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# EFFECTS OF LOCUS COERULEUS INACTIVATION ON ELECTROENCEPHALOGRAPHIC ACTIVITY IN NEOCORTEX AND HIPPOCAMPUS

C. W. BERRIDGE,*† M. E. PAGE,‡ R. J. VALENTINO‡ and S. L. FOOTE*

*Department of Psychiatry, School of Medicine, University of California, San Diego, La Jolla, CA 92093, U.S.A.

‡Department of Mental Health Sciences, Hahnemann University, Philadelphia, PA 19102, U.S.A.

**Abstract**—The effects of inhibition of locus coeruleus neuronal discharge activity on cortical and hippocampal electroencephalographic activity were examined in halothane-anesthetized rats. A combined recording/infusion probe was used to place 35–150-nl infusions of the $\alpha_2$-noradrenergic agonist, clonidine (1 ng/nl) which inhibits locus coeruleus neuronal discharge activity, immediately adjacent to the locus coeruleus. The recording electrode allowed verification and quantification of the electrophysiological effects of these infusions. Simultaneously, electroencephalographic activity was recorded from sites in frontal neocortex and dorsal hippocampus and subjected to power spectrum analyses. Neither cortical nor hippocampal electroencephalographic activity was substantially affected following unilateral locus coeruleus inactivation. In contrast, bilateral clonidine infusions that completely suppressed locus coeruleus neuronal discharge activity in both hemispheres altered cortical and hippocampal electroencephalographic status. The cortical response to bilateral LC inhibition was characterized by a shift from low-amplitude, high-frequency to large-amplitude, slow-wave activity. Additionally, theta-dominated activity in the hippocampus was replaced with mixed frequency activity. The onset of these changes in forebrain electroencephalographic activity was coincident with the complete bilateral inhibition of locus coeruleus neuronal discharge activity. The resumption of pre-infusion electroencephalographic patterns closely followed recovery of locus coeruleus neuronal activity or could be induced with systemic administration of the $\alpha_2$-noradrenergic antagonist, idazoxan.

Clonidine infusions placed 800–1200 $\mu$m from the locus coeruleus were less effective at inducing a complete suppression of locus coeruleus activity. These infusions either did not completely inhibit locus coeruleus discharge (35 nl infusions), or did so with a longer latency to complete locus coeruleus inhibition and a shorter duration of inhibition (150 nl infusions). Changes in forebrain electroencephalographic activity occurred only following the complete bilateral suppression of locus coeruleus neuronal discharge activity. These electroencephalographic responses closely followed or coincided with the onset of complete bilateral locus coeruleus inhibition and persisted throughout the period during which bilateral LC neuronal discharge activity was completely absent (60–240 min). Recovery of electroencephalographic patterns was coincident with the reappearance of locus coeruleus discharge activity.

These results suggest that the clonidine-induced changes in forebrain electroencephalographic activity were dependent on the complete bilateral suppression of locus coeruleus discharge activity, and that under the present experimental conditions the locus coeruleus/noradrenergic system exerts a potent and tonic activating influence on forebrain electroencephalographic state. These results support the hypothesis that this system may be an important modulator of behavioral state and/or state-dependent processes.

Through a highly divergent efferent projection system, the brainstem nucleus locus coeruleus (LC) is the primary source of forebrain norepinephrine (NE) and is the sole source of neocortical and hippocampal NE (reviewed in Ref. 14). This system has been implicated in a wide array of behavioral and cognitive functions, including learning and memory, regulation of sleep and arousal, and affective states such as anxiety and depression. LC neuronal discharge activity fluctuates as a function of the sleep–wake cycle, with higher levels of activity occurring during waking than sleep (e.g. Refs 2, 13, 17). Within the awake state, LC activity is also correlated with the level of alertness or arousal. For example, both monkey and rat, LC neuronal discharge activity increases in response to sensory stimuli that are sufficiently potent or salient to elicit an orienting response.[2,13] These and other observations prompted the proposition that the LC/noradrenergic system mediates certain general processes related to arousal and/or attention.[3,16]

Forebrain electroencephalographic (EEG) activity displays state-dependent patterns.[25,27] That LC neuronal discharge increases immediately prior to neocortical EEG activation in unanesthetized monkeys and rats suggests a possible functional relationship between LC neuronal activity and forebrain EEG. However, earlier attempts to demonstrate a causal link between the LC/noradrenergic system and

†To whom correspondence should be addressed at: Department of Psychiatry (0603), 9500 Gilman Dr., University of California, San Diego, La Jolla, CA 92093, U.S.A.

*Abbreviations*: ECoG, neocortical electroencephalogram; EEG, electroencephalographic, electroencephalogram; HEEG, hippocampal electroencephalogram; LC, locus coeruleus; NE, norepinephrine, PSA, power-spectrum analysis.

forebrain EEG activity using pharmacological or lesion techniques have provided mixed results (for review, see Ref. 27). Such inconsistencies are likely related to inherent limitations of these techniques, such as a lack of pharmacological and anatomical specificity of noradrenergic drugs and the occurrence of lesion-induced compensatory responses that minimize the functional consequences of such lesions.

To more directly test the hypothesis that the LC/noradrenergic system participates in the control of EEG status, we previously examined the effect of LC activation on EEG measures in the halothane-anesthetized rat using an approach that allows selective activation of LC without inducing damage to this nucleus.[5] Electrophysiological recordings were used to guide placement of small infusions of the cholinergic agonist, bethanechol, within 200–500 $\mu$m of the LC to increase discharge activity of LC neurons. In these studies, a robust bilateral activation of neocortical and hippocampal EEG was observed within seconds following unilateral LC activation, the duration of which closely followed the duration of LC activation. These and other observations indicated that the acute enhancement of LC neuronal discharge activity above basal levels exerts potent, global activating actions on forebrain EEG.

Intrabrainstem administration of noradrenergic $\alpha_2$-agonists into the region of the LC has been observed to increase behavioral and EEG measures of sedation.[8,9,10] Because these drugs act to inhibit LC neuronal discharge activity and NE release,[24] these observations are consistent with an action of the LC/noradrenergic system in the maintenance of an activated forebrain. However, interpretation of results obtained utilizing intratissue drug infusions for the study of LC function is complicated by a variety of factors, such as the small size of the nucleus and the close proximity of the LC to other nuclei known to affect behavioral and EEG states.[23,28] These factors, together with an absence of electrophysiological measures documenting the actions of intrabrainstem infusions of $\alpha_2$-agonists on LC neuronal activity (e.g. whether LC inhibition is necessary for changes in EEG state), preclude specific conclusions regarding the site(s) of action for the sedative effects of intrabrainstem-administered $\alpha_2$-agonists.

If in fact intrabrainstem administered $\alpha_2$-agonists enhance EEG measures of sedation through an inhibition of LC neuronal discharge activity, it would be hypothesized that in all instances: (i) infusions that alter forebrain EEG will suppress LC activity; (ii) changes in LC neuronal discharge activity will precede changes in forebrain EEG activity; (iii) the return of EEG activity to the pre-infusion state will follow the recovery of LC neuronal activity; and (iv) infusions that do not suppress LC neuronal discharge activity will not alter forebrain EEG patterns. The purpose of the current study was to test these specific hypotheses.

