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Short communication

Projections from the lateral part of the central amygdalar nucleus to the postulated fear conditioning circuit

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Abstract

The lateral part of the central nucleus projects densely to only three regions: the medial part of the central nucleus, restricted parts of the bed nuclei of the stria terminalis, and the parabrachial nucleus in the pons. The possible role of the lateral central amygdalar nucleus in circuitry mediating conditioned emotional responses is discussed; changing neuropeptide levels in the lateral part may act as a 'gain control' for reversible long-term modulation (LTM) of medial part output.

Keywords: Bed nuclei of the stria terminalis; Conditioned emotional response; Corticotropin-releasing hormone; γ-Aminobutyric acid (GABA); Long-term modulation; Neuropeptide; Parabrachial nucleus

Considerable anatomical, physiological, and behavioral work indicates that the central amygdalar nucleus (CEA) is important for the expression of conditioned autonomic and behavioral responses to at least some aversive stimuli [12,20,24]. However, the CEA has three structurally distinct parts [10,31,56], although its output is usually thought of in terms of the medial part (CEAm). The latter has direct descending projections to areas generating autonomic [19,48,61] and behavioral [43] responses, and stimulation of neurons in this region produces autonomic (e.g. [20]) and behavioral (e.g. [2]) responses that mimic conditioned emotional responses, which are abolished by large lesions of the CEA that include the CEAm [12,24].

The capsular part of the nucleus (CEAc) is interesting because it appears to receive information related to most sensory modalities, either directly from the thalamus [25] and occipital, temporal, and perirhinal cortical areas [30,34,46] (specifically dorsally), or indirectly from the lateral amygdalar nucleus [42] (both dorsally and ventrally). In addition, the cortical [40] and medial [9] amygdalar nuclei, which receive direct inputs from the main and accessory olfactory bulbs, respectively, project to ventral regions of the CEAc, as does the lateral parabrachial nucleus (PB) [4], which relays visceral and/or nociceptive information [5,6,8,11,17]. The CEAc in turn projects to the CEAm (our unpublished observations with PHAL).

Less is known about the lateral part of the central nucleus (CEAl). It receives a direct input from the insular cortex [33,63] and PB [4], and neurons here express various neuropeptides, including corticotropin-releasing hormone (CRH), neurotensin, enkephalin, and somatostatin [10,61]. Non-neuroendocrine CRH neurons have been implicated recently in behavioral responses to stress, fear, and anxiety [13,22]. Intracerebroventricular CRH infusion produces behavioral responses similar to those associated with conditioned fear [13,22,27], whereas CEA electrolytic lesions [26], or CRH receptor antagonists infusion into the region of the CEA [59], reverse these effects. Furthermore, CRH mRNA levels in the CEAl are increased in a dose-dependent way by increasing levels of circulating corticosterone [28,58,62], and CRH peptide levels increase in the CEA area following immobilization stress [41]. Unfortunately, very little is known about CEAl outputs, mainly because its tiny volume has discouraged anterograde tracer experiments. Nevertheless, we decided to examine its axonal projections with the anterograde tracer PHAL because it produces small injection sites and is very sensitive.

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Five adult male Harlan Sprague–Dawley rats (300–350 g) received a single iontophoretic injection of a 2.5% solution of PHAL (Vector Laboratories, Burlingame, CA), prepared in 0.01 M sodium phosphate-buffered saline (NaPBS), into the region of the CEAl through a glass micropipette (15 μm tip diameter) by applying a positive current (5 μA, 7-s off/on intervals) for 10–15 min. Animals were anesthetized for stereotaxic surgery with a mixture of ketamine and xylazine (v/v; 1 ml/kg body weight).

