

Phasic Activation of Locus Ceruleus Neurons by the Central Nucleus of the Amygdala

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The role of the central nucleus of the amygdala (CeN) in modulating output of noradrenaline in the forebrain was evaluated by recording extracellular, single-unit activity from the noradrenergic nucleus locus ceruleus (LC) during stimulation of the CeN. Short high-frequency trains (200 Hz) delivered at 800 μ A in the CeN evoked phasic responses in 90% of the neurons recorded in LC. Single pulses were also effective but less reliably. The responses were complex, multiphasic with an initial latency of 10–20 msec. This early peak was diminished or, in some cases, completely blocked by local or intracerebroventricular application of the corticotrophin releasing factor antagonist α helical CRF (9-41). The later excitatory peak and subsequent inhibition were not effected by the drug treatment. The results underline the reciprocal functional relationship between the amygdaloid complex and the LC and suggest that the LC might be an important “effector” of CeN activation during learning.

Key words: amygdala central nucleus; locus ceruleus; noradrenaline; CRF; neuromodulation; electrophysiology

Introduction

The central nucleus of the amygdala (CeN) has a well established role in emotional learning (Applegate et al., 1982; Pascoe and Kapp, 1985). More recent evidence suggests that this nucleus plays an essential role in regulation of attention (for review, see Gallagher and Holland, 1994; Holland and Gallagher, 1999). Careful behavioral analyses after lesions led Gallagher and Holland (1994) to conclude that the CeN “regulates the processing of cues when predictive relationships between events are first noticed or altered.” This precisely describes the cognitive context that elicits a robust response of the whole population of cells in the noradrenergic nucleus locus ceruleus (LC).

LC neurons are activated both tonically and phasically during a task requiring sustained attention, suggesting a role in vigilance (Aston-Jones et al., 1991). However, the most striking feature of the response patterns of these neurons is that they show phasic responses to novel stimuli followed by rapid habituation and then renew responding whenever the associated reward contingencies are modified, such as during reversal or extinction (Sara and Segal, 1991; Hervé-Minvielle and Sara, 1995; Vankov et al., 1995). Thus, the LC responds to the predictive value or meaning of the stimulus rather than to its physical properties (Sara et al., 1994). These LC response characteristics observed in the rat were later confirmed in the behaving monkey (Aston-Jones et al., 1997). Thus, LC neurons respond “when predictive relationships

between events are first noticed or altered,” in the words of Holland and Gallagher describing the functional role of the CeN.

There is a small but potentially important afferent projection from CeN to LC (Wallace et al., 1989; Luppi et al., 1995). Fibers from CeN terminate in the rostralateral pericereuleus on dendrites identified immunohistochemically as noradrenergic (Van Bockstaele et al., 1996b, 1998). Input from CeN to the LC could be important in regulating LC activity during behavior requiring high levels of attention and stimulus processing. LC might, then, participate in mediating the CeN role in attention, through its ubiquitous projections to the forebrain. The postsynaptic facilitatory effects of norepinephrine (NE) on stimulus processing in all sensory modalities are well documented (Manunta and Edeline, 1997; Waterhouse et al., 1998; Lecas, 2001; Bouret and Sara, 2002).

The role attributed by Gallagher and Holland to the CeN in attention processes, together with the recent anatomical description of the projections of CeN to LC, encouraged us to examine the functional influence of CeN on LC by recording responses of LC neurons to electrical stimulation of the CeN. The role of corticotropin-releasing factor (CRF) in mediating LC responses was evaluated, because it has been shown that a substantial portion of CeN terminals targeting LC dendrites contain CRF (Van Bockstaele et al. 1996a,b) and CRF has an excitatory effect on LC neurons (Siggins et al., 1985).

Materials and Methods

Animals. Electrophysiological recordings were taken from 41 male Sprague Dawley rats obtained from IFFA Credo (L’Arbresle, France). The rats, weighing 320–420 gm at the time of the recording session, were housed for at least 1 week before the experiment in a temperature-controlled vivarium on a 12 hr light/dark cycle. They were weighed and handled regularly and had access to food and water *ad libitum*.