Portions of this study were previously published in abstract form.[4,16]

## EXPERIMENTAL PROCEDURES

### *Animals and surgery*

Male Sprague–Dawley rats (Harlan Sprague–Dawley Inc.) weighing 280–350 g were anesthetized with halothane using a face mask. A tracheotomy was then performed, and halothane (Halocarbon Laboratories; 0.75–1.25% in air) was administered via this route for the duration of the experiment. The animal was placed in a stereotaxic instrument with the incisor bar set 11.5 mm below the ear bars. Body temperature was maintained at 36–38°C.

### *Locus coeruleus recording*

The experimental preparation was similar to that previously described.[1,5] Briefly, two small holes (2–3 mm diameter) centered bilaterally at 1.4 mm lateral to the midline and 4.0 mm caudal to the point at which the lambdoid and midline sutures intersect, were drilled through the skull. A recording/infusion probe, consisting of a stainless steel microelectrode glued parallel to a shorter piece of 26-gauge hyperdomeric tubing, was used to locate each LC. LC neurons were tentatively identified based on previously described criteria.[26] Electrophysiological signals were passed through a high impedance amplifier, a time/amplitude window discriminator, and monitored on an oscilloscope and audiomonitor. Neuronal discharge activity was recorded on magnetic tape as individual trigger pulses and as a ratemeter record of the number of action potentials in successive 10-s intervals.

### *Drugs/peri-locus coeruleus infusions*

In each hemisphere, a recording electrode was positioned such that it traversed a portion of the LC that had a dorsal ventral extent greater than 350 $\mu$m (i.e. the main body of the LC). Once the main body of the LC was located, the probe was raised 2 mm, a 33-gauge infusion needle was inserted into the guide tube, and the probe was again lowered to the depth of the LC. The needle extended to the depth of the electrode tip, with a lateral displacement of 150–500 $\mu$m. The infusion needle was attached to PE20 tubing via a 26-gauge stainless steel sleeve glued to the needle. The other end of the tubing was attached to a 10-$\mu$l syringe, the plunger of which was advanced using an automated infusion pump (Harvard Apparatus). Infusions consisted of 35–150 ng of clonidine (1 ng/nl; Sigma Chemical Co.) dissolved in 0.9% saline containing 2% Pontamine Sky Blue dye given over a 60-s period. Idazoxan (1 mg/ml; Sigma Chemical Co.) was dissolved in saline and administered intraperitoneally at a dose of 0.5 mg/kg.

### *Electroencephalogram recording*

In most experiments, bipolar surface-to-depth electrodes were used to record cortical electroencephalographic activity (ECoG) bilaterally (A + 3.0; L $\pm$ 1.5), as previously described.[5] In some experiments ($n = 4$), hippocampal EEG (HEEG) was also recorded using monopolar electrodes placed in the dorsal hippocampus (A − 4.8; L $\pm$ 2.5), ipsilateral to the ECoG recording electrode as previously described.[5]

### *Electroencephalogram analyses*

Electroencephelogram signals were amplified, filtered (0.1–50.0 Hz bandpass), and recorded on a polygraph and on magnetic tape. Three 8-min EEG segments from each experiment, defined as "pre-infusion", "post-infusion", and "recovery", were selected for power-spectrum analysis (PSA). Each segment was digitized at a sampling frequency of 300 Hz and was tapered at the ends as a cosine function. The pre-infusion segment was defined as beginning 8 min

before the start of the infusion. The post-infusion period began 2 min after the complete cessation of recorded LC neuronal discharge activity. The recovery period was defined as beginning at the point at which the level of neuronal discharge activity of at least one LC had returned to pre-infusion levels. Each segment was subjected to Fast Fourier Transform and PSA. The mean absolute and mean relative power (percentage of total power) were calculated for the frequency bands 0.3–2.3 Hz, 2.3–6.9 Hz, 6.9–13.0 Hz, 13.0–20.0 Hz, 20.0–30.0 Hz, 30.0–40.0 Hz, and 40.0–50.0 Hz. These frequency bands were selected on the basis of visual inspection of EEG patterns as being sensitive to changes induced by sensory stimulation, such as tail-pinch.

*Statistical analyses*

ECoG absolute and relative power for each frequency bandwidth were analysed using a paired $t$-test with Bonferroni correction to compare pre- and post-infusion segments from all effective peri-LC infusion animals. A one-way, repeated measures ANOVA followed by the Duncan's multiple-range test was used to statistically assess EEG recovery in the subset of animals ($n = 14$) for which spontaneous recovery data were collected. HEEG absolute and relative power were analysed using one-way repeated measures ANOVA followed by the Duncan's multiple-range test.

*Histology*

Following each experiment, cathodal current was passed through the LC electrodes (10 $\mu$A/10 s) to mark the recording sites. The animal was deeply anesthetized and then perfused with 50 ml of a solution of 4% formaldehyde and 5% potassium ferrocyanide so that the Prussian Blue reaction could be used to visualize the microelectrode recording site. The brain was removed and placed in 50 ml of the perfusion solution containing 0.5 ml of glacial acetic acid. Following a minimum of 48 h, the brain was frozen and 40-$\mu$m sections were cut and collected through the areas in which the EEG and LC recording electrodes were placed. The sections were stained with Neutral-Red dye for subsequent examination of the LC recording and infusion sites and the placement of the ECoG electrodes.

*Data selection*

Data from a particular animal were included in the analyses for only those cases in which EEG electrode placements were accurate, EEG recordings were electrically adequate, and brainstem recording and infusion sites could be anatomically verified.

## RESULTS

*General observations*

Preliminary observations indicated that bilateral LC inhibition had noticeable effects on ECoG activity only when there was a minimum of large-amplitude, slow-wave activity present before infusion. Thus, in all experiments described below, the level of anesthesia was carefully adjusted such that there was an absence of motor responses to noxious stimulation while high-frequency, low-amplitude (desynchronized) activity predominated in the ECoG and theta activity predominated in the HEEG. Often, several adjustments of the level of anesthesia were necessary to obtain these conditions. In some of the animals, it was not possible to maintain a stable desynchronized state in the pre-infusion ECoG. In these cases, short periods (1–2 min) of large-amplitude, slow-wave activity would appear intermittently. To assure that these fluctuations were accurately represented in the data analyses, at least 30 min of baseline EEG data were collected before any experimental manipulation, and long (8-min) EEG segments were used for PSA analyses. Once data collection was initiated for a particular experiment, the concentration of halothane being administered was not altered.

*Effects of peri-locus coeruleus clonidine on locus coeruleus neuronal discharge activity*

Bilateral peri-LC clonidine infusions produced a complete bilateral inhibition of LC neuronal discharge in 20 of 23 animals (Fig. 1). Of these 20 animals, nine received 35-nl infusions and 11 received 150-nl infusions. In 10 cases, (six = 35 nl; four = 150 nl), the effects of unilateral LC inhibition on ECoG were examined before administering bilateral infusions. In four of these experiments, HEEG was also recorded.