After a 14–16-day survival time, the rats were deeply anesthetized with pentobarbital and perfused transcardially with 150 ml of 0.9% NaCl followed by 300 ml of ice-cold 4% paraformaldehyde in 0.1 M borate buffer (pH 9.5). The brains were removed, post-fixed overnight at 4°C in the same fixative containing 10% sucrose, then frozen, and serial 30-μm-thick sections (1-in-4) were cut in the transverse plane on a sliding microtome. One complete series of sections was processed to detect PHAL using the immunohistochemical procedure described elsewhere [15,40]. PHAL-containing cells (in the injection sites) and fibers were plotted with the aid of a camera lucida onto cytoarchitectonic drawings of adjacent thionin-stained sections, and then transferred onto a series of standard drawings of the rat brain [56] with the aid of a computer (Apple, Macintosh Quadra 700; Adobe Illustrator 5). The rat brain parcellation follows Swanson [56].

In three experiments the PHAL injection labeled many neurons within the CEAl and two injection sites were confined entirely to this part of the CEAl, while one involved the ventral half of the CEAl and the adjacent CEAc. Two control injections were placed just ventral and lateral to the CEAl. Projections labeled in Expt. 12 (Fig. 1) are described in detail because the injection was restricted to the CEAl; the projection pattern is typical of that labeled in the other two experiments with an injection in the CEAl.

Within the amygdala the CEAl projects heavily to the CEAm (Fig. 2E–K, Fig. 3B) and lightly to the most caudal region of the posterior basolateral nucleus (Fig. 2L–M),

