaningful stimuli may also reliably elicit responses of LC cells. To explicitly test this possibility, we have recently begun recording LC activity in unanesthetized primates trained in an "oddball" visual

Fig. 2. Sensory responses of LC neurons are multimodal and state-determined. Panel a. Tone pip-evoked response of LC impulse activity in a behaving rat. Upper part is the oscilloscope recording of impulse activity, middle part is a raster display of activity for tone pip trials ordered consecutively from top to bottom, and lower part is a cumulative post-stimulus time histogram (PSTH) accumulated for 50 tone pip trials (tone pips, 20 msec duration, presented at arrow for all parts of panel). Note phasic activation followed by postactivation inhibition (the latter is due to feedback mechanisms within LC) (Aghajanian, 1978; Aghajanian *et al.*, 1977; Ennis and Aston-Jones, 1986). Panel b. PSTH of LC activity evoked by touching the tail of a behaving rat (touch to rostral tail, approximately as indicated at arrow). Note similarity of this response to that evoked by tone pips in panel a. Twenty-five trials accumulated in the PSTH. Panel c. Bar graph illustrating mean tone pip-evoked response magnitudes for rat LC neurons as a function of behavioral state. Note that responses are greatest for tones that awaken the animal (SWS/W trials), while tones presented during uninterrupted sleep (SWS/SWS trials) elicit substantially reduced responses; tones given during waking (W/W trials) elicit an intermediate response magnitude. Similarly, stimuli that interrupted grooming or consumption behaviors elicited large response in LC activity, and responses were reduced for similar stimuli that did not interrupt these behaviors. Response magnitude for SWS/W trials > W/W trials, $^{**}P > 0.0005$; W/W magnitude > SWS/SWS magnitude, $^{***}P < 0.0005$; SWS/W magnitude > magnitude for all trials combined > SWS/SWS magnitude, $P < 0.0005$ for each; paired t tests were used. (From Aston-Jones and Bloom, 1981b.)

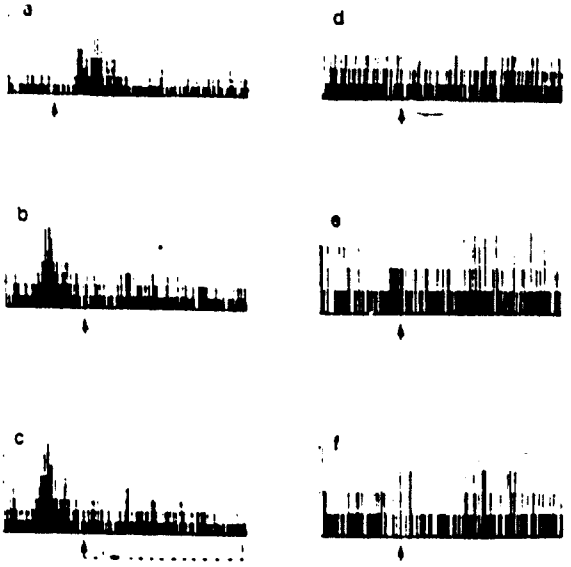


Fig. 3. PSTH displays for a cell in the LC area of a behaving monkey, illustrating selective response to (red) target stimulus. Panels a–c. A response is evoked by the red target stimuli (panel a) but not by correct lever release (panel b) or juice presentation (panel c). Increased activity preceding lever and juice in panels b and c correspond to stimulus presentation. Panels d and e. There is no response to the green non-target light (panel d) or to incorrect lever release to the green stimulus (panel e). Panel f. There is no response to low-intensity tone-pip presentation (see text). All stimuli and lever releases (b) occur at the arrows. Time calibration = 1 sec.

discrimination task (Aston-Jones *et al.* 1988; Alexinsky and Aston-Jones, 1990; Alexinsky *et al.*, 1990). The task involves discriminating differently colored light cues for juice reward. A target stimulus (S +) is presented on 10% of trials, intermixed in a semi-random fashion with non-target lights of a different color (S −). Neurons in the LC area were recorded along with cortical surface slow waves (averaged event-related potentials; AERPs) and behavioral responses (hits, misses, false alarms, and correct omissions). While some cells in the LC area showed responses that were purely sensory or motor in nature, most neurons exhibited activity specifically linked to the target stimulus. That is, responses for most cells were evoked selectively by S + stimuli but not S − stimuli, bar release or reward (Fig. 3).

Recordings during reversal training revealed that these responses were specifically related to the meaningfulness of the stimuli, not to their physical attributes. As illustrated in Figure 4, after reversal training neurons in the LC region reversed their stimulus preference, so that responses were selectively elicited for the new S + (previous S −) while responses for the old S + (new S −) faded. A second period of reversal training rapidly re-established the original stimulus selectivity of primate LC neurons. Interestingly, these changes varied closely with behavioral performance, so that responses to the new S + increased (and responses to the new S − decreased) as the percentage of correct behavioral responses to the new S + increased (and behavioral responses to the new S − decreased).

In addition, cortical activity exhibited a similar set of properties. As shown in Figure 5, AERPs recorded from the frontal and parietal cortices at latencies of 200–300 msec post-stimulation were selectively augmented by S + cues, as reported by

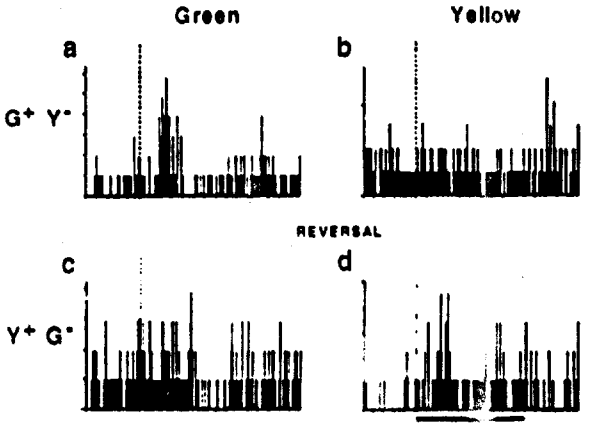


Fig. 4. A reversal procedure in the "oddball" discrimination task reveals responses of a putatively noradrenergic neuron in the LC of a behaving monkey specific to meaningful stimuli. (a,b) PSTHs for response of a neuron to green (target), but not to yellow(non-target), stimuli. (c,d) Similar PSTHs for the same LC neuron but after reversal training, so that target stimuli are now yellow, and non-target stimuli are green. Note that green stimuli (c) no longer elicit responses, while yellow stimuli (d) now elicit a small response. Thus, the response is selectively elicited by meaningful stimuli. Stimuli at dotted lines in all panels; bar = 1 sec.

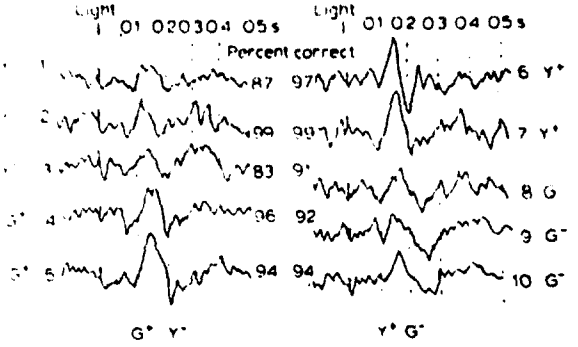


Fig. 5. Averaged event-related potentials (AERPs; 50 trials) for a monkey performing the "oddball" discrimination task, recorded from frontoparietal electrodes. AERPs at left were taken when the target was green (G+) and the non-target was yellow (Y−); those on the right were taken for the opposite stimulus meanings, as indicated. Note that for both yellow and green stimuli, AERPs were larger when the stimulus was a target compared to its use as non-target

others in both human (Hansen and Hillyard, 1984; Hillyard, 1985; Hillyard and Picton, 1979; Ruchkin *et al.*, 1980) and non-human primates (see Foote *et al.*, this volume); there is evidence that these potentials are similar to "P300" potentials in man (Pineda *et al.*, 1987, 1988). During reversal training, the AERPs altered their selectivity in a manner similar to neurons in the LC area, to become selectively responsive to the new S+, and no longer respond to the previous S+ (new S−; Fig. 5). As with the neurons, these changes in cortical-evoked activity followed a time course that closely paralleled behavioral discrimination performance during reversal.

Therefore, there is a close relationship among neurons in the LC area, cortical activity, and behavioral discrimination during a task requiring sustained attention to sensory cues (Alexinsky and Aston-Jones, 1990; Alexinsky *et al.*, 1990). These results are consistent with the hypothesis that LC neurons function to promote attentiveness and adaptive behavioral responding to changing stimuli (Aston-Jones, 1985). These results also support the proposal that LC neurons may be responsible in part for the attention-related AERPs recorded in this paradigm. Additional evidence for this possibility is found in the contribution by Foote *et al.* (this volume). Further studies are underway to better define the role of the LC system in such adaptive behavioral capacity.