For all effective peri-LC infusions (summarized in Fig. 2), the microelectrode recording site was localized to the main body of the LC; the infusion site was generally centered within a 100–500-$\mu$m radius of the LC; and no infusion-related damage to the LC was observed. The latency from the start of the peri-LC clonidine infusions to the onset of LC inhibition ranged from 15 to 180 s (median = 40 s). The latency from the start of the infusion to complete LC inhibition ranged from 40 to 520 s (median = 220 s). These infusions completely inhibited LC neuronal discharge for ~30–180 min, after which time neuronal discharge levels slowly increased to pre-infusion levels over the subsequent 30–60 min.

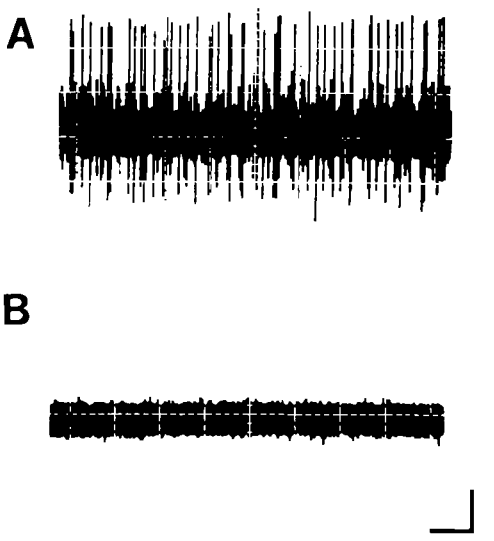


Fig. 1. LC neuronal discharge activity before and after a peri-LC clonidine infusion. Oscilloscope trace of a filtered multiunit LC recording. A was taken 2 min before the infusion. B was taken 10 min after the infusion, at which time no neuronal discharge was evident. Scale bars = 1 s/div, 50 $\mu$V/div.

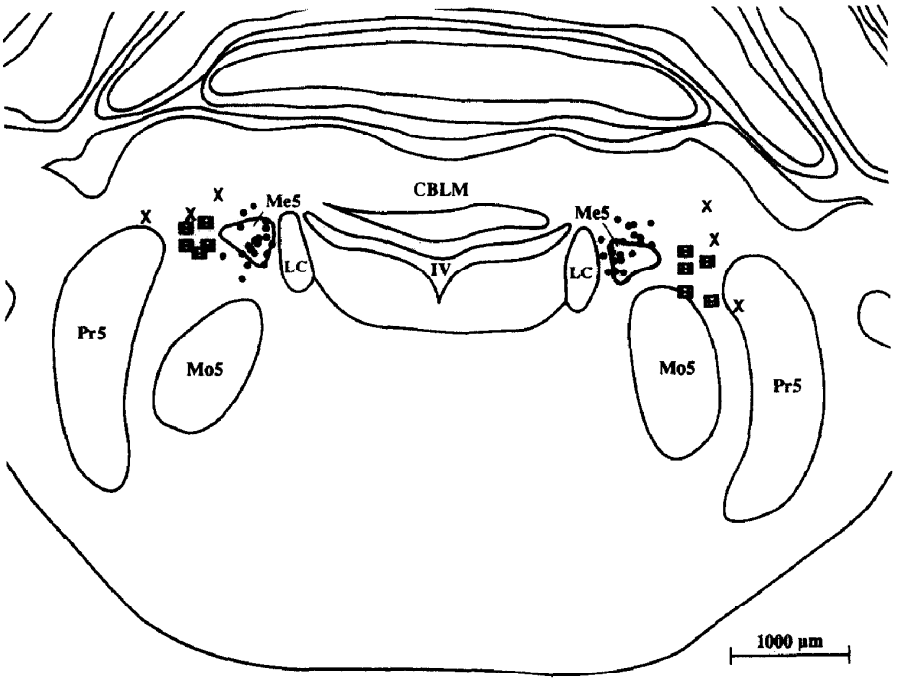


Fig. 2. Schematic depiction of effective and ineffective clonidine infusion sites. Infusion sites are indicated on a tracing of a representative tissue section. Distances between the infusion sites and the LC were measured in fixed and dried tissue sections. Solid circles indicate sites where either 35-nl ($n = 9$) or 150-nl ($n = 11$) clonidine infusions completely inhibited LC neuronal discharge activity and altered ECoG and HEEG activity. Boxes indicate sites where 35-nl clonidine infusions did not completely suppress LC activity and did not alter ECoG or HEEG activity. Subsequent 150-nl infusions at these sites were either ineffective at suppressing LC activity ($n = 1$) or completely inhibited LC activity only after a delay of many minutes with coincident EEG responses ($n = 4$). Xs indicate sites where only 150 nl infusions were made. At these sites, the infusions were either ineffective ($n = 1$), or completely inhibited LC activity only after a delay of many minutes with coincident ECoG and HEEG responses ($n = 2$). CBLM, cerebellum; Me5, mesencephalic nuclues of the trigeminal nerve; Mo5, motor nucleus of the trigeminal nerve; Pr5, principal sensory nucleus of the trigeminal nerve.

*Effects of unilateral locus coeruleus inhibition on neocortical electroencephalogram and hippocampal electroencephalogram*

The effects of unilateral LC inactivation on ECoG activity were examined in 10 animals 10–15 min before bilateral LC inhibition. In the majority of cases ($n = 6$), unilateral LC inhibition had no obvious effects on ECoG activity as determined by visual inspection of the EEG traces. However, in the four remaining animals unilateral LC inhibition produced a moderate apparent increase in the incidence and amplitude of low-frequency (~1 Hz) and mid-frequency (~8–18 Hz) activity (data not shown). In two of the four experiments, the EEG response was observed bilaterally whereas, in the other two, the effect was observed only in the cortex ipsilateral to the inhibited LC. Since these results were neither consistent nor striking, they were not subjected to statistical analyses.

*Effects of bilateral locus coeruleus inhibition*

Alterations in both ECoG and HEEG activity patterns were consistently observed following bilateral LC inhibition. ECoG changes consisted of a shift from predominantly desynchronized (high-frequency, low-amplitude) to large amplitude, slow-wave activity (Fig. 3). In the HEEG, bilateral LC inhibition resulted in a transition from theta-dominated activity to mixed-frequency activity. In general, this effect occurred simultaneously with the increase in ECoG slow-wave activity (Fig. 4). The magnitude of the EEG response was substantially larger following bilateral LC inhibition than following unilateral inhibition. The shift in ECoG and HEEG activity always occurred either simultaneously with, or within seconds of, the complete inhibition of LC neuronal discharge activity bilaterally, regardless of the length of time between the onset of unilateral and bilateral LC inhibition.