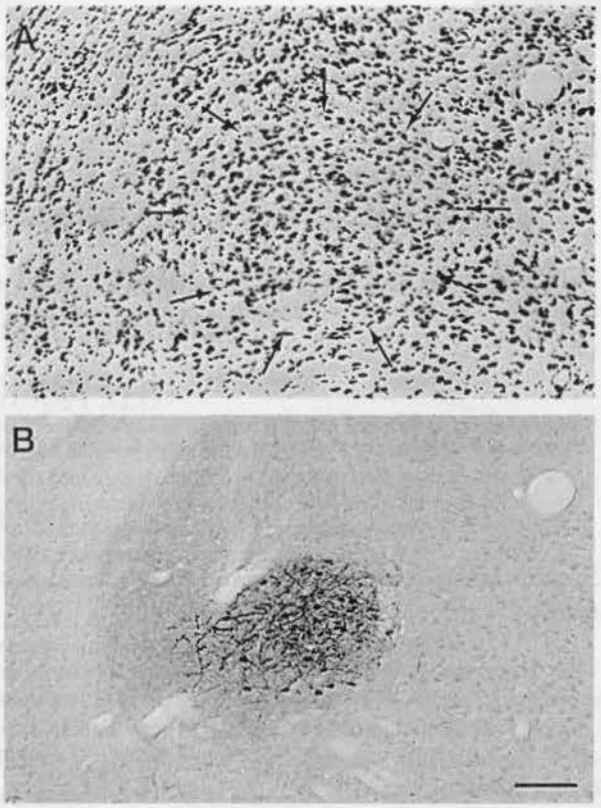
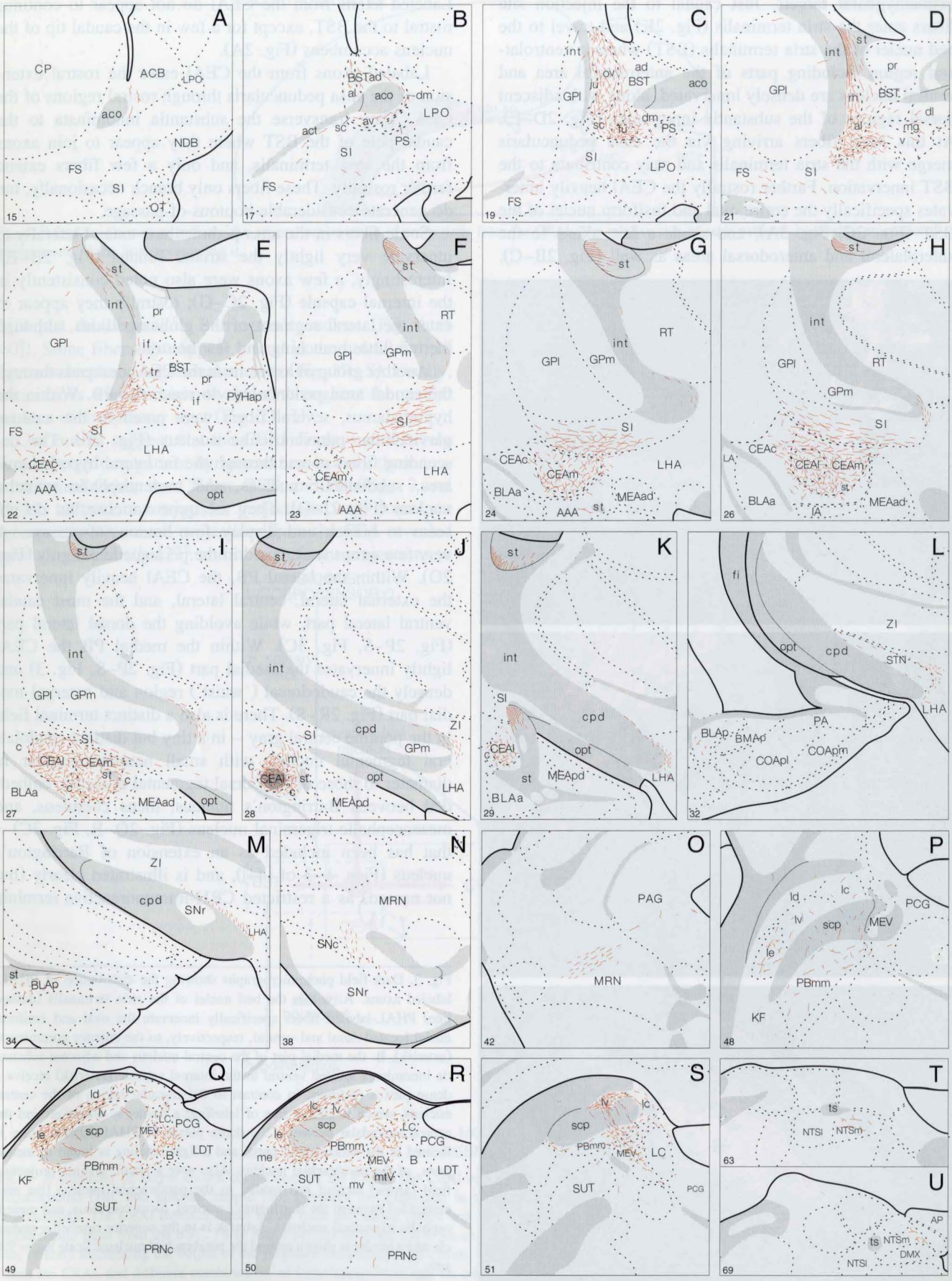


Fig. 1. Bright-field photomicrographs illustrating the appearance of the CEAl PHAL injection site in Expt. 12 (B), and the caudally adjacent thionin-stained section (A). The injection in this experiment was restricted to the caudal third of the CEAl. Scale bar = 100 μm.

and its fibers avoid the rest of the amygdala except for very sparse labeling in the anterior amygdalar area. The CEAm (Fig. 2F–J) is very densely innervated by highly branched, bouton-laden fibers, whereas the CEAc contains only a few anterogradely labeled fibers (Fig. 2E–K). Many branching fibers and boutons are also present within the CEAl itself, rostral and caudal to the injection site.