Afferent circuits responsible for discharge properties of LC neurons

We have recently described the major afferents to the LC in rodent (Aston-Jones *et al.*, 1986, 1990); we detail these results elsewhere in this volume (Aston-Jones *et al.*). In brief, major afferents are found in two rostral medullary nuclei: the paragigantocellularis (PGi) in the ventrolateral rostral medulla, and the area of the prepositus hypoglossi (PrH) in the dorsomedial rostral medulla. Our stimulation and pharmacological analyses have revealed that the PGi predominantly excites LC cells via an excitatory amino acid projection, though inhibitory adrenergic projections exist as well. Conversely, the PrH potently and purely inhibits the LC by way of GABA projections.

As a major excitatory input to LC, the PGi is a natural candidate for relaying the multimodal sensory-evoked activation of LC neurons described above. This possibility is supported by the parallel pharmacological sensitivity of LC responses evoked by PGi or by footpad (or sciatic nerve) stimulation. Broad spectrum EAA antagonists simultaneously attenuate both PGi- and sciatic-evoked responses, while antagonists of NMDA or cholinergic receptors have no effect on either response (Ennis and Aston-Jones, 1988). These results have now been replicated by several groups (Chen and Engberg, 1989; Rasmussen and Aghajanian, 1989a; Svensson *et al.*, 1989; Tung *et al.*, 1989). To test the hypothesis that sciatic-evoked activation of LC is mediated through PGi, we (Chiang and Aston-Jones, 1989) recorded LC neurons while stimulating the contralateral footpad subcutaneously to activate the sciatic nerve, and slowly infused lidocaine (100–400 nl) into the PGi region. Such lidocaine infusions consistently blocked responses of LC neurons to sciatic nerve

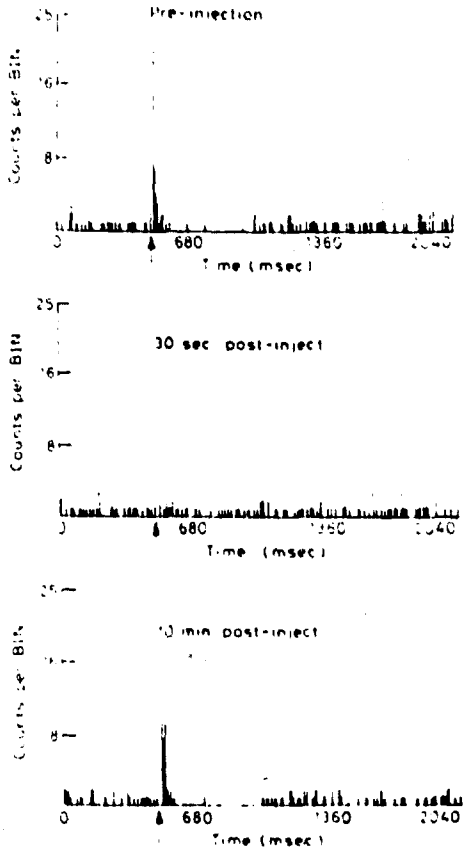


Fig. 6. Local microinfusion of lidocaine into paragigantocellularis (PGi) attenuates sensory response of an LC neuron. Upper panel, PSTH taken 2 min before microinjection. Note typical excitation elicited by stimulation of the rear footpad (for sciatic nerve activation, at arrow) followed by postactivation inhibition, which typically follows all LC neuronal excitation. Middle panel, PSTH of this neuron 30 sec after lidocaine (125 nl, 2% solution) was microinfused into PGi. Note abolition of response to footpad stimulation. Lower panel, PSTH of this LC neuron 10 min after microinfusion. Note partial recovery of response to footpad stimulation. Stimulation for all PSTHs = 2 mA, 0.5 ms pulses, presented at 0.5 Hz. Each PSTH is a collection of 50 stimuli. Similar attenuation of sciatic-evoked LC activity was obtained with microinjections of GABA or a Mn/Cd "synaptic decoupling" solution into PGi. (From Chiang and Aston-Jones, 1989.)

activation (Fig. 6). Similar infusions of GABA, or of a synaptic decoupling solution, 10 mM Cd^{++} plus 20 mM Mg^{++} (to antagonize Ca^{++} effects and prevent transmitter release), produced similar attenuation of footpad responses in LC neurons. These results indicate that PGi forms a critical synaptic link in this sensory response. It is noteworthy that electrolytic lesions of the PGi area by others have failed to block sciatic-evoked activation of the LC (Rasmussen and Aghajanian, 1989a). This result may reflect topographic specificity within the PGi for LC-projecting neurons that mediate responses to sciatic stimulation. Indeed, infusions of lidocaine, GABA or the Cd^{++}/Mn^{++} solution were all most effective when injected into the ventromedial retrofacial PGi area (Chiang and Aston-Jones, 1989). Therefore, electrolytic lesions of the PGi that spared this ventral, juxtaolivary region may fail to attenuate this sensory response of LC neurons.

Further experiments are underway to test the hypothesis that other modalities of sensory responses in LC are also mediated by EAA inputs from the PGi. Indeed, experiments by others have found that the EAA pathway from PGi to LC mediates the LC response to systemic nicotine administration (Chen and Engberg, 1989; Engberg, 1989), while we (Akaoka *et al.*, 1990, 1991) and others (Rasmussen and Aghajanian, 1989b) have found that hyperactivity of LC cells during opiate withdrawal is also mediated by this medullary afferent.

Effects of excitatory amino acid antagonists on morphine withdrawal behaviors

It has long been known that LC neurons are hyperactive during morphine withdrawal (Korf *et al.*, 1974; Aghajanian and Wang, 1987). In 1983 it was found that this hyperactivity does not occur in a slice preparation of LC neurons (Andrade *et al.*, 1983). This indicated that withdrawal hyperactivity of LC neurons was not a consequence of altered opiate mechanisms within the LC, but instead may reflect a change in afferent control of the LC. Our recent studies revealing major afferents to the LC from the PGi and PrH suggested that one of these inputs may generate opiate withdrawal hyperactivity in the LC (Aston-Jones *et al.*, 1990). Indeed, Rasmussen and Aghajanian (1989b) have found that lesions of the PGi, or antagonism of the amino acid

pathway from the PGi to the LC, blocked the morphine withdrawal-induced activation of the LC. We (Akaoka *et al.*, 1990, 1991) and others (Tung *et al.*, 1990) have confirmed their results.

As LC may play a role in certain opiate withdrawal symptoms, these results indicated that EAA antagonists may be effective in attenuating the opiate withdrawal syndrome. With this possibility in mind, we studied the effects of several EAA antagonists during withdrawal from morphine. Rats were pretreated continuously for 6 days with morphine delivered from chronically implanted osmotic minipumps (Alza Corp.; 34 mg/kg/day). Animals were then given an EAA antagonist or vehicle and subsequently administered naltrexone (1 mg/kg, ip) to precipitate withdrawal. Several behavioral indices of opiate withdrawal were scored, including jumping, wet dog shakes, head shakes, teeth chattering, chewing, diarrhea, rhinorrhea, lacrimation, ptosis, and