In some instances, although the first obvious changes in ECoG activity occurred virtually simultaneously with the onset of bilateral LC inhibition, the maximal EEG response was slightly delayed. The latency between the initial and the maximal ECoG response, as estimated from visual inspection of the EEG trace, ranged from 10 to 240 s (median = 40 s). These ECoG and HEEG responses were stable and persisted throughout the entire period during which both LCs remained completely inhibited. For the

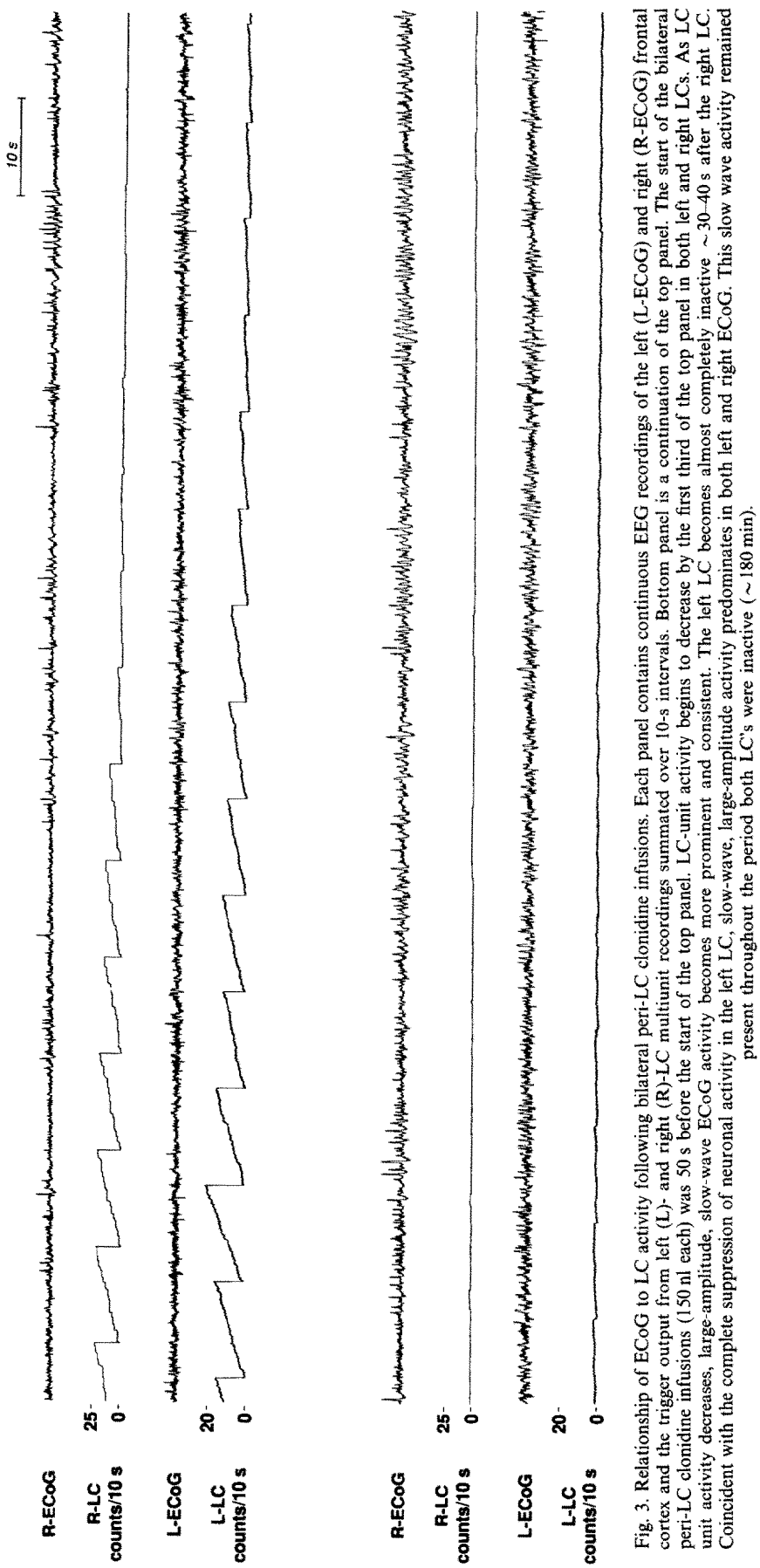


Fig. 3. Relationship of ECoG to LC activity following bilateral peri-LC clonidine infusions. Each panel contains continuous EEG recordings of the left (L-ECoG) and right (R-ECoG) frontal cortex and the trigger output from left (L)- and right (R)-LC multiunit recordings summated over 10-s intervals. Bottom panel is a continuation of the top panel. The start of the bilateral peri-LC clonidine infusions (150 nl each) was 50 s before the start of the top panel. LC-unit activity begins to decrease by the first third of the top panel in both left and right LCs. As LC unit activity decreases, large-amplitude, slow-wave ECoG activity becomes more prominent and consistent. The left LC becomes almost completely inactive ~30–40 s after the right LC. Coincident with the complete suppression of neuronal activity in the left LC, slow-wave, large-amplitude activity predominates in both left and right ECoG. This slow wave activity remained present throughout the period both LC's were inactive (~180 min).