PHAL-labeled fibers from the CEAl travel through both the stria terminalis and ansa peduncularis to innervate

Fig. 2. A summary of CEAl projections. The distribution of PHAL-labeled axons in Expt. 12 was plotted onto a series of standard drawings of the rat brain derived from an atlas [56], arranged from rostral (A) to caudal (U). The dark gray area in the CEAl (J) indicates the injection site (see Fig. 1). The number in the lower left corner of each drawing refers to the corresponding rostrocaudal level of the atlas. AAA, anterior amygdaloid area; ac, anterior commissure; ACB, n. accumbens; aco, anterior commissure, olfactory limb; AP, area postrema; B, Barrington's n.; BLAa, -p basolateral amygdalar n., anterior, posterior parts; BMAp, basomedial amygdalar n., posterior part; BSTad, -al, -av, -dl, -dm, -fu, -if, -ju, -mg, -ov, -pr, -rh, -tr, -v, -sc, bed nuclei stria terminalis anterodorsal, anterolateral, anteroventral areas, dorsolateral, dorsomedial, fusiform, interfascicular, juxtacapsular, magnocellular, oval, principal, rhomboid, transverse, ventral nuclei, subcommissural zone; CEAc, -l, -m, central amygdalar n., capsular, lateral, medial parts; COApl, -pm, posterior cortical amygdalar n., lateral, medial parts; CP, caudoputamen; cpd, cerebral peduncle; CU, cuneate n.; DMX, dorsal motor n. of the vagus nerve; FS, fundus of the striatum; GPl, -m, globus pallidus, lateral, medial segment; IA, intercalated amygdalar n.; int, internal capsule; IVn, trochlear nerve; KF, Kölliker-Fuse subnucleus (of PB); LA, lateral amygdalar n.; LC, locus coeruleus; LDT, laterodorsal tegmental n.; LHA, lateral hypothalamic area; LPO, lateral preoptic