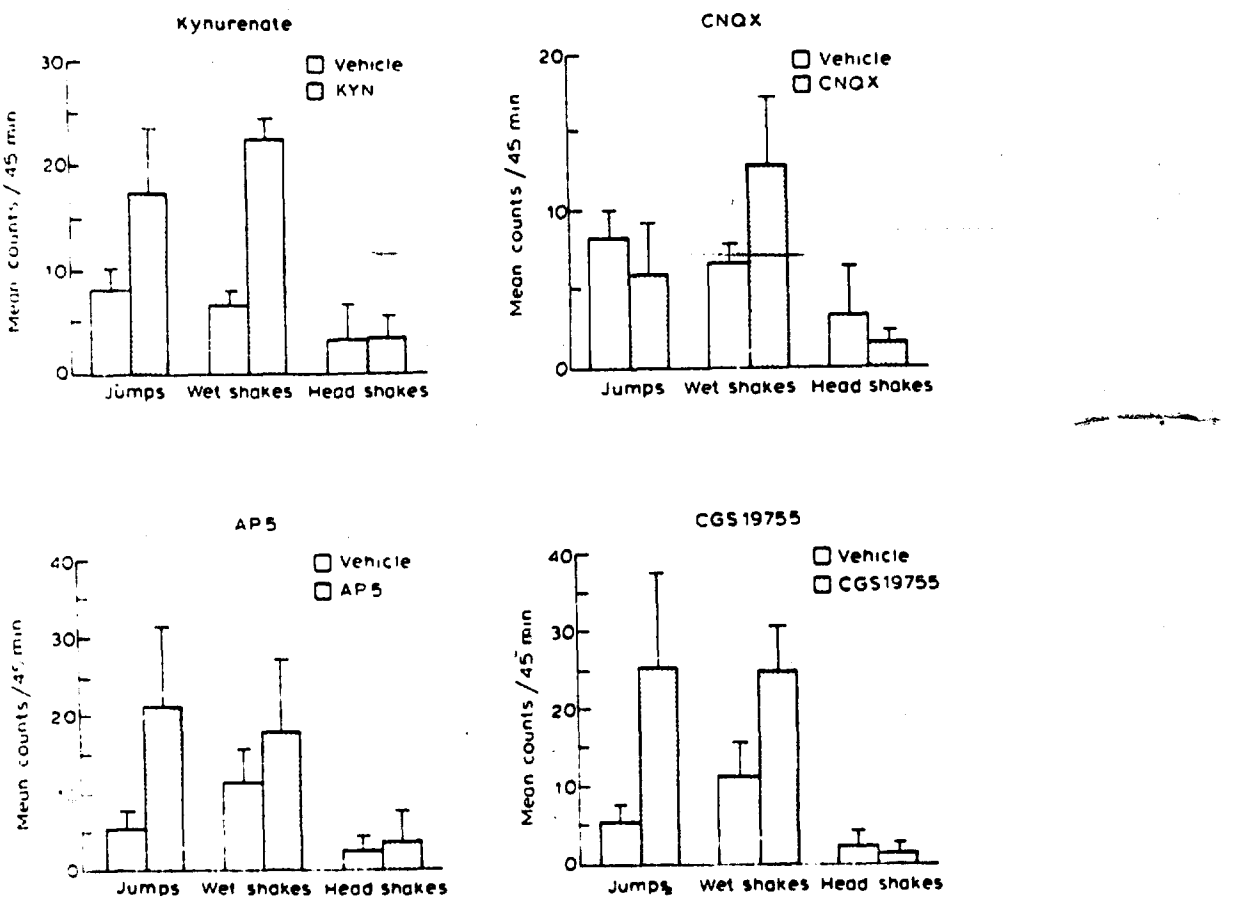


Fig. 7. Effects of EAA antagonists on naltrexone-precipitated morphine withdrawal behaviors. Rats ($n = 2$ for each drug tested) were administered morphine continuously over 6 days via osmotic minipump implanted subcutaneously. All drugs were administered icv 5 min before naltrexone injection (1.0 mg/kg, ip). The broad spectrum EAA antagonist kynurenate (11 mmol to produce about 32 mM in CSF), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (3.5 nmol to produce about 10 mM in CSF), the specific NMDA antagonist 2-amino-5-phosophonopentanoate (AP5) (20 nmol to produce about 57 mM in CSF), and the more potent NMDA antagonist CGS19755 (1.2 nmol to produce about 3.5 mM in CSF), as indicated, all showed the ability to increase escape behavior and wet shakes above control levels, but did not affect other withdrawal signs such as head shakes, oral behavior, diarrhea, rhinorrhea, lacrimation, ptosis, and piloerection. Higher doses of each drug usually produced ataxia. Overall, none of these antagonists were effective in reducing signs of behavioral withdrawal. Approximate concentrations in CSF were calculated using a value of 350 ml for CSF volume. (From Ennis and Aston-Jones, 1988.)

piloerection. Results for some of these measures are summarized in Figure 7. Naltrexone alone, or with the vehicle control instead of an EAA antagonist, consistently elicited robust withdrawal in all of the behavioral measures. However, none of the EAA antagonists tested by intracerebroventricular (icv) (kynurenate, 2-amino-5-phosophonopentanoate (AP5), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), or CGS19755 (Lehmann *et al.*, 1988)) or intraperitoneal administration (CGS19755) had consistent effects on most of the withdrawal signs. The only signs that were affected in these studies were jumping and wet dog shakes, both of which appeared to be increased by each of the EAA antagonists given icv (Fig. 7); further studies are needed to confirm these early results.

Overall, these results clearly indicate that EAA antagonists of either the NMDA or non-NMDA receptor do not prevent naltrexone-precipitated withdrawal from morphine. Thus, central EAA neurotransmitters may not be importantly involved in these withdrawal signs. Also, LC hyperactivity during this state is attenuated by kynurenate (Rasmussen and Aghajanian, 1989b; Akaoka *et al.*, 1990; Tung *et al.*, 1990) or CNQX (Akaoka and Aston-Jones, 1991); this indicates that at least these components of the morphine withdrawal syndrome are not dependent on LC hyperactivity. This is consistent with observations (Britton *et al.*, 1984) that lesions of the ascending NE projections from LC do not attenuate such behavioral signs of opiate withdrawal. Although LC may not mediate these behavioral manifestations of opiate withdrawal, its hyperactivity may be important for other withdrawal phenomena. Additional studies are needed to determine what components of opiate withdrawal are induced by, or are dependent upon, the hyperactivity seen in LC neurons.

Effects of prefrontal cortex stimulation on LC activity

While these results seem adequate to explain certain properties of LC neurons, other characteristics do not fit easily into this framework. In particular, it is difficult to understand how inputs from only two medullary structures could be responsible for the selective responsiveness of primate LC neurons to meaningful stimuli during discrimination behavior (described above). Such complex behavior is generally associated with cortical structures, yet there were no cortical inputs to LC in our tract-tracing analysis.

The prefrontal cortex (PFC) has been linked previously with high level cognitive and attentional processes. Others (Arnsten and Goldman, 1984) have reported projections to the LC area of primates from the PFC. Although there were no retrogradely labeled cells in cortex following injections of tracers into LC, we examined descending projections of the PFC in rat using anterograde transport of WGA-HRP from the medial PFC (Chiang *et al.*, 1987). Such injections yielded remarkably specific innervation patterns in the dorsal pons, with dense innervation of the central grey rostral and medial to LC but no fibers or terminals within central LC proper. As described in Aston-Jones *et al.* (this volume), this region is densely innervated by extranuclear dendrites of LC neurons. These results confirmed those reported for monkey (Arnsten and Goldman, 1984), and indicated that PFC could influence LC neurons via innervation of distal extranuclear dendrites, or less directly by innervation of neurons in the pericoerulear region that, in turn, may innervate LC or its extranuclear dendrites (see Aston-Jones *et al.*, this volume).

To examine the effect of PFC activity on LC neurons, we (Chiang *et al.*, 1987) stimulated the medial PFC while recording single LC neurons in anesthetized rats. The most consistent response of LC neurons following cortical stimulation was antidromic activation (9 of 27 cells), as expected. In subsequent subjects, 6-hydroxydopamine was infused into the midbrain dorsal noradrenergic bundle 1 week prior to experiments to lesion ascending LC projections and eliminate this confounding effect. As illustrated in Figure 8a, low-frequency stimulation of PFC in such animals

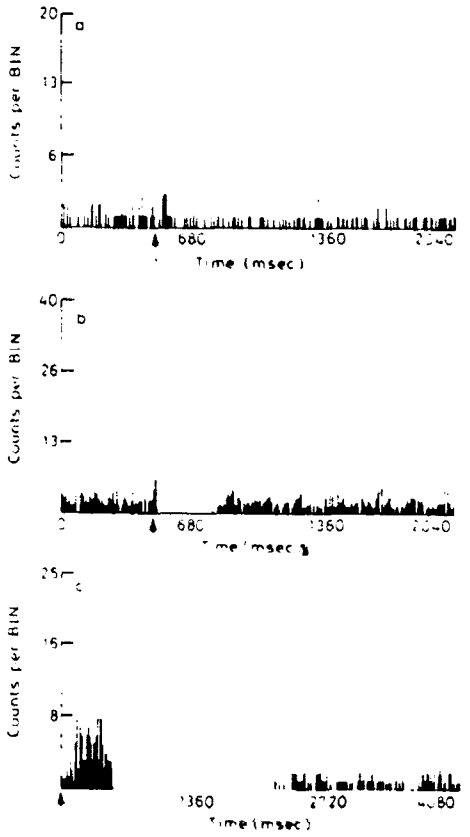


Fig. 8. Train, but not low-frequency stimulation, of prefrontal cortex potently activates LC neurons. PSTHs illustrating responses of LC (a and c) and lateral dorsal tegmental neurons (b) to stimulation of prefrontal cortex (PFCx) in rats. Panel a. PSTH showing response of an LC neuron to PFCx stimulation at 0.5 stimuli/sec (1.0 mA). Twenty-three of 58 cells exhibited weak excitation at relatively long latencies (mean onset = 41 msec). In addition, 9/58 cells were weakly inhibited at long latencies (mean onset = 63 msec, not shown). Forty-four percent of LC neurons exhibited no response to PFCx stimulation. Panel b. PSTH using similar stimulation but recording from a cell in the lateral dorsal tegmental nucleus (LDT) where anterograde label is seen from PFCx injections. Note the more robust, shorter latency excitatory response. Panel c. Train stimulation of PFCx (10 pulses at 20 Hz) consistently produced significant activation of LC neurons (55/64 cells; mean onset = 120 msec, mean duration = 227 msec). Taken together, these data are consistent with an indirect or distal dendritic influence of PFCx on LC neurons. Stimulation at arrows in all PSTHs, 50 sweeps in panels a and b, and 25 sweeps in panel c.