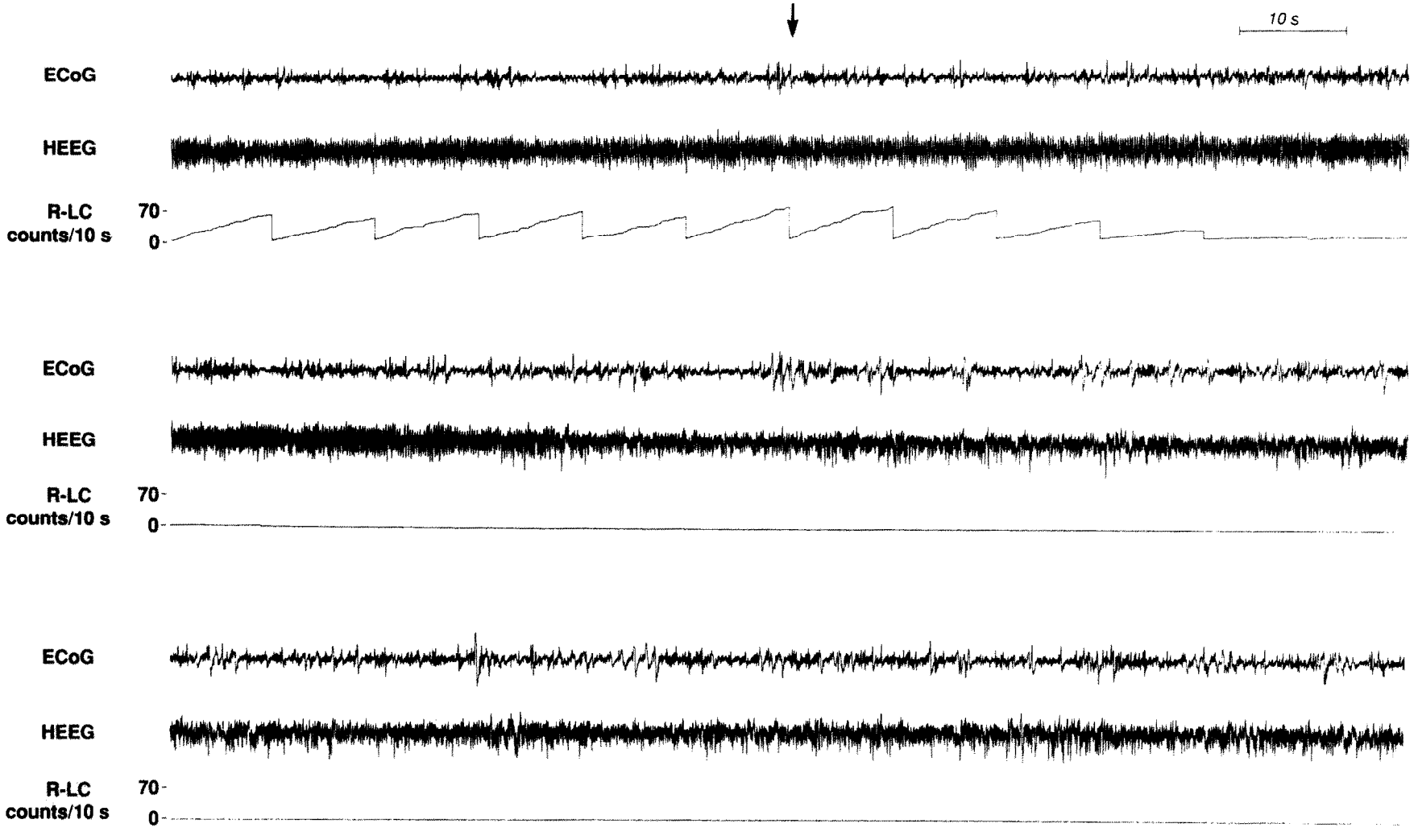


Fig. 4. Effect of bilateral LC-inhibition on ECoG and HEEG activity. Left ECoG activity is shown in the top trace, left HEEG activity in the middle trace, and the trigger output from the right LC summated over 10-s intervals in the third trace of each panel. Subsequent panels are continuations of the previous panel. Approximately 15 min before an infusion on the right side, a 35-nl peri-LC clonidine infusion was made on the left side which completely eliminated LC neuronal discharge activity and had minimal effects on ECoG and HEEG activity. The end of the 35-nl peri-LC infusion (over 60 s) on the right side is indicated by the arrow. In the top panel, LC activity is seen to decrease, until by the beginning of the middle panel, recorded activity ceased. ECoG slow-wave activity begins to increase once LC unit activity is nearly absent and predominates following the complete inhibition of LC activity. Within 30–40 s after the complete suppression of LC neuronal discharge activity, theta activity in the HEEG is substantially diminished and high-frequency activity begins to appear. Shortly following this, mixed-frequency activity predominates. These ECoG and HEEG patterns persisted throughout the entire period when both LCs were completely inhibited (~40 min).

ECoG, the duration of the response ranged from 30–180 min (median = 40 min).

In the majority of cases, the first indications of ECoG recovery appeared coincident with the resumption of a low level of neuronal discharge activity in either LC. As LC neuronal activity progressively recovered, the ECoG returned to the pre-infusion state in which high-frequency, low-amplitude activity predominated. In some cases, large-amplitude, periodic activity was observed at the beginning of the ECoG recovery (Fig. 5A). Often a very low level of LC neuronal activity (1–2 counts/s, multiunit recording) would be observed for many minutes before a noticeable change in ECoG activity. However, in these cases, an abrupt increase in LC activity would then occur. At this point, the ECoG would also abruptly change to a state in which high-frequency, low-amplitude activity predominated (Fig. 5B). Similar results were observed for HEEG recovery. Complete recovery of the ECoG/HEEG coincided with the return of at least one LC recording to pre-infusion levels of neuronal discharge activity (ECoG; range 60–240 min, median 80 min).

*Power spectrum analyses*

PSA demonstrated statistically significant effects of bilateral LC inhibition on ECoG activity, consistent with the qualitative observations described above (Fig. 6; Table 1). LC inhibition significantly increased the mean absolute power of the 0.3–2.3 Hz, 2.3–6.9 Hz, 6.9–13.0 Hz and 13.0–20.0 Hz frequency bands. This increase ranged from 284% of pre-infusion levels in the lowest frequency band to 147% of pre-infusion levels in the 13.0–20.0 Hz frequency band. There was a small, statistically nonsignificant increase in mean power of the highest frequency bands. Relative power was significantly increased in the 0.3–2.3 Hz frequency band (154% of pre-infusion levels), not altered in the 2.3–6.9 Hz, 6.9–13.0 Hz, and 13.0–20.0 Hz frequency bands, and significantly decreased in the 20.0–30.0 Hz, 30.0–40.0 Hz, and 40.0–50.0 Hz bands. After recovery of LC unit activity, the absolute and relative power of all frequency bands returned to pre-infusion levels.

ANOVA indicated significant effects of bilateral LC inhibition on HEEG power spectra for the four cases in which HEEG was recorded (Table 1). Mean absolute power significantly increased in the 0.3–2.3 Hz, 6.9–13.0 Hz, 13.0–20.0 Hz, and 30.0–40.0 Hz frequency bands. Relative power was significantly increased in the 0.3–2.3 Hz and 6.9–13.0 Hz frequency bands and significantly decreased in the 2.3–6.9 Hz and 40.0–50.0 Hz bands.

*Effects of systemic idazoxan*

The effects of the $\alpha_2$-antagonist, idazoxan (500 $\mu$g/kg; ip), on the bilateral LC inhibition-induced increase in ECoG slow-wave activity were examined in six animals. When administered 10–15 min following the onset of the maximal ECoG response, idazoxan increased LC neuronal discharge activity and, coincident with this increase, decreased the incidence of ECoG slow-wave activity (data not shown).

*Effects of clonidine infusions outside the immediate region of the locus coeruleus*

Clonidine infusions were made at distances of 800–1200 $\mu$m from the LC in eight animals (Fig. 2). In five cases, bilateral 35-nl infusions were made ~30–45 min before a second set of bilateral 150-nl infusions. In all cases, the 35-nl infusions were ineffective at inducing a complete inhibition of LC neuronal discharge activity, and these infusions did not have any noticeable effects on ECoG activity. Of the eight 150-nl bilateral infusions, two were ineffective at inhibiting LC neuronal discharge, and did not alter the ECoG. In the six remaining cases, LC activity was completely inhibited. However, in these cases, the latencies between the start of the infusion and the occurrence of complete LC inhibition were significantly longer (range, 60–700 s; median, 450 s) than those observed for 150-nl peri-LC infusions (range, 40–340 s; median, 140 s). In these six cases, as with peri-LC infusion, an increase in ECoG slow-wave activity was observed virtually simultaneously with the complete bilateral inhibition of LC neuronal discharge activity (data not shown), and LC recovery began shortly before the onset of ECoG recovery. The times for the ECoG response to fully recover in these six cases (range, 25–80 min; median, 35 min) were substantially shorter than those observed with peri-LC clonidine infusions (range, 60–240 min; median, 90 min).