Surgery. Rats were anesthetized with urethane, 1.2 gm/kg, which was usually sufficient for the entire recording session, but it was supplemented if there was any sign of discomfort. The rats were mounted in a stereotaxic apparatus with the head positioned so that bregma was 2 mm

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below lambda, making an angle of approximately -14° from the head level position. Burr holes were drilled over the CeN and LC, the dura was removed, and electrodes were implanted under electrophysiological control. A bipolar stimulating electrode assembly consisted of two tungsten electrodes glued together (0.1–0.5 M Ω) with 500 μ m separating the tips. This was aimed at the CeN: -1.8 mm posterior to bregma, 3.8 mm lateral to the midline, and ~ 7.6 mm ventral to the surface of the brain. The LC electrode was lowered at -3.9 mm posterior to the lambda suture and 1.15 mm lateral to the midline. LC neurons were usually found at 5.2–5.8 mm ventral to the surface of the brain, just under the fourth ventricle. They were identified by their broad action potentials, slow firing rate (1.2 Hz), and distinctive excitatory–inhibitory response to contralateral paw pinch.

Pharmacology. In five experiments, the effect of the CRF antagonist α helical CRF (9-41) (α hCRF) (Sigma, St. Quentin Fallavier, France) was examined. In two experiments, α hCRF was injected into the ventricles (intracerebroventricular injection). A 26 gauge guide cannula was implanted above the lateral ventricle contralateral to the recording site (~ 1 mm posterior to bregma and ~ 1.5 mm lateral to midline), 1 mm dorsal to the ventricle (3.4 mm below brain surface), and cemented in place with dental cement. Injection was made through a 33 gauge cannula extending 1 mm ventrally from the edge of the guide to reach the ventricle. In three subsequent experiments, a 33 gauge cannula was glued to the recording electrode so that the edge of the cannula was ~ 200 μ m anterolateral to the tip of the recording electrode. The cannula was attached to flexible tubing into which a 2 μ l Hamilton microsyringe was inserted. The electrode–cannula assembly was lowered into the LC as described above.

Two hundred micrograms of α hCRF was dissolved in 190 μ l of distilled water and stored as 10 aliquots of 19 μ l at -20° C. Just before the injection, the solution was completed with 1 μ l of hypertonic saline to make an isotonic solution at a concentration of 1 μ g/ μ l with a neutral pH. For intracerebral injections, 1 μ l of this solution was slowly infused into the LC. Three to 4 μ l were injected in intracerebroventricular experiments.

Stimulating and recording. The electrophysiological signal was filtered (400–3000 Hz bandpass), amplified (10,000 \times) (amplifier model # P511; Grass Instruments, West Warwick, RI), and displayed on an oscilloscope and an audio monitor. Wave forms were discriminated online using the Cambridge Electronic Design (CED) (Cambridge, UK) CED1401 digital converter and Spike2 software (CED). Data were stored on a personal computer for additional offline analysis. Stimulation was delivered through an isolation unit in single pulses (200 μ sec) or in trains of three pulses at 200 Hz. Stimulation intensities included 200, 500, and 800 μ A. Each series consisted of 40–60 stimulations.

Data analysis. Single units were isolated wherever possible, using the Spike2 software. If the spikes were not clearly separable, the file was treated as a multiunit recording. Poststimulus time histograms (PSTHs) and raster displays were generated for neuronal activity 500 msec before and 500 msec after the stimulation, using 2 msec bins. The mean and SD of neuronal firing activity was calculated for the 500 msec prestimulation baseline. A firing rate increase to 2 SDs above the mean of the baseline, sustained over at least four bins, was considered an excitatory response. A decrease to 2 SDs below the mean was considered an inhibitory response. Response latencies were thus calculated for each unit or multiunit record.

To quantify baseline and evoked activity, the firing rate was calculated by summing the number of spikes per bin for two 50 msec windows on each PSTH, from 60 to 10 msec before stimulus onset and from 20 to 70 msec after stimulus onset. The mean firing rate in Hertz during these two periods was obtained by multiplying each count by 20 and dividing it by the number of trials (40–60).

To quantify the effect of CRF antagonist injections, spike counts during a baseline and two response windows were computed for successive trials, before and after injection. These windows were 15 msec in duration, starting 15 msec after stimulus onset for the early response window and 30 msec after stimulus onset for the late response window. The 15 msec baseline window ended 10 msec before stimulation. Spike counts obtained before and after α hCRF injection were compared with two-way ANOVA or with a nonparametric multiple comparison (Newman–Keuls analog test) when necessary (when spike counts were very low or not

normally distributed). One factor was defined as CeN stimulation with two levels: baseline and response (either early or late). The other factor was α hCRF injection with two levels: before and after injection. A significant interaction between these two factors or a significant effect of the response factor in only one condition (before or after injection) was used as statistical criteria to define an influence of α hCRF on the response to CeN stimulation. For each cell, mean spike count within the defined window, before and after injection, was calculated. The magnitude of early and late phases of the response after α hCRF injection could then be expressed as a difference from the baseline and converted to Hertz. To compare the effect of α hCRF on early and late phases of the response, an average value (across trials) of spike count difference was obtained for each cell for early and late response counts, respectively. A two-way ANOVA was applied to the population of recorded cells, with one factor being defined as response phase (two levels, early and late). The other factor was α hCRF injection with two levels: before and after injection.