yielded only weak effects on 23/58 LC neurons, and no significant effect on the remaining cells. In contrast to this weak influence on LC neurons, Figure 8b shows that similar stimulation of PFC potently activated cells in the laterodorsal tegmental nucleus, in the same area that was heavily labeled by anterograde transport of WGA-HRP (described above). This indicated that the PFC was preferentially influencing neurons in pericoerulear areas that are densely innervated with PFC fibers and terminals, and only weakly (perhaps indirectly) influencing LC neurons.

Consistent with this possibility, in additional experiments we found that train stimulation of the PFC activated LC cells much more potently than single-pulse stimulation (Fig. 8c). In addition, we found that infusions of the local anesthetic, lidocaine, into the PGi partially attenuated the influence of PFC stimulation on LC (Chiang *et al.*, 1987). These results suggest that cognitive activity at least partially accesses the LC indirectly through the PGi; additional influence may also arise through possible connections onto distal dendrites or local "interneurons" in the pericoerulear region.

These results, together with those indicating that PGi mediates at least some sensory responses of LC cells, indicate that brainstem connections play an extremely important and broad integrative role in regulating the outflow of the LC broadcast system. In addition, the cellular characteristics of these major afferents (summarized below) shed additional light on LC function, and how adaptive behavioral responses to a changing sensory environment are generated.

A view of LC function based on cellular attributes: the vigilance / response initiation hypothesis

As outlined at the beginning of this article, a cellular anatomic and physiological understanding of the LC system requires knowledge of (1) the efferent projections of LC neurons, (2) the effects of NE released from LC terminals on target neuron activity, (3) the conditions under which LC neurons are active and releasing their transmitter, and (4) the factors controlling LC

discharge. When viewed together, these cellular properties have broad functional implications.

First, the widespread efferent trajectory of the LC system implies that its function is a very global one, with physically distant and functionally disparate brain areas receiving innervation from (often individual) LC neurons. This notion is underscored by our physiological studies, revealing that LC neurons are markedly homogeneous in their discharge characteristics; for example, LC neurons throughout the nucleus exhibit very similar rates and patterns of spontaneous or sensory-evoked impulse activity (Aston-Jones and Bloom, 1981a,b). Thus, our data, in combination with the efferent anatomic results reviewed above, indicate that robust LC discharge results in globally synchronized release of NE onto target neurons located throughout the neuraxis.

Postsynaptically, NE influences target cells so as to relatively promote responses to other, strong afferent input while reducing spontaneous or low-level activity. Such an enhancement of postsynaptic "signal-to-noise" ratios can lead to increased selectivity of target cell discharge to favor specific aspects of their response profiles, as discussed in this volume by Waterhouse, Woodward and others.

In the context of these previous findings, the specific conditions of LC activation in unanesthetized behaving animals lead us to an hypothesis for LC function, suggesting a role of this system in the control of vigilance and initiation of adaptive behavioral responses (Aston-Jones and Bloom, 1981a,b; Aston-Jones *et al.*, 1984). We have proposed that the LC is strongly influenced by two general classes of extrinsic afferents (each possibly derived from two or more separate groups of neurons): excitatory inputs mediating sensory-evoked (or state transition-related) activity in LC neurons, and a more tonically active set of inhibitory afferents serving to modulate overall LC excitability in accordance with the vigilance state associated with the concurrent behavior. The more recent findings that PGi and PrH are major excitatory and inhibitory afferents to LC suggest that they may provide these two classes of inputs; however, further work is needed to test this possibility.

The level of LC activity at any time may be a consequence of the relative influence of each of these two classes of inputs. Strong tonic inhibition (such as found during PS) could serve to prevent LC neurons from responding to environmental stimuli during this state, so that LC inactivity permits PS to occur. Conversely, we propose that intense LC activity interrupts automatic, internally driven or vegetative behaviors (such as sleep, grooming, or consumption) that are incompatible with phasic, adaptive behavioral responding and instead engages a mode of activity characterized by a high degree of vigilance and interaction with diverse environmental stimuli. This theoretic framework is consistent with our observation that LC activity is most intense just before interruption of low-vigilance behaviors such as sleep, grooming or consumption, giving rise to alert orienting behaviors.

Intense LC activation may occur when either tonic inhibition of LC neurons (engaged for automatic or vegetative behaviors) has suddenly decreased, or when excitation impinges on these cells (in response to a strong, unexpected sensory event) that is sufficiently intense to overcome concurrent tonic inhibitory inputs. Conversely, low vigilance programs may predominate in behavior when either LC discharge is effectively inhibited from responding to unexpected external stimuli, or when strong unexpected stimuli are not present in the environment. In this way, the LC may serve as a gate to determine the relative influences of two mutually exclusive sets of behavioral programs. In general terms, the LC may function to influence the overall orientation of behavior or mode of sensorimotor activities, to favor either automatic, vegetative behavioral programs, or phasic adaptive responding to salient environmental stimuli.

An alternative, but equivalent, expression of this proposed role for the LC in the regulation of vigilance is a role in the initiation of adaptive

behavioral responses. Pronounced LC activity is associated with abrupt attention to external stimuli, which itself immediately precedes, and is a necessary component of, initiation of adaptive motoric response to salient external stimuli. Thus, in our analysis, the LC can logically fit into either sensory or motor functions, as it is not solely or directly related to either. Analysis of LC function in terms of state regulation is an alternative, and perhaps more appropriate, framework.

This overall hypothesis of LC function can be stated in more abstract terms of nervous system operation. One view of heightened vigilance (*e.g.*, startle, awakening, or stimulus-evoked disruption of ongoing behavior) is that this state is associated with conflicting patterns of neural activity, brought about, for example, by a disrupting stimulus that is inconsistent with (conflicts with) the set of stimuli that are predicted or expected to accompany the ongoing behavioral paradigm. The ensuing state of heightened vigilance consists, in this view (Fuller and Putnam, 1966), of a set of behaviors aimed at reducing or resolving this conflict, so that impinging stimuli are once again predicted by behavior. The mode of achieving this resolution involves investigating or exploring different behaviors in the animal's repertoire (that may have had a weak relationship to a similar stimulus in the past). This "internal exploration" activity can be likened to a random search process, exploring the field of possible behavioral responses to the unexpected stimulus event. In our hypothesis, illustrated in Figure 9, robust LC discharge accompanying such a stimulus would engage a random search process by terminating ongoing low-vigilance activity and rearranging neural priorities via enhancement of signal-to-noise ratios of target neurons. This latter effect would result in preferential transmission of nervous information concerning salient stimuli and events, thereby favoring responses to the most urgent current stimuli. This proposed role of the LC as a random search generator is consistent with (and is simply a restatement of) the proposed roles in vigilance regulation and adaptive response initiation.