In three additional cases, bilateral peri-LC clonidine infusions (150 nl) were made that were ineffective in inducing complete bilateral LC inhibition, and that did not alter ECoG activity. Histological examination revealed substantial damage in the area of the infusion needle/LC, involving the LC, and indicated that the infusate possibly entered the fourth ventricle. Due to the questionable nature of the infusion site, the degree to which the LCs were damaged, and the ineffectiveness of the infusion with regard to LC activity, these cases are not depicted in Fig. 2 and were excluded from data analyses.

## DISCUSSION

The above-described results support the specific hypotheses tested in these studies. These results indicate that the enhancement of EEG measures of sedation observed following intrabrainstem administered $\alpha_2$-agonists is dependent on inhibitory actions of these drugs on LC neuronal discharge activity. Thus, clonidine infusions made immediately adjacent to or distant from LC enhanced EEG measures of sedation only when the complete suppression of bilateral LC neuronal activity was observed: infusions that did not completely suppress LC neuronal discharge activity

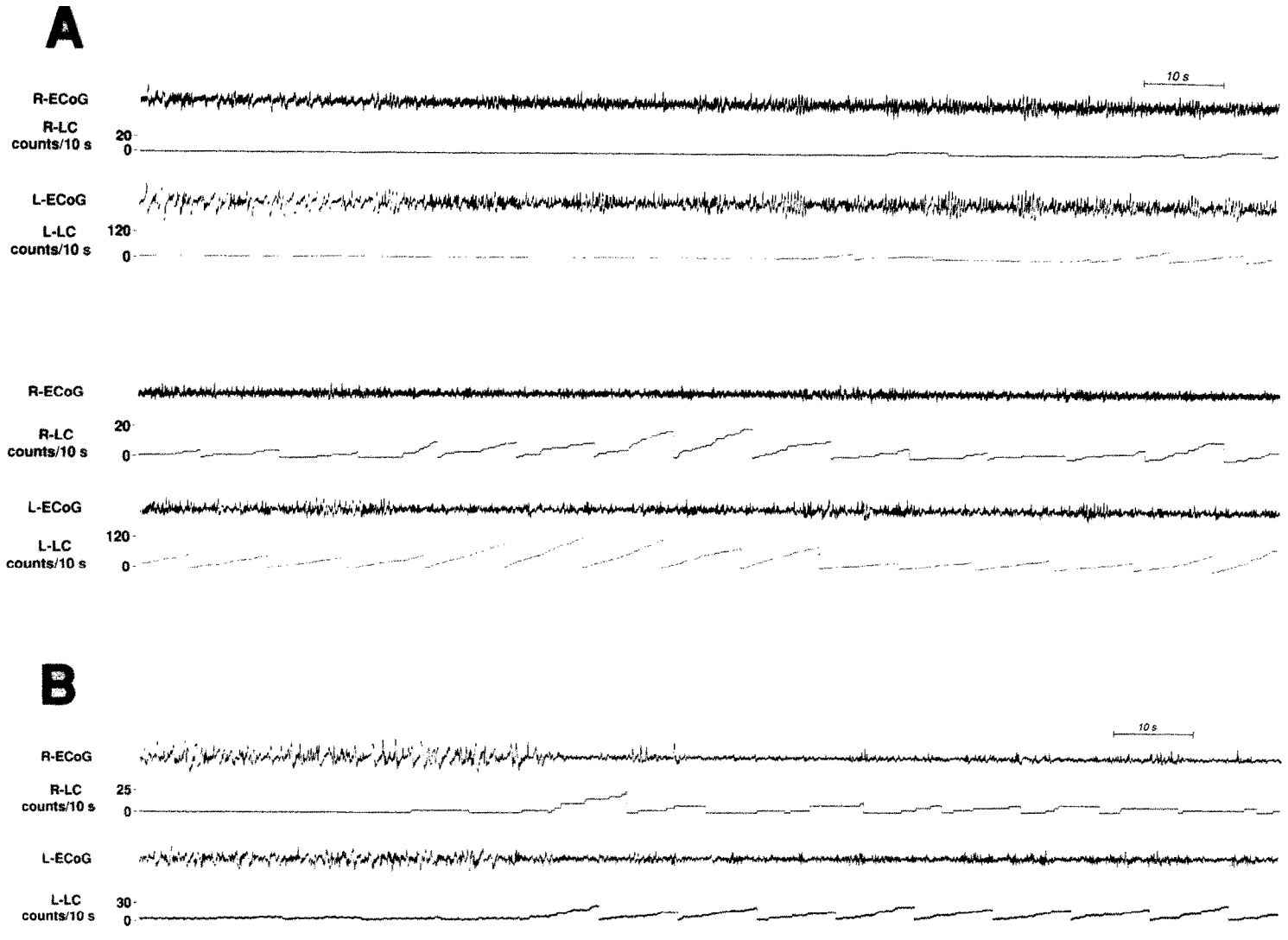


Fig. 5. Relationship between LC unit activity and ECoG during recovery of LC unit discharge activity following bilateral peri-LC clonidine infusions. A and B represent separate experiments. (A) Left and right ECoG recordings and LC multiunit trigger output, summated over 10-s intervals. In both the left and right ECoG, slow-wave activity persists as long as both LCs are inactive. When LC discharge activity becomes apparent, large-amplitude, periodic ( ~ 2 Hz) activity appears in the ECoG. With sustained increases in LC activity, high-frequency, low-amplitude ECoG activity appears. (B) Additional example of relationship between LC unit activity and ECoG during recovery from clonidine infusions. As in A, when both LCs are nearly completely inactive, large-amplitude, slow-wave activity dominates the ECoG. In both the left and right LCs, a single action potential was detected occasionally for a few minutes prior to the segment shown. In this experiment, an abrupt increase in neuronal discharge rate occurred and coincident with this, desynchronized ECoG activity appeared.