area; MEAad, -pd, medial amygdalar n., anterodorsal, posterodorsal parts; MEV, mesencephalic n. of the trigeminal; NTSl, -m, solitary tract n., lateral, medial parts; opt, optic tract; OT, olfactory tubercle; PA, posterior amygdalar n.; PAG, periaqueductal gray; PBlc, -ld, -le, -lex, -lv, -me, -mm, -mv, parabrachial n., central lateral, dorsal lateral, external lateral, extreme lateral, ventral lateral, external medial, medial medial, ventral medial parts; PCG, pontine central gray; PPN, pedunculopontine n.; PRNc, pontine reticular n., caudal part; PS, parastrial n.; PVHap, paraventricular hypothalamic n., anterior parvicellular part; RT, reticular thalamic n.; scp, superior cerebellar peduncle; SI, substantia innominata; st, stria terminalis; STN, subthalamic n.; SNc, -r, substantia nigra, compact, reticular parts; SUT, supratrigeminal n.; ts, solitary tract; ZI, zona incerta.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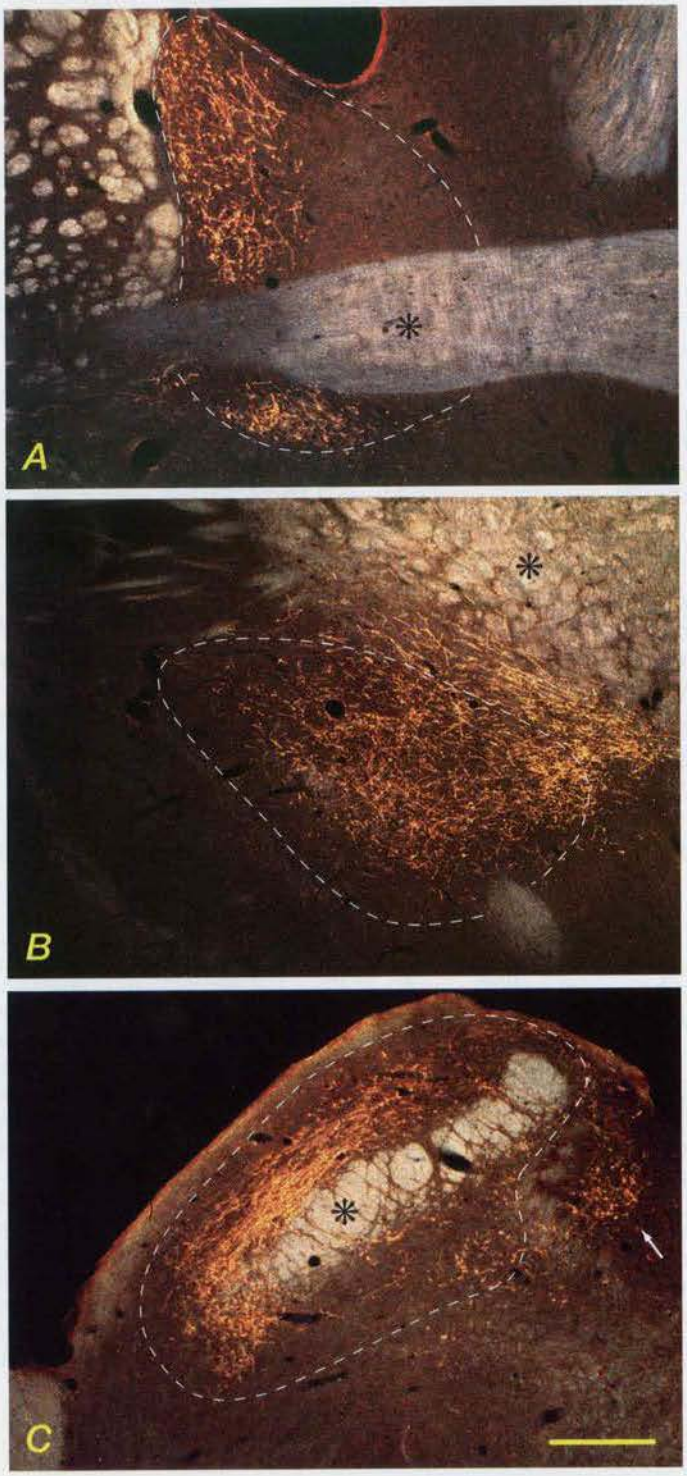
extraamygdalar targets. Just caudal to the injection site fibers enter the stria terminalis (Fig. 2K) and travel to the bed nuclei of the stria terminalis (BST) where a ventrolateral region including parts of the anterolateral area and ventral nucleus are densely innervated, along with adjacent dorsal regions of the substantia innominata (Fig. 2D–E). At this level fibers arriving via the ansa peduncularis merge with the stria terminalis and may contribute to the BST innervation. Farther rostrally the CEAl heavily innervates specifically the entire oval and fusiform nuclei of the BST (Fig. 2C, Fig. 3A), and sends a few axons to the anterolateral and anterodorsal areas as well (Fig. 2B–C).