Histology. At the end of the stimulation session, the rats were injected with the noradrenergic $\alpha 2$ receptor agonist clonidine (40 μ g/kg, i.p.) as a pharmacological control for the recording electrode placement. Clonidine binds to inhibitory autoreceptors in the LC, and this systemically administered dose completely inhibits firing of the noradrenergic neurons with a latency of onset of ~ 10 min (Dyon-Laurent et al., 1994). Thirty minutes after injection of clonidine, electrolytic lesions were made in the stimulating and recording sites by passing constant current (9 V) between the tips and the ground for 5 sec. Rats were perfused intracardially with saline and then Formalin (10%). Brains were removed and stored in Formalin. Sixty micrometer sections were cut, stained with cresyl violet, and examined for lesions in the appropriate structures.

Results

Histology

Placement of the stimulating electrode in the CeN is shown in Figure 1A. Placement of the recording electrode in the LC was determined by inspection of the histology as shown in Figure 1B, and the response to clonidine is illustrated in Figure 1C. Thirty-five rats had recording electrodes in the LC, and all of the cells were inhibited after clonidine injection. Of these, 27 also had stimulating electrodes correctly positioned in the CeN. Four stimulating electrodes were situated in the internal capsule, just lateral to CeN, two in the basolateral nucleus of the amygdala (BLA), one in the striatum, and one in the ventral hippocampus.

Two recording electrodes were located anterior and medial to LC in the lateral dorsal tegmental nucleus (LDT), three were anterior and dorsolateral to LC in the parabrachial nucleus, and one was in the cerebellum. None of the cells recorded from these sites responded to clonidine. All six of these rats had accurately placed stimulating electrodes in the CeN.

Responses of LC neurons to stimulation of CeN

Fifty-three single LC units and 13 multiunit recordings were taken from the 27 rats that had both stimulating electrodes in the CeN and recording electrodes in the LC. Ninety percent (48 of 53) of isolated single units showed a significant response to the CeN stimulation; 44 of these were orthodromically driven. Responses were seen to single pulses at 500 and 800 μ A in 20 of 53 single units (38%), with a mean latency of 11.7 ± 0.7 msec, latency being determined as described in Materials and Methods. More reliable responses were elicited by the short train of three pulses delivered at 200 Hz at an intensity of 800 μ A. Forty-eight single units responded to the train, with a latency of mean 20.3 ± 0.5 msec from the first pulse. Figure 2 shows an example of two single units recorded from the same electrode (Fig. 2A), one response being clearly biphasic, with a latency of 17 msec (Fig. 2B), and the other short lasting, monophasic, with a latency of 20 msec (Fig. 2C).