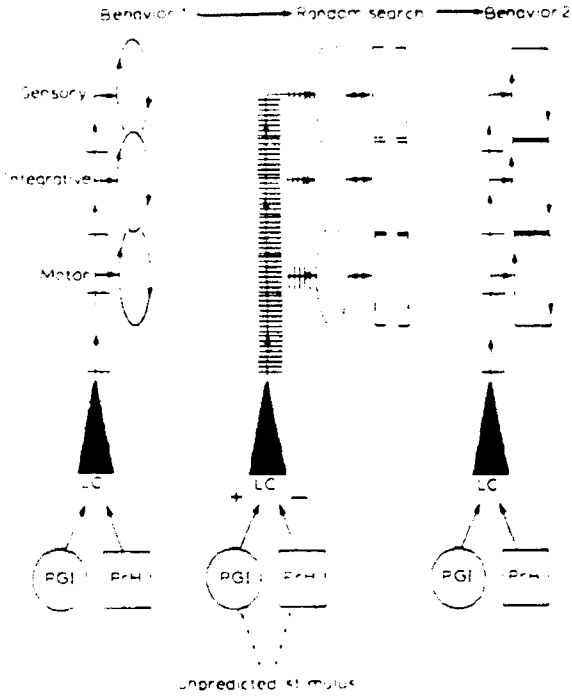


Fig. 9. Schematic illustrating proposed role of LC system as a random search generator. Left panel. Stable behavior represented by self-reinforcing (self-consistent) neural loops representing different aspects of a sensory-motor ensemble for a particular set of stimuli. Middle panel. An unexpected, meaningful stimulus occurs that activates LC (via PGi or prepositus hypoglossi (PrH)). The global release of norepinephrine from LC terminals suppresses low-level non-driven activity in neural loops or relatively enhances responses to phasically strong inputs, destabilizing and disrupting current ongoing behavior. This destabilized state by default "searches" for circuits that are sufficiently self-reinforcing and self-consistent to establish a new set of stable neural loops. Right panel. Activity driven by the currently strongest sensory events (and associated motor acts) establishes a new steady state condition of stable neural loop activity representing a new behavior or state.

Functional implications of major innervation of LC by PGi and PrH

The new findings concerning afferent control of LC described here and in Aston-Jones *et al.* (this volume) have led to advances in understanding other properties of this system. One example is from the work of Engberg and colleagues who

found several years ago that systemic nicotine potently activates LC neurons via an unknown, indirect influence (Engberg and Svensson, 1980; Svensson and Engberg, 1980). Recent work has found that the PGi is the critical link in this response. Their evidence indicates that nicotine stimulates peripheral sensory (presumably visceral) afferents which in turn activate the excitatory amino acid pathway from PGi to LC (Hajos and Engberg, 1988; Chen and Engberg, 1989; Engberg, 1989). These results, and the close connection of PGi to visceral stimuli (via, e.g., vagal inputs to NTS to PGi) indicates that other drugs may affect the LC in a similar way, and suggests a new pathway for some psychopharmacological effects. One other example of potent drug effects on LC neurons that has been found to be mediated through the PGi is hyperactivity of LC neurons during withdrawal from morphine, described above. Such knowledge of the mechanisms of drug influences on LC neurons is a significant advance as it opens the way for modulation of these effects, which are thought to be important for the psychological and behavioral impact of many systemically administered drugs.

The recent findings for major afferents to LC from the PrH and PGi have also prompted us to extend our theoretic framework to include functional attributes of these rostral medullary regions, as described below (Aston-Jones *et al.*, 1990).

The PrH is classically known to be a preoculomotor area. It has strong projections to oculomotor nuclei of the brainstem and many of its cells discharge closely in relation to eye movements (Baker, 1977; McCrea *et al.*, 1979; Brodal, 1983; McCrea and Baker, 1985). However, as illustrated in Figure 10, this nucleus also has connections to pinnae motor areas (Henkel, 1981) and to vestibular nuclei that influence head movement (Cazin *et al.*, 1982, 1984). Also, many PrH neurons exhibit activity that does not readily fit into a strict oculomotor framework (Lopez-Barneo *et al.*, 1982; Lannou *et al.*, 1984). It is important to note that the LC-projecting neurons in PrH are

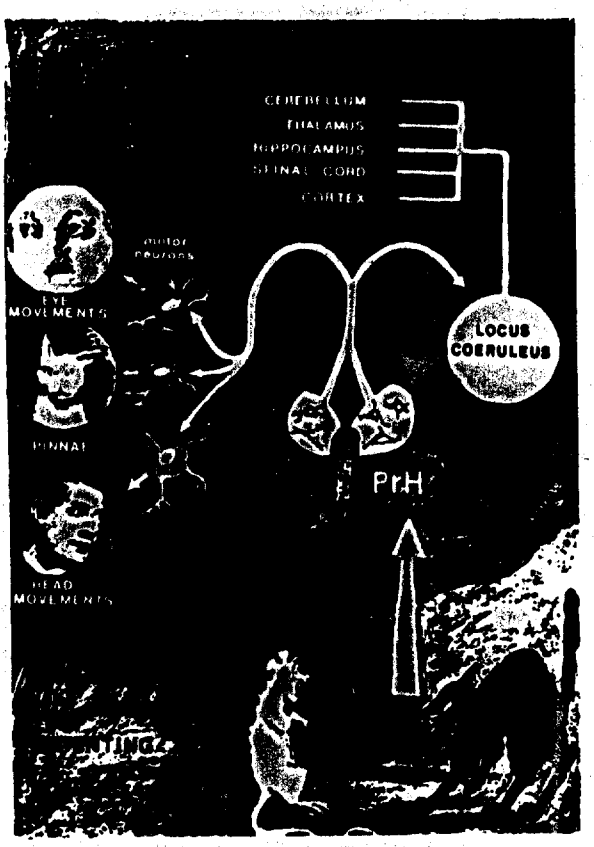


Fig. 10. Functional attributes of the PrH. Many PrH neurons project to brain areas associated with ocular, pinnae and head movements, all components of orienting behavior. Thus, stimuli that are sufficiently intense to elicit an orienting response may do so in part through PrH circuitry, which at the same time may participate in the activation of LC at such times. This model predicts that LC-projecting neurons in PrH would decrease activity during orientation and release LC from tonic inhibition. Thus, LC would be disinhibited and prepotent for responding to stimuli at the same time that sensoria are oriented towards the most salient stimuli, helping to increase attentiveness to such stimuli. It is important to note that many aspects of this model remain to be tested

restricted to the medial and perifasicular aspect of this nucleus, and that the PrH has received little attention in the rat. These properties, and the fact that the medial PrH is a major input to the LC, may indicate a somewhat broader function for the PrH (or, in particular, those PrH neurons that innervate the LC) than only oculomotor control. In an admittedly conjectural view, the PrH may be concerned with the initiation and

coordination of holistic orientation responses, rather than just the ocular components. In this framework (illustrated in Fig. 10), unexpected, urgent stimuli may influence the PrH to (i) orient the sensoria towards salient stimuli, and (ii) coordinate other central processes important in generating adaptive responses to imperative stimuli (*e.g.*, increase LC excitability). As the PrH potently inhibits the LC via a GABA pathway (Ennis and Aston-Jones, 1989), this model predicts that the robust LC activity accompanying orienting behaviors results, at least in part, from disinhibition of LC from PrH (Aston-Jones *et al.*, 1990).

The PGi is a key sympathoexcitatory brain region, sending strong projections to directly innervate preganglionic sympathetic neurons in the spinal cord (Milner *et al.*, 1988; Ross *et al.*, 1981; Ruggiero *et al.*, 1985). Thus, it is an important brain region for preparing the body to respond to urgent stimuli in the environment (defense response, "fight or flight" response) as sympathetic responses to such stimuli may be mediated, at least in part, through this area. As such unexpected or urgent stimuli are also the most reliable stimuli for activating LC neurons in rats or monkeys (described above), this function for the PGi suggests that it may be involved in activating LC neurons as well as peripheral sympathetic neurons in response to such stimuli. In fact, there is a remarkable temporal correlation between evoked LC discharge and sympathetic nerve activity (Elam *et al.*, 1981, 1984, 1985, 1986; Reiner, 1986). This led us to test whether sensory responses of LC neurons are mediated through the PGi. Indeed, as described above, we found that blockade of the EAA pathway from the PGi eliminated responses to sciatic nerve activation (Aston-Jones and Ennis, 1988; Ennis and Aston-Jones, 1988), as did disruption of synaptic transmission within the PGi (Aston-Jones and Ennis, 1988; Chiang and Aston-Jones, 1989; Aston-Jones *et al.*, 1990). Thus, as illustrated in Figure 11, it appears that the peripheral sympathetic system is activated in parallel with the central LC system