Labeled axons from the CEAl do not appear to continue rostral to the BST, except for a few in the caudal tip of the nucleus accumbens (Fig. 2A).

Labeled axons from the CEAl enter the rostral extension of the ansa peduncularis through rostral regions of the CEA. They transverse the substantia innominata to the caudal pole of the BST where they appear to join axons from the stria terminalis, and only a few fibers extend farther rostrally. These fibers only branch occasionally, but do generate considerable boutons-of-passage.

Some fibers in the ansa peduncularis extend laterally to innervate very lightly the striatal fundus (Fig. 2B–E). Interestingly, a few axons were also noted consistently in the internal capsule (Fig. 2C–G); rostrally they appear to enter the lateral segment of the globus pallidus, although there is little branching and few boutons.

Another group of axons from the CEAl descends through the caudal ansa peduncularis to reach the PB. Within the hypothalamus, several fibers were noted in the anterior parvicellular paraventricular nucleus (Fig. 2E). The descending fibers course through the far lateral hypothalamic area, subthalamic nucleus, and mesencephalic reticular nucleus (Fig. 2J–O). They are quite varicose but do not seem to branch and display few boutons-of-passage. A very few axons were noted in the periaqueductal gray (Fig. 2O). Within the lateral PB, the CEAl heavily innervates the external lateral, central lateral, and the most caudal ventral lateral part, while avoiding the dorsal lateral part (Fig. 2P–S, Fig. 3C). Within the medial PB the CEAl lightly innervates the medial part (Fig. 2P–S, Fig. 3) and densely the caudodorsal ('waist') region and external medial part (Fig. 2R–S). There is also a distinct terminal field in the pontine central gray – in a tiny but distinct caudolateral tegmental nucleus with small neurons (similar in diameter to those of the dorsal tegmental nucleus), embedded between Barrington's nucleus, locus coeruleus, and mesencephalic trigeminal nucleus (Fig. 2Q–R, Fig. 3C) – that has been included as an extension of Barrington's nucleus (Figs. 1–3 of [44]), and is illustrated clearly (but not named) as a restricted CRH-immunoreactive terminal

Fig. 3. Dark-field photomicrographs showing the distribution of PHAL-labeled axons. A: within the bed nuclei of the stria terminalis (dashed line) PHAL-labeled fibers specifically innervate the oval and fusiform nuclei located dosal and ventral, respectively, to the anterior commissure (asterisk). B: the medial part of the central nucleus and adjacent substantia innominata located ventral to the internal capsule (asterisk) receive a dense plexus of fibers, in contrast to the capsular region of the central nucleus, which is almost free of labeling; a border is drawn around the central amygdalar nucleus. C: a dense plexus of PHAL-labeled fibers is located in the ventrolateral parts, and a light labeling is found in medial parts, of the parabrachial nucleus. Also note a small, distinct projection field (arrow) located just medial to the parabrachial nucleus (the area embedded between the Barrington's nucleus, locus coeruleus, and mesencephalic trigeminal nucleus). Asterisk is in the superior cerebellar peduncle and a border is shown around the parabrachial nucleus. Scale bar = 500 μm.

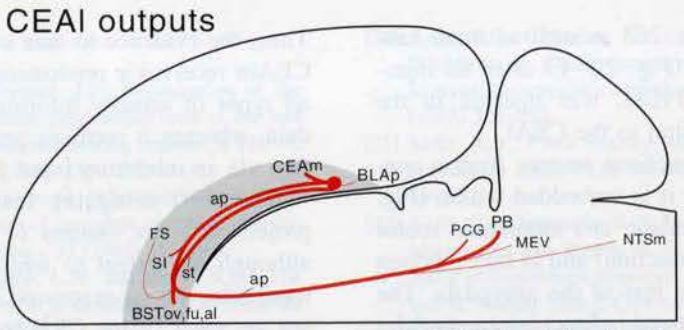


Fig. 4. Summary diagram to indicate the general organization of projections from the CEAl. The relative size of each pathway is roughly proportional to the thickness of the line representing it.

field just dorsolateral to Barrington's nucleus (Fig. 1 of [60]). Some fibers also appear to end in the caudal half of the mesencephalic trigeminal nucleus (Fig. 2R–S). Finally, some axons were consistently noted throughout the medial nucleus of the solitary tract (Fig. 2T–U).

Our results suggest that the CEAl displays a relatively simple projection pattern by innervating heavily three major terminal fields: the CEAm, BST, and PB (Figs. 3 and 4). No cortical or amygdalar projections were found, in agreement with overall projections from the CEA revealed autoradiographically [23]; and no significant projections to the lateral hypothalamic area, periaqueductal gray, or dorsal vagal complex were observed, in agreement with retrograde tracer studies [53,61]. Our results are in agreement with the topography of CEAl projections to the PB previously demonstrated with anterograde tracers [36]. In addition, previous retrograde tracer injections in the 'anterolateral' and 'posterolateral' parts of the BST resulted in labeling of the caudal and rostral CEAl, respectively [52]. Our results, however, do not provide evidence for a rostro-caudal topography in projections from the CEAl to the BST, because labeled terminals were observed in the