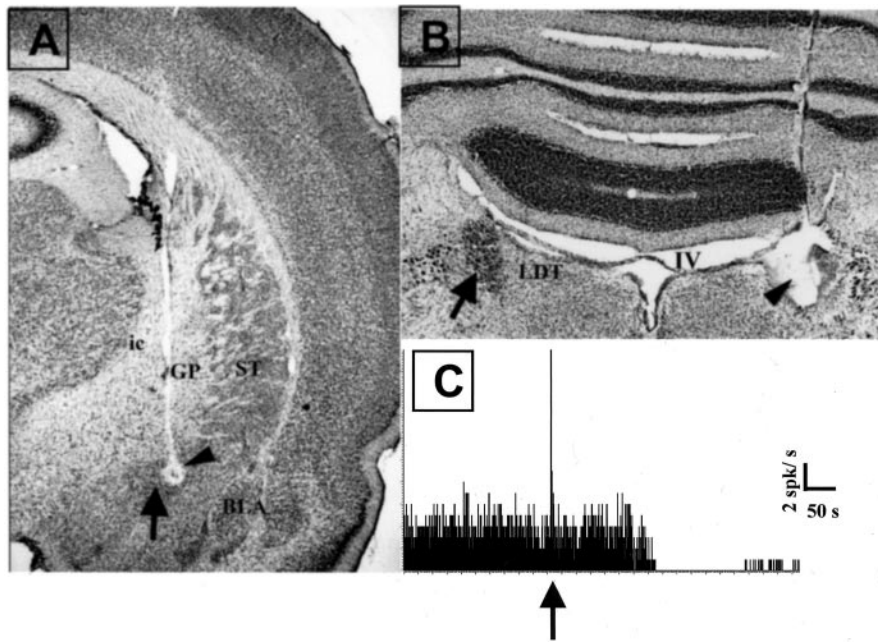


Figure 1. Histological and pharmacological control of electrode placement. *A*, Histological verification of the recording site in the CeN (arrow). The small lesion (arrowhead) in the CeN was made by passing DC current between the tips of the stimulating electrodes. Note the descending tract of the electrode through the internal capsule (ic) and the globus pallidus (GP). ST, Striatum. *B*, Histological verification of the recording site in LC. Note on the left the intact LC, the darkly stained nucleus just under the fourth ventricle (IV), as indicated by the arrow. On the contralateral side is the lesioned site of the LC (arrowhead) made by passing DC current between the tip of the recording electrode and the ground. The electrode tract can be seen above the lesion in the cerebellum. *C*, Example of a pharmacological verification of the recording from a noradrenergic neuron. Note the total inhibition of activity of the unit after systemic injection of clonidine (40 $\mu\text{g}/\text{kg}$) indicated by the arrow. All LC units and multiunit activity responded with total but transient inhibition after clonidine injection. The peak reflects the phasic activation of LC neurons during the injection.

All 13 multiunit recordings taken from LC showed an excitatory response to train, with a latency of 21.3 ± 0.4 msec. An example of a multiunit response is shown in Figure 3.

Antidromic responses were seen in four neurons. Response latency ranged from 21 to 24 msec, with a response to every stimulation trial and within cell response latencies being the same on every trial.

One unit responded with a total inhibition lasting for nearly 300 msec; this was the only purely inhibitory response seen in the entire series, and it should be noted that a neighboring unit, recorded from the same electrode, was antidromically driven by the stimulation. Thus, the inhibition seen in the other neuron was probably attributable to release of NE by the antidromically driven cell, which responded to every stimulation.

Responses of LC neurons to stimulation outside of the CeN

Stimulation of the BLA produced excitatory responses in two LC multiunit recordings with latencies of 28 and 32 msec.

In four experiments, the stimulating electrode was located in the internal capsule, and, in two of four multiunit records, there was a response in LC to the 800 μA train with a 28 msec latency. There was no response to the stimulation of the striatum ($n = 1$) or the ventral hippocampus ($n = 1$).

Responses to CeN stimulation in non-LC recordings

Verified placements in parabrachial nucleus in three rats (two single units and two multiunit) revealed reliable excitatory responses to CeN stimulation at 500 μA with a variable latency (range of 18–31 msec).

Two multiunit recordings were taken from the LDT, both showing excitatory responses with 25 and 18 msec latencies.

One recording electrode was located in the cerebellum, and there was no response to the CeN stimulation.

Effect of CRF antagonist on LC response to CeN stimulation

Intraventricular ($n = 2$) and intracereular ($n = 3$) injections of αhCRF were made. Eleven single units were recorded from the five animals. The spontaneous firing rate of LC neurons was not affected by either intracerebroventricular or local injection, (Fig. 4, Table 1), enabling a clear comparison of the phasic responses to CeN stimulation before and after αhCRF injection.

All cells showed a significant response to CeN stimulation (500 μA trains). There were no differences in effect of the CRF antagonist whether it was injected into the ventricle or into LC. Figure 5 (left) shows a response of a cell having very little spontaneous activity and a reliable phasic response to the stimulation. The response was significantly decreased after the intracerebroventricular administration of the CRF antagonist (Fig. 5, right).

When the whole response window (50 msec) was considered, the injection of αhCRF had no significant effect on re-

sponse magnitude (Table 1), because the treatment only affected the early phase of the response. For four of the six units that had only presented an early response, this response was completely blocked (Fig. 6*A*). This early response was significantly decreased for the other two units. Five units showed a biphasic response that consisted of an early peak at ~ 18 msec and a later peak at ~ 30 msec from stimulus onset; an example is seen in Figure 6*B*. For these cells, clearly only the early phase of the response was altered by αhCRF , the late phase being unaffected.