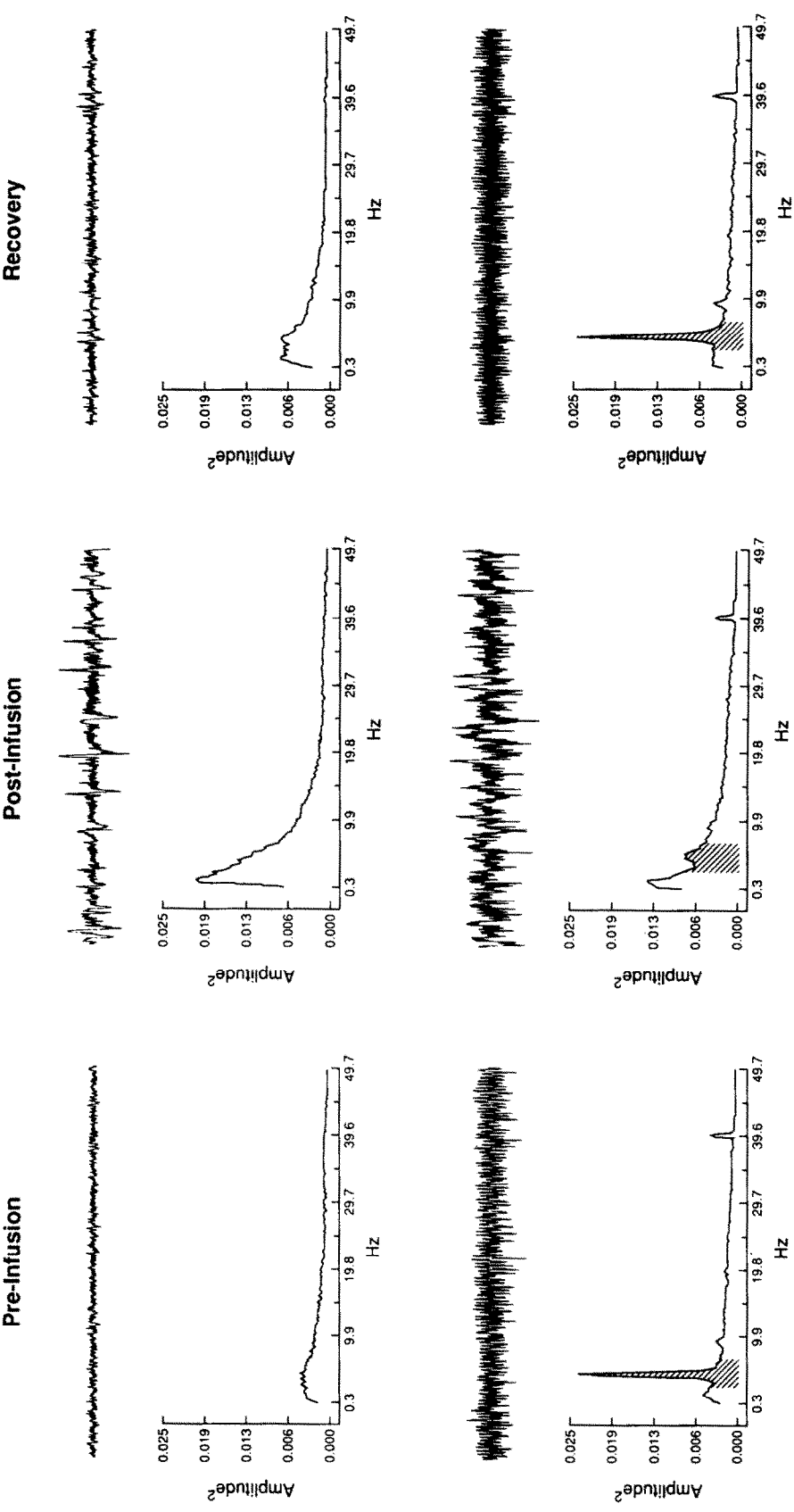


Fig. 6. PSA of ECoG and HEEG samples from pre-infusion, post-infusion, and recovery periods from a bilateral LC-infusion experiment. Data are from a separate experiment from those displayed in Fig. 5. A 25-s raw EEG trace representative of the entire 8-min period from which the PSA was computed is shown above each power spectrum. The most striking post-infusion changes in the ECoG are the increase in power of the slowest frequencies and in the HEEG the appearance of mixed-frequency activity. Shading indicates the theta frequency band (2.3–6.9 Hz) in the HEEG power spectra.

Table 1. Mean absolute and relative power of post-infusion and recovery segments of cortical (ECoG) and hippocampal (HEEG) electroencephalographic activity (expressed as percentage of pre-infusion means)

| Frequency (Hz) | Absolute Power Post | Recovery | Relative Power Post | Recovery |
|---|---|---|---|---|
| ECoG | | | | |
| 0.3–2.3 | 284 ± 33** | 95 ± 6 | 154 ± 9** | 94 ± 4 |
| 2.3–6.9 | 239 ± 61** | 101 ± 6 | 102 ± 5 | 98 ± 4 |
| 6.9–13.0 | 155 ± 8** | 110 ± 5 | 99 ± 6 | 106 ± 2 |
| 13.0–20.0 | 147 ± 8** | 98 ± 6 | 84 ± 4 | 99 ± 7 |
| 20.0–30.0 | 129 ± 5 | 106 ± 5 | 79 ± 4** | 105 ± 4 |
| 30.0–40.0 | 122 ± 5 | 109 ± 6 | 76 ± 3** | 109 ± 5 |
| 40.0–50.0 | 119 ± 5 | 109 ± 8 | 70 ± 4** | 109 ± 6 |
| HEEG | | | | |
| 0.3–2.3 | 250 ± 5** | 114 ± 1 | 172 ± 3** | 102 ± 2 |
| 2.3–6.9 | 111 ± 1 | 108 ± 2 | 72 ± 2* | 97 ± 1 |
| 6.9–13.0 | 191 ± 4** | 89 ± 4 | 131 ± 1** | 103 ± 1 |
| 13.0–20.0 | 163 ± 4** | 116 ± 2 | 108 ± 2 | 102 ± 1 |
| 20.0–30.0 | 143 ± 3** | 115 ± 3 | 98 ± 1 | 101 ± 1 |
| 30.0–40.0 | 123 ± 2 | 119 ± 3 | 90 ± 1 | 83 ± 6 |
| 40.0–50.0 | 108 ± 1 | 107 ± 2 | 82 ± 1* | 99 ± 2 |

Absolute and relative power are expressed as percentage of pre-infusion means (±S.E.M.) for the post-infusion (ECoG, $n = 20$; HEEG $n = 4$) and recovery (ECoG, $n = 14$; HEEG $n = 4$) segments. *$P < 0.05$; **$P < 0.01$ significantly different from pre-infusion means.

did not alter forebrain EEG patterns. Further, there was a close temporal relationship between the onset and recovery of the clonidine-induced changes in LC neuronal discharge activity and the ECoG/HEEG responses. In all cases, the changes in forebrain EEG closely followed the changes in LC neuronal discharge rates.

*Site of action*

A combined recording/infusion method was used in the present study in order to permit stronger inferences to be made about the site of action of clonidine infusions. This is necessary because the use of intratissue infusions for the study of LC function is complicated by a number of factors. First, given the small size of the nucleus, care must be taken to avoid placement of an infusion needle directly into the LC and consequent damage to the nucleus. Second, the close proximity of the LC to the fourth ventricle and various nuclei associated with modulation of behavioral/EEG states necessitates the use of small infusion volumes that minimize spread of drug beyond the LC. Unfortunately, the size of the LC makes the consistent placement of an infusion needle immediately adjacent to this nucleus virtually impossible using standard stereotaxic techniques. Finally, because drugs can dramatically differ in the degree to which they diffuse through tissue, the appropriate infusion volume for a given drug is generally unknown (see below).

To minimize these confounding factors, the current study used electrophysiological recordings to guide placement of clonidine infusions within 150–500 $\mu$m of the LC to suppress LC neuronal discharge activity. The close proximity of the needle to the LC permits the use of small infusion volumes, and thus minimizes the spread of clonidine outside the immediate vicinity of the LC. Importantly, electrophysiological recordings permit: verification and quantification of alterations in LC activity levels; analysis of the temporal relationship between the onset and offset of LC activation and any observed physiological effects; and determination of the distance over which a given drug exerts effects on neuronal discharge rates. This information gained through the use of electrophysiological recordings is essential for determining whether intrabrainstem clonidine infusions enhance EEG measures of sedation through actions at the LC or nearby brainstem nuclei.