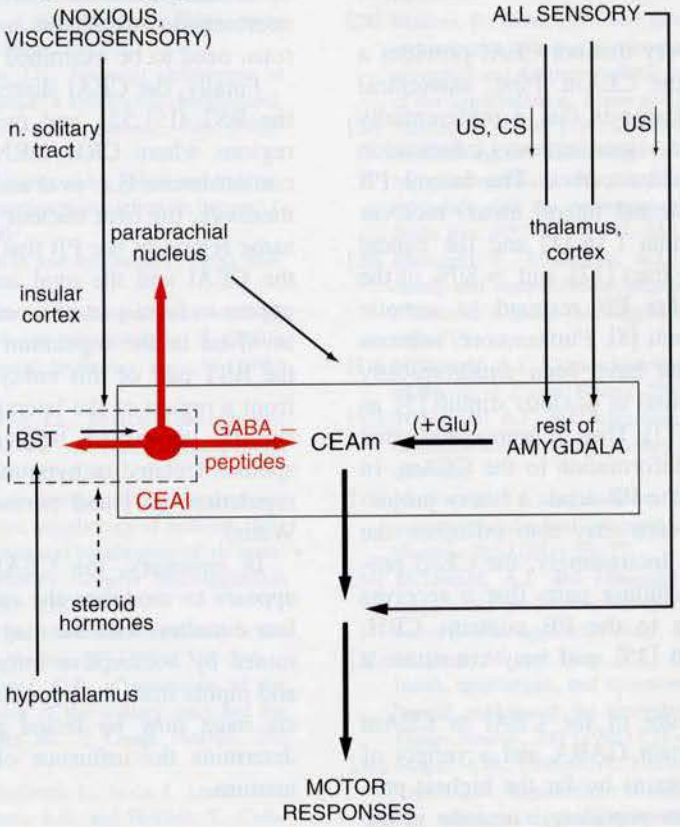


Fig. 5. A summary of major known connections of the CEAl. The CEAl receives hormonal and neuronal inputs. High levels of mineralocorticoid [3], glucocorticoid [18], and gonadal steroid [50] receptors are expressed in the CEAl, and its peptide expression is regulated at least by circulating corticosterone. The CEAl receives direct inputs from the insular cortex and PB relaying noxious and/or viscerosensory information, as well as indirect hypothalamic input from the BST. Three major outputs from the CEAl are to the PB and BST (including areas it receives inputs from), and to the CEAm, thus, directly influencing the output of the proposed conditioned fear circuit. Large amounts of GABA and various peptides including CRH are expressed within the CEAl, and different combinations of these transmitters may be contained in its outputs.

oval and fusiform nuclei (Fig. 2C) as well as more caudolateral regions of the BST (Fig. 2D–E) after all injections. In a previous study, PHAL was injected in the rostrolateral CEA [16] just rostral to the CEAl.

Suggestions about CEAl functions emerge from a consideration of the circuitry that it is embedded within (Fig. 5). The CEAm projects to somatic and autonomic motor regions of the brainstem (Introduction) and in turn receives inputs from the CEAl, and the rest of the amygdala. The latter is a complex set of direct and indirect intraamygdalar projections that may relay information from all sensory modalities to the CEAm (Introduction and below). Much of this information reaches the amygdala via the thalamus and cerebral cortex (or directly in the case of the olfactory system) (e.g. [25,30,34,46]). A simple model of fear conditioning postulates that at least some conditioning stimuli (e.g. a tone) are paired with noxious somatosensory information (the unconditioned stimulus) in the lateral amygdalar nucleus [45], which modulates CEAm output via a relay through the basal amygdalar nuclei [38,40,42,47]. The neurotransmitter(s) in these amygdalar inputs to the CEAm is not certain although glutamate is likely because basal nuclei form asymmetric synapses on CEAm neurons in the cat [38], there is little GABA or neuropeptides [32], and there is some evidence for glutamate in certain other amygdalar projections [14].