This differential effect of αhCRF on early and late phases of the response is clearly seen when the entire sample is taken into consideration. Figure 7 depicts the mean early and late responses, before and after drug treatments. The drug \times phase interaction was significant ($F_{(20,1)} = 6.4$; $p = 0.02$), confirming that effect was limited to the early phase of the response.

For those cells ($n = 7$) that still responded to CeN stimulation after αhCRF injection, a significant increase in latency was observed as a consequence of the effect on the early phase of the response ($t_{(6)} = -2.6$; $p = 0.04$) (Table 1).

Discussion

Responses of LC units and multiunits to CeN stimulation were consistent and present in the large majority of recorded cells. Although the anatomical projections of CeN to LC have been well described, with some neurochemical definition (Van Bockstaele et al., 1996a,b), this is the first direct demonstration of a functional influence of CeN on LC activity *in vivo*. The responses elicited were for the most part excitatory, with little variability in

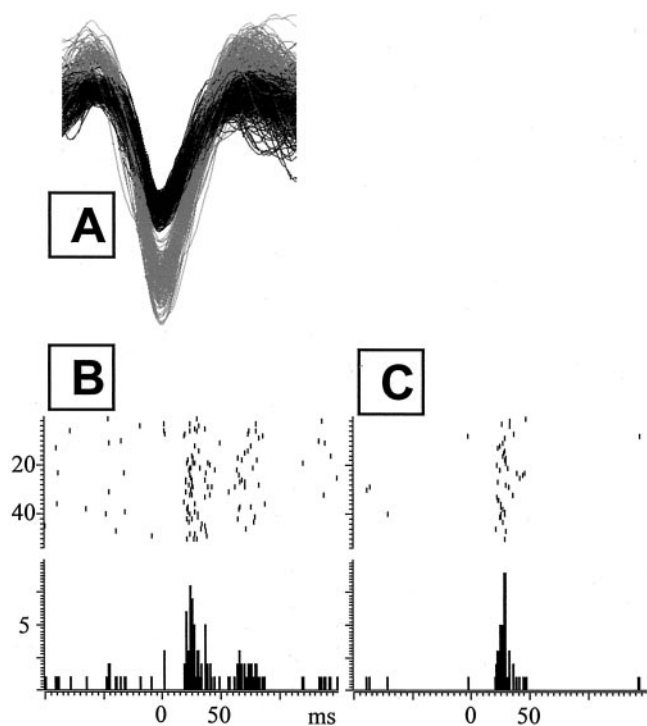


Figure 2. Responses to a train of 800 μ A of two single units in LC recorded simultaneously from the same electrode. *A* shows superimposed waveforms of the two units. The unit depicted in *B* shows biphasic response with the initial phase latency at 17 msec. In the unit depicted in *C*, the initial latency is 20 msec, and second phase is absent. Top, Raster display of trial-by-trial responses to the stimulation. Bottom, Cumulative PSTH with 2 msec bins.

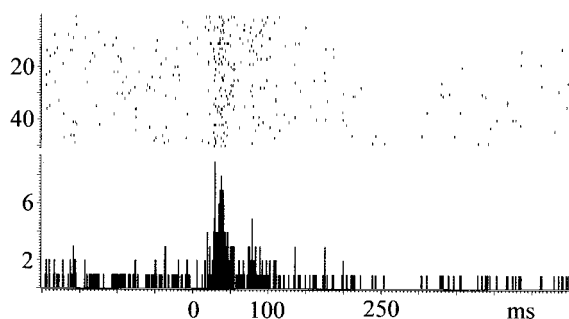


Figure 3. Multiunit response LC to a train of 800 μ A applied to CeN. Note the relatively low baseline activity, which suggests that only a few neurons are included in this recording. The response consists of a biphasic excitation followed, 200 msec after stimulus, by a long-lasting inhibition. This postexcitation inhibition is probably attributable to an α 2 receptor-mediated autoinhibition.

initial latency, but most responses were complex, with biphasic excitation (Fig. 2) or excitation followed by inhibition (Fig. 3).