The observations obtained using this approach strongly suggest that clonidine infusions enhance forebrain EEG measures of sedation through inhibition of LC neuronal discharge rates. Regardless of whether infusions were placed in close proximity or distant to the LC, EEG responses were observed only after profound suppression of LC neuronal discharge rates. Thus, 35-nl infusions placed outside the immediate vicinity of the LC (800–1200 $\mu$m) that moderately suppressed, but did not completely eliminate, LC neuronal discharge activity had no obvious effects on EEG activity patterns. Larger infusions (100–150 nl) placed distant to the LC were less effective than peri-LC infusions in producing a complete suppression of LC neuronal activity. When effective, the latency to complete LC inhibition was longer and the duration of inhibition shorter, consistent with the relatively large distance over which the drug had to travel to reach the LC. Importantly, in these cases infusions altered EEG activity only when a complete inhibition of LC activity was obtained. Regardless of distance of infusion site from LC, EEG responses closely followed the complete bilateral suppression of LC neuronal discharge activity. Further, the return of ECoG and HEEG activity to pre-infusion activity patterns closely followed the reappearance of low levels of LC neuronal discharge activity.

Taken together the present observations are incompatible with an action of clonidine at a brainstem site other than the LC. For example, if clonidine were acting at a site(s) lateral to the LC, it would be expected that infusions placed distant to the LC would have elicited ECoG responses prior to affecting LC neuronal activity or in the absence of a complete suppression of LC activity. The consistently short latency between the complete inhibition of LC activity and EEG responses, whether the infusions were placed immediately adjacent or distant to the LC, further suggests that clonidine was not acting at a site medial to the LC, such as the dorsal raphe nucleus, to elicit changes in EEG activity. Also, given the limited effective range of 35-nl clonidine infusions and the fact that effective infusions were made at a site adjacent to the main body of the LC, it is unlikely that clonidine was acting at a site anterior to the LC, such as the lateral dorsal tegmental nucleus. Finally, the current observation that clonidine-induced inhibition of LC neuronal discharge activity increased EEG synchronous activity is entirely consistent with our previous observation[5] that bethanechol-induced activation of LC elicits EEG desynchronization, and that this EEG activation can be blocked by intracerebroventricular administration of the $\beta$-noradrenergic antagonist, propranolol.

Our previous observations indicated that 100–150 nl infusions of the cholinergic agonist, bethanechol, do not alter levels of LC discharge activity when placed greater than 500–600 $\mu$m from the LC.[5] Therefore, it is notable that in the present study infusions of similar volumes of clonidine altered LC neuronal activity levels even when placed 1000–1200 $\mu$m distant to the LC. This suggests that factors other than infusion volume, such as the chemical nature of a drug, can substantially affect the volume of tissue over which a given drug exerts physiological effects. The above observations highlight the necessity for verification of the physiological effects of drug infusions and the distance over which infusions exert such effects.

*Sedative/anesthetic effects of $\alpha_2$-agonists*

The sedative/anesthetic properties of $\alpha_2$-agonists are well documented.[8,9,10,11,12,20,21,29] For example, systemically administered $\alpha_2$-agonists are commonly used clinically as adjuncts to surgical anesthesia since they decrease the effective anesthetic dose.[6,19] The present observations that selective LC inhibition substantially enhances EEG indices of sedation in anesthetized animals suggest that the LC is a major site of action for the anesthesia-enhancing effects of $\alpha_2$-agonists. However, it remains to be determined if the anesthetic properties of systemically administered $\alpha_2$-agonists are solely due to inhibitory actions on LC/noradrenergic neurons. For example, Segal *et al.*[21] obtained evidence suggesting that at least some of the anesthesia-enhancing effects of these drugs are mediated through actions at postsynaptic $\alpha_2$-receptors on non-noradrenergic cells. Further, inhibitory actions of these drugs on noradrenergic cell bodies and/or terminals other than those of the LC could also have anesthesia-enhancing effects.

Previous work suggests that the conclusion that $\alpha_2$-agonists enhance sedation in anesthetized animals through inhibitory actions on LC neurons may generalize to unanesthetized preparations. Thus, systemically administered $\alpha_2$-agonists, which would be expected to reduce LC activity among other actions, have sedative properties in unanesthetized animals. In more restricted manipulations, enhanced EEG and behavioral signs of sedation have been observed following injection of $\alpha_2$-agonists into the LC area of the brainstem in unanesthetized rats.[8,9,10,11] However, as mentioned, the large infusion volumes of clonidine (200–500 nl) used in these previous studies and the absence of LC electrophysiological observations complicate conclusions regarding the critical site of action for this effect. This is particularly true in light of the documented ability of clonidine, which when infused at a dose less than those used in the above listed studies, exerts profound electrophysiological effects over distances greater than 1000 $\mu$m from the site of infusion (see above). Note that one difference between the current study and the previous studies[10,11] is that we did not observe any major effects of unilateral LC inhibition on EEG. This difference might result from the use of larger infusions in their studies, and the consequent action of clonidine at the contralateral LC or other brainstem sites. Alternatively, there may exist significant differences in the physiological effects of unilateral LC inhibition in halothane-anesthetized vs unanesthetized rat.

## CONCLUSION

Together with our previous observations, the current results suggest that neuronal discharge activity within one LC is capable of sustaining an activated EEG state bilaterally throughout the forebrain in the halothane-anesthetized rat. The absolute level of LC neuronal discharge activity required for maintenance of this EEG activation appears to be dependent on the level of anesthesia. In the lightly anesthetized animal in which a spontaneously activated forebrain EEG was observed, the EEG state was significantly affected only following complete bilateral LC inhibition. These observations indicate that under these experimental conditions, minimal levels of activity within the LC/noradrenergic system are necessary for maintenance of this activated EEG state. Observations during the recovery phase following peri-LC clonidine further support this interpretation; EEG recovery was generally observed at levels of LC activity that were substantially below those that characterized pre-infusion conditions. Further, the level of LC activity at which EEG recovery began was, in general, quite similar to the levels below which synchronous activity was observed during the onset

of LC inhibition. It remains to be determined whether the bilaterally distributed, LC-dependent changes in forebrain EEG are due to actions of the relatively small number of LC axon collaterals that project contralaterally, or to bilateral projections of an intermediate site through which the LC acts to alter forebrain EEG status.

That the maintenance of an activated forebrain is dependent on LC neuronal discharge activity is consistent with the hypothesis that the LC/noradrenergic system is involved in global regulatory processes related to arousal and/or attention. However, other neural systems, such as brainstem cholinergic and serotonergic systems, are also thought to modulate forebrain EEG status.[7,18,22,23,28] The above-described results are compatible with the hypothesis that numerous ascending systems regulate EEG state. The actions of one or more of these other systems are likely involved in the maintenance of ECoG desynchronization and HEEG theta activity during rapid eye movement sleep, a state during which LC neurons are virtually silent. The degree to which the LC/noradrenergic system participates in the regulation of forebrain EEG across varying behavioral states in the unanesthetized animal remains to be determined.

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