In contrast, the tiny but very distinct CEAl provides a highly specialized input to the CEAm. First, anatomical and physiological evidence suggests that it preferentially receives noxious (and perhaps viscerosensory) information from the PB and rostral insular cortex. The lateral PB (central lateral and outer external lateral areas) receives inputs from spinal cord lamina I [6,11] and the caudal medial nucleus of the solitary tract [17], and $\approx 80\%$ of the neurons in this region of the PB respond to somatic and/or visceral noxious stimuli [8]. Furthermore, neurons in this region of the PB that have been antidromically activated from the CEAl respond to noxious stimuli [5], as do neurons in the CEAl itself [7]. Thus, it seems likely that the CEAl transmits noxious information to the CEAm. In addition, the same region of the PB sends a heavy projection to the CEAc which in turn may also influence the CEAm output (Introduction). Interestingly, the CEAl projects back to the the PB, including parts that it receives inputs from. This projection to the PB contains CRH, neurotensin, and somatostatin [35], and may constitute a feedback loop in the circuit.

A second interesting feature of the CEAl to CEAm projection is that it may contain GABA and a variety of neuropeptides. The CEAl contains by far the highest proportion of GABAergic and/or peptidergic neurons in the amygdala [32,51], and it has been suggested that the very low spontaneous firing rate of CEAm neurons [39] is due to tonic inhibition via local GABAergic neurons [37,53]. Our results suggest that the CEAl provides at least one major local source of GABAergic input to the CEAm. Thus, the evidence to date suggests the possibility that the CEAm receives a predominantly excitatory input relaying all types of sensory information from most of the amygdala, whereas it receives predominantly noxious information via an inhibitory input from the CEAl.

The most intriguing feature of the CEAl to CEAm projection is its content of neuropeptides (Introduction), although the extent to which GABA and particular neuropeptides are co-expressed in individual CEAl neurons is not yet clear (some CRH/neurotensin co-localization has been reported [49]). Nevertheless, it has been shown that circulating corticosterone produces a dose-dependent increase in CRH and neurotensin mRNA levels in the CEAl [28,58,62]. This raises the possibility that the stress associated with fear conditioning produces reversible, relatively long-lasting changes in the synthesis of neuropeptides in CEAl neurons, which could act as a 'gain control' for the long-term modulation (LTM) of CEAm output initiated by the rest of the amygdala. The magnitude and time course of such modulation or 'biochemical switching' first suggested for neurons expressing CRH and other peptides in the hypothalamic paraventricular nucleus [54,55,57], would depend on the pattern of changes in circulating corticosterone. The rate and time course of neuropeptide synthesis in the CEAl, and the electrophysiological effects of CRH, neurotensin, enkephalin, and somatostatin on CEAm neurons, need to be examined in detail.

Finally, the CEAl shares bidirectional connections with the BST ([51,52], and present results), specifically with regions where CRH mRNA is regulated by circulating corticosterone (i.e. oval and fusiform nuclei; [29,62]). Furthermore, the oval nucleus receives a dense input from the same region of the PB that projects to the CEAl [1]. Thus, the CEAl and the oval and fusiform nuclei of the BST appear to form part of a corticosterone-sensitive subsystem involved in the regulation of CEAm output. In addition, the BST part of this subsystem receives a selective input from a region of the lateral hypothalamic area involved in specific motivated behavioral and/or homeostatic responses (related to hypovolemic thirst and/or body water regulation; [21] and personal communication from A.G. Watts).

In summary, the CEAl forms part of a circuit that appears to modulate the output of amygdalar conditioned fear circuitry, with the magnitude of this modulation determined by nociceptive information, adrenal steroid levels, and inputs from the hypothalamus. However, this hypothesis must now be tested physiologically, specifically to determine the influence of CEAl projections on CEAm neurons.

Acknowledgements

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