Van Bockstaele et al. (1998) provided strong evidence that a substantial portion of the CeN terminals in the pericereulus region make asymmetric, excitatory synaptic specializations on target cells staining positively for tyrosine hydroxylase. There are abundant synapses on unidentified cells and dendritic processes as well. This region contains a large population of GABAergic neurons that locally regulate LC activity, thus providing an additional mediating mechanism for CeN influence on LC. A substantial number of CeN axon terminals located in the pericereulus region are CRF immunoreactive (Van Bockstaele et al., 1998). Previous studies showed a marked decrease in CRF in LC region after lesions of the CeN, suggesting that this nucleus is

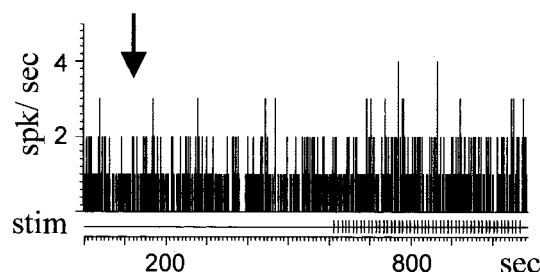


Figure 4. Local injection does not alter the firing rate of LC neurons. The firing rate of a single unit is shown as a function of time. Neither local α hCRF application (arrow) nor CeN stimulation (lines) modify the baseline activity of the cell.

a major source of CRF in LC (Koepler-Muly et al., 1993). Moreover, recent studies using a combination of cellular and molecular techniques clearly confirm the existence of CRF receptors in LC neurons (Fox et al., 2002). Electrophysiological studies, *in vivo* and *in vitro*, have indicated that CRF has an excitatory effect on LC neurons (Siggins et al., 1985; Curtis et al., 1995), so CRF should be a strong candidate for mediating at least part of the LC response to CeN stimulation. The present results confirm this by showing that the short latency early part of the response is significantly attenuated or completely blocked after local application of the CRF antagonist in the LC. The presence of vesicles in these terminals that were not CRF immunoreactive suggests that other peptides and neurotransmitters are likely to be colocalized with CRF in CeN terminals (Van Bockstaele et al., 1998). Indeed, immunohistochemical studies revealed large populations of neuropeptide Y, somatostatin, substance P, cholecystikinin, and vasoactive peptide containing neurons within the CeN (Cassell et al., 1986). The residual early response sometimes seen after the CRF antagonist could be mediated by any of these. The late part of the response, spared by the injection, is most likely polysynaptic.

The majority of extrinsic projections from CeN are GABAergic (Swanson and Petrovich, 1998); their short-latency inhibitory influence might delay expression of the excitatory response in LC. It should be noted that the stimulus artifact associated with the train stimulation had a duration of 13 msec, so it could be masking an early inhibitory response.

Specificity of CeN stimulation

Stimulating electrodes located in BLA and the internal capsule evoked responses in LC. The BLA does not project to LC but does have a small direct projection to the CeN and another via the lateral amygdala (LA) (Pitkanen et al., 1997). Thus, the polysynaptic influence would account for the longer-latency response of LC cells to this stimulation. Responses in LC were also elicited, less reliably, from the internal capsule. Data from our laboratory suggest that fibers originating in the FR2 region of frontal cortex and projecting to the region of the LC pass via the internal capsule. Electrical stimulation of the FR2 produces an excitatory response in LC (Briois and Sara, 1997; Jodo et al., 1998) that is abolished by cutting this fiber system (L. Briois and S. J. Sara, unpublished observations). It is important to note that stimulation of other electrode placements, near but outside of the CeN, namely in the ventral hippocampus or the ventral striatum, did not evoke responses in LC.

It is possible that there is current spread to other structures in the vicinity that could mediate the effect of the CeN stimulation. The most likely candidate is the bed nucleus of the stria terminalis (BNST), just adjacent to the CeN and a major output pathway

Table 1. LC unit firing rate (in Hertz) around CeN stimulation before and after α hCRF injection

| | Spontaneous | Evoked | Early | Late | Latency (msec) | Resp Cells |
|---------------|-------------|------------|------------|------------|----------------|------------|
| Control | 1.8 ± 0.4 | 14.7 ± 3.1 | 16 ± 4.5 | 14.2 ± 3.1 | 23.8 ± 1.8 | 11/11 |
| α hCRF | 1.8 ± 0.6 | 10.3 ± 2.5 | 4.5 ± 2.6* | 13.3 ± 3.4 | 37.4 ± 4.1* | 7/11 |

Spike count expressed in Hertz, averaged across 11 LC units. Mean ± SEM spontaneous activity (50 msec before stimulus onset). Mean evoked activity (between 20 and 70 msec after stimulus). Early, Mean early response (between 15 and 30 msec after stimulus). Late, Mean net late response (between 30 and 45 msec after stimulus). For these last two variables, spike count during baseline in an equivalent window was subtracted from the evoked spike count. The effect of α hCRF is restricted to the early phase of the evoked activity, which also results in a significant increase in response latency for cells that still showed a response. Resp cells, Number of units responding to the CeN stimulation before and after drug treatment. * $p < 0.05$.