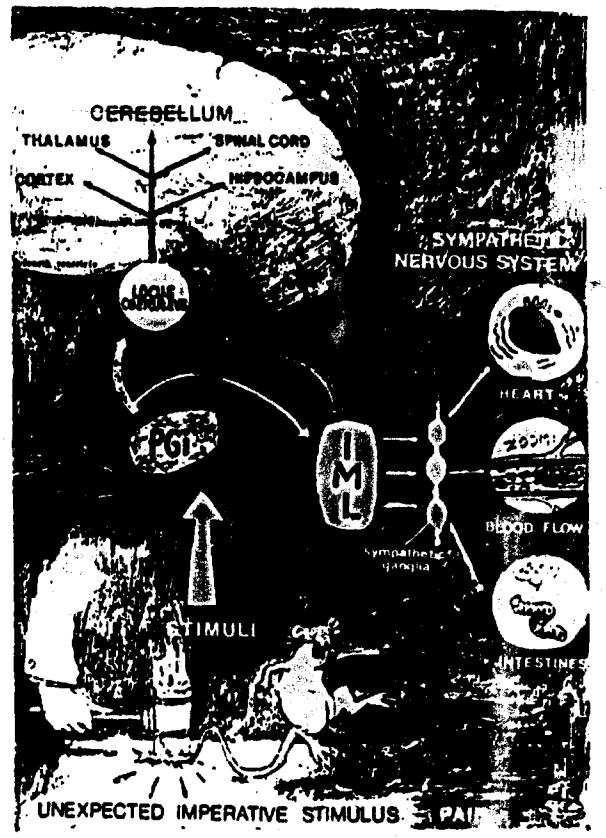


Fig. 11. Functional attributes of the PGi. The PGi is a key sympathoexcitatory region, reflecting its strong connections to sympathetic preganglionic neurons of the intermediolateral cell column (IML) of the spinal cord. It is known that stimuli that activate the peripheral sympathetic system also activate the LC (see text); we propose that this co-activation reflects parallel projections from PGi to IML and LC. Thus, activation of the peripheral sympathetic system prepares the animal physically for adaptive phasic responses to urgent stimuli, while parallel activation of LC increases vigilance and attentiveness, preparing the animal cognitively for adaptive responsiveness to such stimuli. It is proposed that LC serves as the cognitive limb of the global sympathetic nervous system, and that cognitive and peripheral sympathetic responses are integrated and coordinated through PGi.

by projections to both from the PGi area. Preliminary studies indicate that projections to sympathetic neurons and LC arise from separate but intermingled cells in PGi. Nonetheless, the PGi appears to be a key area for integration and coordination of activities in the LC and the sympathetic systems.

This analysis of the PGi has led us to extend our hypothesis of LC function, from serving to control vigilance to acting as the cognitive limb of a global sympathetic system, serving to optimize the animal's behavioral state (via heightened attention to environmental stimuli) for making adaptive decisions concerning phasic behavioral responses at the same time that the peripheral sympathetic system prepares the animal physically to execute phasic responses to urgent stimuli (Fig. 11).

One possible, though admittedly speculative, extension of our recent research concerns the nature of neural processing necessary to specify unexpectedness or novelty. The stimuli that best activate LC neurons possess the attributes of unexpectedness or novelty, and typically cause both a sympathetic and behavioral orienting response. By investigating the circuits whereby sensory stimuli are processed and transferred to PGi for activation of LC, we will begin to elucidate the neural mechanisms that are used to compute the stimulus qualities of expectedness, novelty and urgency. This has broad implications for neurobiological studies involving attention, stimulus-gating and preparatory behavioral set.

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Acute morphine induces oscillatory discharge of noradrenergic locus coeruleus neurons in the waking monkey

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Neurons were recorded extracellularly from the locus coeruleus (LC) of a waking, chair-restrained cynomolgus monkey before and for 0.5–4 h after intramuscular injections of morphine sulfate (0.3–3.0 mg/kg). Tonic discharge of each LC neuron tested ($n = 11$) decreased after morphine injection. This effect appeared to be dose-dependent for the range of 0.3–3.0 mg/kg. Unexpectedly, these same doses of morphine also induced a pronounced burst-pause discharge pattern in all LC neurons recorded. Thus, whereas in the naive animal pauses in discharge longer than 3 s were rare during waking, after morphine injection LC neurons frequently exhibited pauses in impulse activity of 10 s or longer during non-drowsy waking. The bursts in activity following morphine corresponded to orienting behaviors or apparent alertness, whereas pauses were associated with eye closure or slowly drifting gaze. Closer analysis revealed that this burst-pause activity pattern was somewhat regular, with a period of about 15–35 s. This observation was confirmed by autocorrelogram analysis. In view of previous findings in rodent LC, we suggest that acute morphine elicits a dual effect on primate LC neurons, inhibition of discharge by direct effects on opiate receptors located on LC cells, and periodic phasic activation mediated by excitatory afferents to the LC.

Several findings indicate that the noradrenergic neurons of the brain nucleus locus coeruleus (LC) may be important mediators of the effects of exogenous opiates. LC neurons are heavily invested with opiate receptors (predominantly of the μ subtype; [12, 25, 30]) and exogenously applied opiates potently inhibit impulse activity of LC neurons [3, 6, 14, 16, 32, 34]. These findings, combined with the many roles proposed for this ubiquitous noradrenergic system [7, 9–11, 16], have led to proposals that the LC system mediates several effects of opiates. For example, the LC has been proposed to play an important role in analgesia [23], so that opiate actions on LC neurons may be one avenue for opiate-induced analgesia. Similarly, LC has been proposed to play a central role in regulation of behavioral state and alertness [7, 9], functions markedly altered by opiates in a manner consistent with mediation through the LC system. Finally, a host of studies indicate that heightened LC discharge during opiate withdrawal may mediate various symptoms of the opiate withdrawal response [18, 19].

While several experiments have found that morphine directly applied to LC neurons hyperpolarizes these cells [6, 14, 34] or inhibits their impulse activity [4, 16, 32], studies using systemic morphine in unanesthetized animals have been less consistent. In unanesthetized rat, Valentino et al. [32] found that intraventricular morphine inhibited LC discharge while Rasmussen et al. [27] reported that intravenous morphine increased tonic LC discharge in cat. However, there have been too few studies of morphine effects on LC neurons in unanesthetized animals to resolve such conflicts, and no such studies in primates. Therefore, we investigated the effects of acutely administered morphine on discharge of LC neurons in the waking, chair-restrained cynomolgus monkey. Our results reveal that morphine acutely decreases LC discharge but simultaneously increases periodic bursting of these neurons [26].

A male cynomolgus monkey (*Macaca fasicularis*, approx. 5 kg) was habituated to chair restraint and trained to perform a visual discrimination task [5, 8]. Subsequently, during sterile surgery chronic electrode holders were affixed to the skull bilaterally for recording impulse activity of LC neurons, along with a post for fixing head position. The electrode holders consisted of a cylinder which held a guide tube (18 gauge) stereotaxically directed at the LC. One or two microwire recording elec-

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trodes (25 μm diameter, factory preinsulated) were placed in a 26 gauge inner cannula and inserted through one of the guide cannulae to record impulse activity of LC neurons. The recording electrode inner cannula was attached to a screw-driven microdrive which allowed dorsoventral movement in increments of approximately 40 μm. The recording microwire extended approximately 7 mm below the guide and inner cannulae when positioned in the LC. An FET-preamplifier attached to the microdrive provided a low-impedance microwire recording signal, which was fed via overhead cables to conventional filters and amplifiers. LC neurons were tentatively localized during recording sessions by their characteristic discharge properties, including slow impulse activity (about 1–4 spikes/s), markedly decreased tonic activity during periods of drowsiness, and biphasic excitatory–inhibitory responses to novel stimuli [15, 16, 20]. The position of the guide tube could be adjusted after surgery, so that multiple penetrations through different rostrocaudal or mediolateral positions were possible with one implant.

During recording sessions, the animal was placed in a customized comfortable primate chair inside a large sound-attenuating chamber, and his head was fixed in place by an overhead arm attached to the head-mounted fixation post. The animal adapted rapidly to this restraint, never showed signs of resistance or antagonistic behavior, and usually climbed voluntarily from his cage to the chair. Pupil size and eye movements were observed via an infrared video camera positioned near the orbit and video monitor outside the environmental chamber. Before morphine injections, the animal performed a visual discrimination task for another experiment [5, 8] (data not shown). The animal typically stopped performing this task shortly after morphine injection.

Morphine sulfate was dissolved in saline and injected intramuscularly (in 0.4–0.8 ml, i.m.; into neck muscles) while the animal was distracted by presentation of orange-flavored juice. Injections elicited little or no distress from the animal. Discharge of a single LC neuron was recorded for at least 30 min before each morphine injection, and for 30 min–4 h afterwards. One injection only was made per day and per cell, and injections were spaced apart by at least 5 days (usually longer; average interval between injections = 20.5 days). The order of doses was varied in a semi-random fashion.

Spike data were continuously collected on computer disk and analyzed off-line (Cambridge Electronic Design 1401 interface, Spike 2 software). Mean tonic discharge rate was calculated in 500 s bins. Other data were analyzed from discharge frequency plots; these are plots of the mean discharge rate for an individual neuron during the 10 s interval preceding each spike.