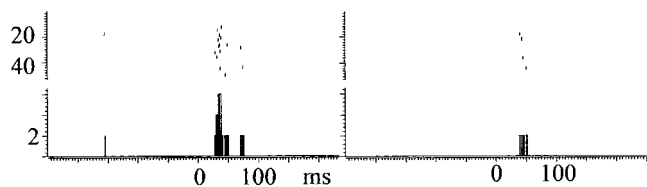


Figure 5. Intracerebroventricular injection of CRF antagonist decreases LC response to CeN stimulation. PSTHs (2 msec bin) and rasters constructed around CeN stimulation (500 μ A trains) for an LC unit with very little spontaneous activity. Before α hCRF injection (left), the cell exhibits a clear response to CeN stimulation. After intracerebroventricular injection of α hCRF (right), the response is greatly decreased (nonparametric Newman–Keuls analog test; $p < 0.05$).

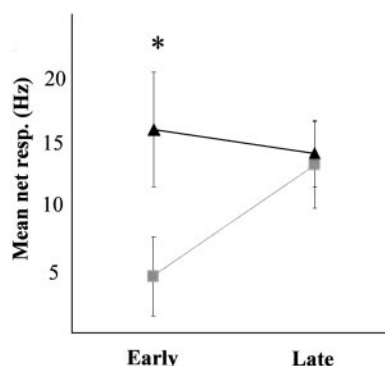


Figure 7. Mean ± SEM early and late responses of 11 LC units to CeN stimulation before and after injection of α hCRF. Only the early phase of the response was affected by the treatment. Triangles, Control; squares, α hCRF. An ANOVA reveals a significant interaction between response phase and treatment factors ($F_{(20,1)} = 6.4$; $p = 0.02$).

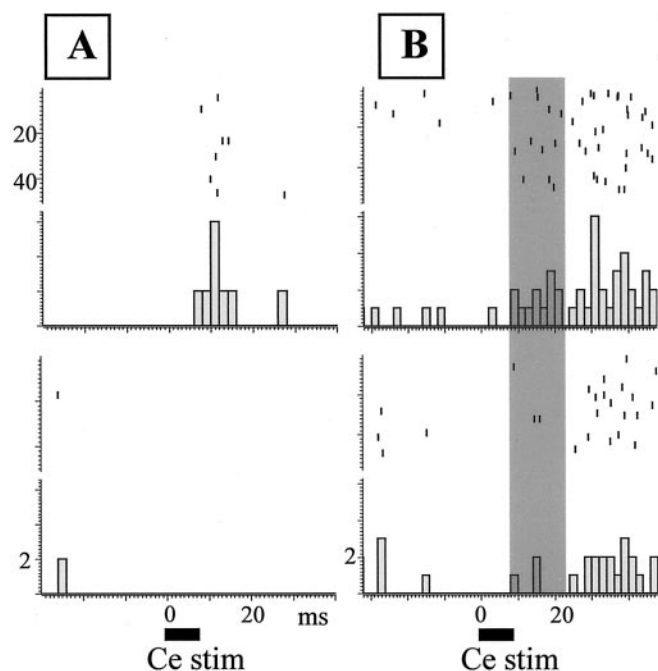


Figure 6. Effects of local injection of CRF antagonist on LC response to CeN stimulation. *A*, PSTHs and rasters constructed around CeN stimulation (Ce stim) (500 μ A trains; horizontal bar) for an LC unit showing a monophasic response (top). This response is completely blocked by the CRF antagonist (bottom; nonparametric Newman–Keuls analog test; $p < 0.05$). *B*, Same representation for another single unit showing a biphasic response to CeN stimulation. Before α hCRF injection (top), the cell exhibits a biphasic response with an early (shaded area) and a late phase. After the injection (bottom), the early response is greatly attenuated (30% of its preinjection value; nonparametric Newman–Keuls analog test; $p < 0.05$), whereas the late phase is spared.

from the amygdala. This nucleus does send a large projection to the pericereuleus region. Recent studies by Van Bockstaele et al. (1996a) have shown that, like CeN projections, BNST terminals synapse on both noradrenergic and non-noradrenergic dendrites in the region. The major difference is that the BNST synapses are mostly symmetrical, i.e., of the inhibitory type. Thus, current spread to the BNST could be contributing to the response in LC, but it most likely does not account for the consistent excitatory

responses observed. In fact, current spread to the BNST might account for the puzzling observation that LC responses to short trains had significantly longer latencies than responses to single pulses of the same intensity. According to the ultrastructural studies of Van Bockstaele, activation of the BNST, more likely during a train than during a brief pulse, would provide a competing inhibitory influence to LC, delaying the appearance of the excitatory response.

The relatively sparse number of antidromically driven responses was initially unexpected, because the CeN receives a substantial noradrenergic input. A recent report has shown, however, that the majority of the noradrenergic fibers in the CeN originate, not in LC as originally suggested by Fallon et al. (1978), but in the medulla oblongata catecholamine cell groups (Asan, 1998). This corroborates a previous study using double labeling to show that only ~5% of LC neurons were retrogradely labeled from the CeN (Petrov et al., 1993).

Parabrachial and LDT responses to CeN stimulation

Responses to CeN stimulation in the parabrachial nucleus and in the LDT are not surprising, because the CeN projects directly to both of these regions (Hopkins and Holstege, 1978); the responses had slightly longer latencies than those in LC, but there were not enough data to evaluate the reliability of this difference.

Functional significance

There has been much discussion concerning the relative roles of the various nuclei of the amygdala in learning and memory processes. There is a general consensus that the amygdala plays an important role in pavlovian fear conditioning and inhibitory avoidance conditioning (Kapp et al., 1979; Pascoe and Kapp, 1985; Davis, 1994; McGaugh et al., 1996; LeDoux, 2000) and that the LA is involved in processing information concerning the conditioned stimulus (CS), whereas the CeN governs reactive responses (Amorapanth et al., 2000). There is, however, disagreement as to whether the amygdala is the site of plasticity

underlying the associative fear learning or whether the role of the amygdala is to modulate memory formation in other brain regions (Fanselow and LeDoux, 1999; Cahill et al., 1999; Vazdarjanova and McGaugh, 1999; Wilensky et al., 2000). Consideration of the functional consequences of the CeN influence on LC might put the question in a new light. Activation of CeN during conditioning will cause an increase in firing of LC cells, which in turn will release NE in the BLA and LA, because there is a projection from LC to both of these regions (Fallon et al., 1978). As we know from the extensive work of McGaugh et al. (1996), this increase in NE in the amygdala will enhance memory consolidation by interaction with hormones and neurotransmitters. The CeN excitation of LC will also release NE in forebrain regions. This should result in increased vigilance and attention (Berridge and Foote, 1991) and enhanced sensory processing (Manunta and Edeline, 1997; Waterhouse et al., 1998; Lecas, 2001; Bouret and Sara, 2002), thereby facilitating learning.

Several studies have shown that stimulation of the amygdala enhances synaptic transmission, plasticity, and long-term potentiation in the dentate gyrus (DG) of the hippocampus (Ikegaya et al., 1995, 1996; Akirav and Richter-Levin, 1999). The activation of LC by stimulation of CeN directly or via BLA should result in a massive release of NE from LC terminals, found profusely in the DG. There is a large literature showing noradrenergic-induced enhancement of cellular excitability (Harley and Sara, 1992), synaptic transmission, (Sara and Bergis, 1991; Kitchigina et al., 1997), and synaptic plasticity (Neumann and Harley, 1983) *in vivo* in the rat DG. If activation of the amygdala occurs during learning, then its influence on LC should promote synaptic plasticity in the hippocampus, thought to be necessary for memory formation.

There are surprisingly few electrophysiological studies of the involvement of CeN in learning to lend support to the view of CeN function put forth by Gallagher and Holland (see Introduction). The extensive work of Kapp et al. (1994) has, however, clearly shown that this nucleus is activated by CSs in pavlovian aversive conditioning (Kapp et al., 1994). It is evident that CeN activity during learning can influence forebrain structures through several different pathways. Gallagher and Holland (1994) have, indeed, provided evidence from lesion and pharmacological studies that the cholinergic system is involved in mediating the CeN role in attention. In those studies, however, the conditioned orientating response, suppressed after CeN lesion, was spared by lesions of the cholinergic system (Holland and Gallagher, 1999), suggesting the involvement of other outputs. Our electrophysiological studies showing that responses are evoked in LC cells under similar cognitive conditions as for CeN (see Introduction) together with the present results showing the strong excitatory influence of CeN on LC makes a strong case for considering LC to be another effector of CeN activity. The widespread LC projections on both sensory and limbic areas, particularly the BLA and the hippocampus, should facilitate neuronal processing across these areas, as well as the formation of a distributed memory trace.

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