At the end of the last recording session for each hemisphere, cur. + (10 μA, 30 s) was passed through the tip of the microwire recording electrode to mark its position of LC. The monkey was sacrificed by perfusion under deep nembutal anesthesia 2 and 10 days after marking lesions. Frozen sections (30 μm thickness) were taken through the LC; alternate sections were stained for Nissl (Cresyl violet) or with an antibody against tyrosine hydroxylase (TH; Eugene Tech mouse monoclonal; ABC-peroxidase). Recording sites were estimated by comparing the depths noted during recording sessions with the locations of the marking lesions in each hemisphere. All data reported here are from recordings presumed to have been obtained from noradrenergic LC neurons based on (i) histologic location near or within the group of TH-positive LC neurons, and (ii) discharge features characteristic of noradrenergic LC neurons in previous studies (see above).

The discharge of 11 LC neurons was recorded before and after various doses of morphine ($n = 3$ for 0.3, 1 and 3 mg/kg; $n = 2$ for 10 mg/kg). The effects of morphine were similar from cell to cell in this population, and the results given below are typical.

Morphine decreased the rate of tonic discharge of each LC neuron tested. The rate-depressing effect of morphine appeared to be time- and dose-dependent in the range of 0.3–3 mg/kg (Fig. 1). There was an overall significant effect of time and dose on mean tonic discharge rate ($F = 34.2$, $P < .001$; ANOVA with repeated measures).

Morphine injections had profound effects on behavior, which varied with the dose given. The lowest dose (0.3 mg/kg) arrested performance on the discrimination task and gave rise to epochs (10–60 s long) of fixed or slowly drifting gaze while the pupil diameter oscillated widely. The highest dose (10 mg/kg) caused periods of apparent drowsiness, when the animal closed his eyes for 10–30 s and was immobile. Doses of 1 mg/kg and 3 mg/kg caused episodic behavioral sedation intermediate between these states, with prolonged periods of immobility and fixed or drifting gaze and pupil fluctuations, but no or only occasional epochs of eye closure.

In addition to decreased average discharge, we were surprized to observe that after morphine LC neurons consistently exhibited marked bursts of activity intermixed with prolonged (10 s or longer) pauses in discharge; LC neurons did not exhibit such burst-pause activity patterns in the absence of morphine (Fig. 2). While difficult to quantify, this burst-pause activity pattern appeared to be most intense with the 3 mg/kg and 10 mg/kg doses of morphine (Figs. 2 and 3), but was present with all doses tested. In addition, this burst- pause activity pattern occurred with some regularity. Observations of discharge frequency plots and autocorrelation analysis

revealed that bursts and pauses in activity tended to occur with a period of between 15 and 35 s (Fig. 3). In contrast to the amplitudes of fluctuations in activity, this periodicity of fluctuations appeared to be approximately the same for different doses of morphine.

The burst-pause activity pattern of LC neurons after morphine was closely related to abrupt changes in apparent behavioral state and activity. Behavioral state was categorized using distinct patterns of eye movement observed on the ocular video monitor, as follows: apparent sedation or inattentiveness was scored for epochs of fixed or slowly drifting gaze, while apparently increased alertness and attentiveness was scored for periods of phasic eye movements and visual exploration. As noted above, after morphine the animal exhibited various degrees of episodic sedation, dependent upon the dose administered. We noted that all LC neurons recorded after morphine consistently exhibited an activity burst with (often in advance of) abruptly increased alertness, and a pause in discharge with sedation or inattentiveness. This relationship was most apparent with high doses of morphine: when the animal closed his eyes, LC neurons were inactive (generating the pauses in activity), but became active again when the monkey opened his eyes and explored his environment (yielding the bursts in activity). With lower morphine doses, the same relationship was observed between discharge and behavior except that eye closure did not occur. Instead, the monkey periodically attained a fixed or slowly drifting gaze which was consistently associated with LC inactivity; such periods usually lasted 10–30 s. Typically, the monkey then abruptly resumed active ocular movement and exploration of his environment, and this behavioral change was closely associated with a burst of LC activity and continued tonic discharge.

The changes in LC discharge and behavior induced by morphine persisted throughout the period during which individual LC neurons were recorded (up to 4 h). Thus, there was no apparent recovery from acute morphine effects within the time frame examined here.

The present results demonstrate that systemic morphine in the awake monkey decreases tonic LC discharge but simultaneously induces LC neurons to exhibit oscillatory burst-pause activity with a period of 15–35 s. Furthermore, these discharge patterns of LC neurons were closely related to morphine-induced behaviors. Decreased discharge corresponded with apparent sedation or inattentiveness, while bursts of LC activity were closely associated with apparently increased arousal and alertness. Thus, the periodic oscillation of LC discharge after morphine corresponded with periodic changes in behavioral state.

The effects of morphine were very similar for each LC neuron examined. All cells exhibited decreased activity after morphine. All doses of morphine also induced periodically oscillatory discharge of LC neurons, with greater doses inducing apparently more intense fluctuations in activity. Nonetheless, the period of these oscillations was surprisingly uniform (15–35 s) between doses and neurons.

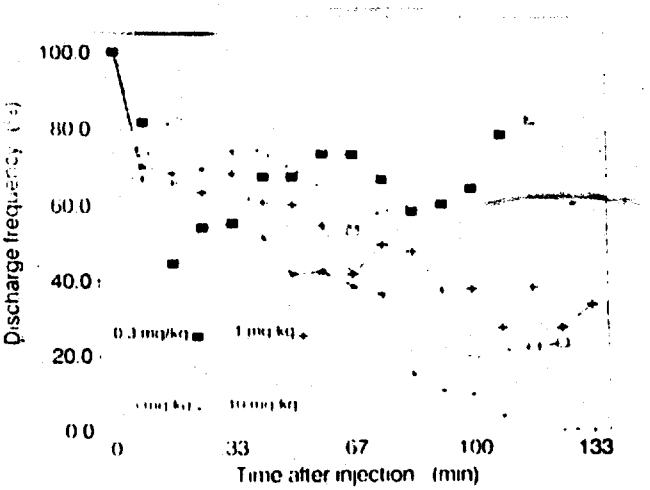

Fig. 1. Mean percentage of pre-drug (baseline) spontaneous discharge rate for LC neurons following i.m. morphine at different doses, as indicated. Data are averaged for 3 cells for each dose, except 10 mg/kg, which is for 2 cells. Note apparent time- and dose-dependent decrease in activity. Vertical bars attached to dose-symbols are mean values of the standard errors of the mean for the points in each corresponding dose curve.

The small number of cells, repeated injections into the same animal, and the single monkey studied are limitations of the present study. However, these limitations are offset to some extent by the similarity among monkey LC neurons studied here and in previous reports [5, 8, 10, 15, 16, 20, 21].

Doses were separated by at least 5 d (average = 20.5 d) to minimize the possible development of tolerance; this is an important consideration as LC neurons have been found to exhibit profound tolerance to morphine with frequent or continuous exposure [6, 13]. However, with the temporally separated injections used here tolerance to morphine was not apparent in either behavioral observations or cellular activity. Nonetheless, it will be important to confirm these results in additional subjects.

Another concern in the present study is the route of administration employed. Morphine reaches the brain more slowly by the intramuscular route employed than when given intravenously, and previous work has shown that the rate of drug access to brain is an important factor in determining behavioral effects [22]. In addition, opiate abusers typically administer drugs intravenously. However, morphine given intramuscularly is not sub-

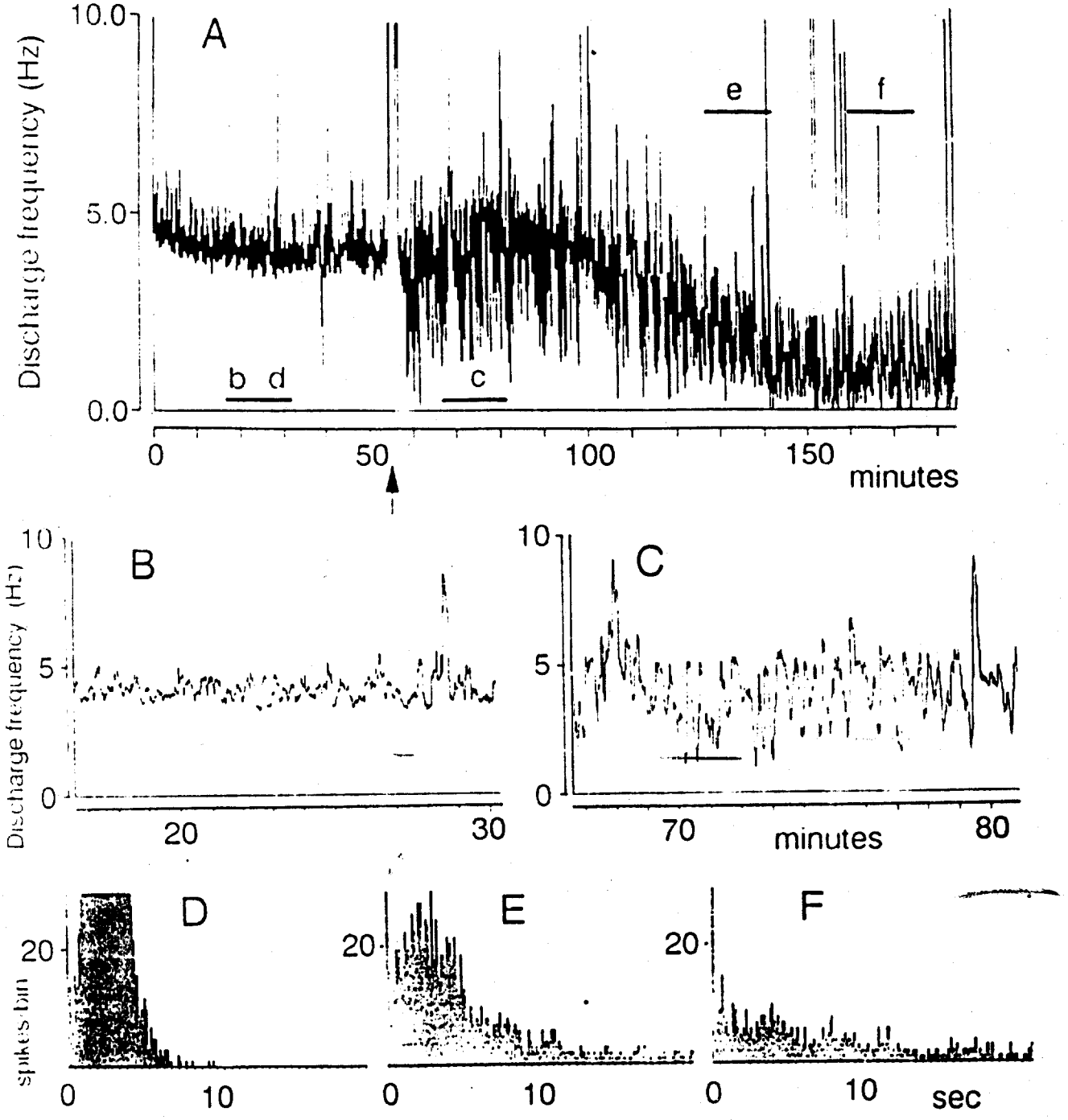

Fig. 2. A: discharge frequency plot for a typical LC neuron before and after 10 mg/kg morphine, i.m. (injection at arrow). Plot is of the mean frequency of discharge averaged over the 10 s interval preceding each spike. Note that in addition to an overall decrease in mean discharge with time, activity fluctuates markedly after morphine, with pauses interspersed among bursts of discharge. Bars marked b and c correspond to epochs expanded in time in B and C, respectively. Bars marked d–f correspond to epochs during which interspike interval histograms were taken, shown in D–F, respectively. B: discharge frequency plot of the same LC neuron as in A, but at higher temporal resolution, before morphine. This plot corresponds to the epoch marked b in A. Compare to a similar resolution plot after morphine, shown in C. C: discharge frequency plot of the same LC neuron as in A, but at higher temporal resolution, after morphine. This plot corresponds to the epoch marked c in A. Compare to a similar resolution plot before morphine, shown in B. D–F: interspike interval histograms (ISHs) of activity for this neuron before (D; taken from epoch d in A), or after morphine (E and F; taken at epochs e and f in A). Note the increased proportions of short and long interspike intervals after morphine compared to before morphine.

jected to 'first-pass' liver metabolism, and reaches the brain substantially faster than after oral administration [22]; the monkey in our study typically began to show clear behavioral signs of opiate intoxication within 2 min

of injection. It will be of interest to determine if results differ compared to intravenous injections in future studies.

The present results are consistent with but extend previous reports of opiate effects on LC neurons. Studies in vitro have clearly shown that opiates act at μ receptors to directly inactivate LC neurons by hyperpolarization [3, 34]. Similarly, local application of opiates onto LC neurons in vivo has consistently been found to potently decrease their spontaneous discharge [1, 2, 4], as has intraventricularly administered morphine [31, 33]. However, effects of systemic morphine on LC cells have differed for studies in anesthetized vs. unanesthetized animals. Investigators have consistently found that morphine given intravenously to anesthetized rats rapidly and potently inhibits LC discharge [4, 16, 24, 31]. However, in waking cats intravenous morphine is reported to activate LC neurons [27]. This discrepancy may indicate that a different population of LC neurons was recorded in cat, a species whose neurochemically heterogeneous LC nucleus makes identification of noradrenergic neurons uncertain. In addition, cats typically exhibit a different behavioral response to opiates than other species: opiates often activate rather than sedate these animals [17, 29]. Such behavioral activation may give rise to higher LC activity, as LC neurons are well-known to be tightly linked with behavioral state in this manner [7, 9, 15, 16, 20, 28]. Indeed, this would be consistent with the present results showing a close association between morphine-induced behaviors and oscillations in LC activity.

The periodically oscillatory activity observed was unexpected but pronounced after morphine. However, preliminary analysis indicates that similar oscillations may also occur spontaneously without morphine, though much smaller in amplitude. This may be related to results of similar ultradian fluctuations in the EEG and behavior of monkeys previously reported [14].

The mechanism by which morphine induces these changes in monkey LC activity is not known. However, previous work in other species allows reasonable hypotheses to be generated. One possibility is that the decrease in tonic LC discharge is induced by the direct action of morphine on LC neurons, consistent with previous studies described above. However, systemically administered morphine in waking monkeys may also induce changes in the activity of afferents to LC which may be responsible for the periodically oscillatory discharge observed. We propose that morphine may potentiate a periodically active excitatory afferent to the LC, either by increasing the activity or transmitter release of these afferent neurons, or by altering the sensitivity of LC neurons to such inputs. The presently observed effects of morphine may then result from the superimposition of such potentiated

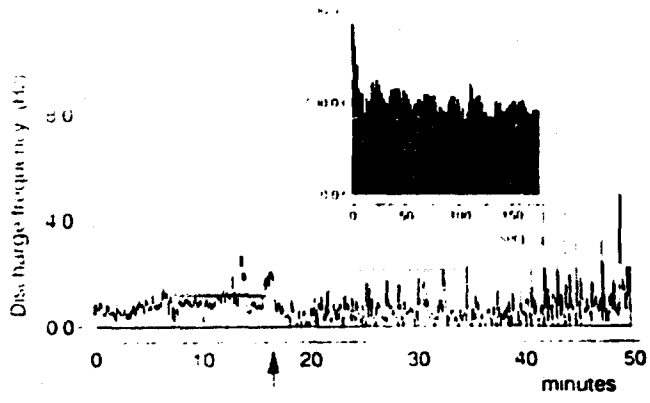

Fig. 3. Discharge frequency plot for a typical LC neuron before and after injection of morphine (3 mg/kg, i.m., at arrow). Note decreased average discharge frequency but increased fluctuations in discharge rate following morphine. Note also that such fluctuations appear to occur somewhat regularly, with a period of about 20 s. Inset: autocorrelogram of this cell's activity for 30 min, beginning 10 min after morphine injection. Note oscillatory activity with a period of about 20 s.

phasic afferent activation of LC neurons and tonic direct inhibition of these cells by morphine. The apparent correlation of changing behavioral state with this oscillatory discharge of LC cells, in view of our preliminary results for such periodic LC activity (of much smaller amplitude) in the undrugged animal and other findings of ultradian fluctuations in EEG and state of waking monkeys [14], suggests that burst-pause fluctuations in LC discharge after morphine may be partially responsible for the corresponding fluctuations in behavioral state. Similar periodic changes in state occur in humans after opiate administration which appear as 'the nods', as a person 'nods' in and out of awareness of his surroundings.

The present results may indicate, therefore, that morphine potently influences LC neurons not only by direct actions at the membrane level, but also by actions upon circuits afferent to LC neurons. These results point out the importance of studying drug effects at the system level in intact animals to fully appreciate the effects on neural activity. These results also indicate that afferents to LC may be additional targets for pharmacologically manipulating morphine effects on LC discharge in clinical treatment of drug abuse.

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