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Research Report

Neurotrophic factors in the central nucleus of amygdala may be organized to provide substrates for associative learning

Khristofor Agassandian, Matthew Gedney, Martin D. Cassell*

Department of Anatomy and Cell Biology, The University of Iowa, 51, Newton Road, BSB, Iowa City, IA 52245, USA

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ABSTRACT

The central nucleus of amygdala was examined to identify the ultrastructural distribution of neurotrophins responsible for the complex of neuronal signaling processes which regulate synaptic transmission and neuronal plasticity, and possibly underlie memory formation. We investigated at the electron microscopic level the cellular organization of brain-derived neurotrophic factor (BDNF) and its receptor, tyrosine kinase receptor B (TrkB), in the extended amygdala (CE). We also investigated the interaction between cortical inputs to CE and BDNF and TrkB. Our results indicate the presence of pro-BDNF and BDNF in terminals in the CE which show a strong association with immunoreactive postsynaptic densities. TrkB receptor immunoreactivity was localized to postsynaptic densities of asymmetric synapses on dendrites and dendritic spines. Cortical terminals formed asymmetric synapses with dendritic shafts and spines, but were not BDNF immunoreactive. TrkB receptors were observed opposed to cortical terminals. These data also suggest that one potential substrate for associative learning may be the interaction of different cortical inputs with neurotrophin-containing terminals ending on dendritic spines and other neuronal structures of CE.

1. Introduction

Current models of the amygdala's role in associative learning generally propose that the key sensory–sensory interactive events, and the establishment of the permanent link between conditioned (CS) and unconditioned (US) stimuli occurs in the lateral and basolateral (L/BL) nuclei (Cardinal et al., 2002; Ledoux, 2000; Sah et al., 2003). Interactions between modality-specific cortical and thalamic inputs to the L/BL are thought to provide the anatomical basis for associative learning (Cardinal et al., 2002; Rogan et al., 1997). The acquisition and expression of conditioned responses (CR) in turn are thought to be mediated by co-opting preexisting connections between L/BL and the central nucleus component of the extended amygdala (CE) (Savander et al., 1997). However, it has been suggested that the L/BL is not the site where "memories" of CS-US association are stored during fear conditioning, but instead the L/BL may modulate the consolidation of such associations in other brain structures (Cahill et al., 1999). Further, physiological evidence suggests that plasticity in *both* the lateral nucleus and CE is necessary for acquisition of conditioned fear (Pare et al., 2004).

The CE is generally considered to be the output nucleus of the amygdala (Pitkanen et al., 2003) and is targeted by L/BL projections (Savander et al., 1997). However, the CE itself

* *Corresponding author*. Fax: +1 319 335 7198.
E-mail address: khristofor-agassandian@uiowa.edu (K. Agassandian).

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receives inputs from gustatory and visceral regions of the insular cortex (Shi and Cassell, 1998), the ventromedial and posterior thalamus (Aoki et al., 2000; Drake et al., 1999; Linke et al., 2000), and the parabrachial area (Bernard et al., 1993; Cassell et al., 1999), suggesting that it has direct afferent systems capable of supporting sensory–sensory associations. The lateral CE shares many features with the striatum, including non-reciprocated cortical inputs, medium-sized spiny neurons containing GABA, and a dense dopaminergic innervation (Cassell et al., 1999). Dendritic spines on striatal neurons are thought to be a possible substrate for synaptic changes associated with stimulus–response (S–R), or procedural, learning (Gubellini et al., 2004). Similar structures in the CE may act as a substrate for CS–CR learning or simple S–R learning.

Learning and memory substantially depend on the level of brain-derived neurotrophic factor (BDNF). In hippocampal neurons, BDNF appears to be moved through a regulated pathway that secretes it in response to neuronal activity (Farhadi et al., 2000; Goodman et al., 1996). BDNF easily distributes through a cell body to its branching structures, i.e. axons and dendrites, which make contact with neighboring neurons, and plays a role in episodic memory in humans. Furthermore, BDNF can be secreted in small amounts near the cell body through the constitutive pathway (Egan et al., 2003). Long-term memory may be mediated by protein synthesis-dependent, late-phase long-term potentiation (L-LTP) and the conversion of precursor pro-BDNF to the mature BDNF is critical for L-LTP expression in mouse hippocampus (Pang et al., 2004). Pro-BDNF mutants and wild-type (non-transgenic) pro-BDNF animals display the same TrkB pattern and pro-BDNF phosphorylates the TrkB receptor (Fayard et al., 2005). Of direct relevance, BDNF and TrkB interactions change within the basolateral amygdala 2 h after fear conditioning, whereas levels of several other trophic factors do not as it does in hippocampus-dependent functions (Rattiner et al., 2004). This is a clear demonstration that BDNF may have a similar role in amygdala-dependent learning and memory.

BDNF immunoreactivity (IR) has been identified in the BL, medial, and basomedial amygdaloid nuclei, which contain many immunoreactive cell bodies but very few immunoreactive fibers. In contrast, the central nucleus of amygdala (especially the lateral subdivision) contains no BDNF-immunoreactive cells but does contain high densities of immunolabeled fibers, which often form pericellular baskets around perikarya (Conner et al., 1997; Meredith et al., 2002). Pro-BDNF immunoreactivity has been reported in the CE and medial amygdaloid nucleus using light microscopic methods (Zhou et al., 2004). In view of this fact, and the fact that BDNF and its receptor TrkB play a critical role in activity-dependent synaptic plasticity (Patterson et al., 1996) and have been implicated as mediators of amygdala-dependent learning and memory, we investigated at the electron microscopic level the distribution of pro-BDNF, BDNF, and receptor TrkB in the CE and intercalated cell mass (ICM) and their relationship to cortical inputs. These morphological data could provide evidence that the cellular substrates of associative learning are present in the central nucleus.

2. Results

2.1. *Light microscopy*

At the light microscopic level, BDNF and TrkB IR were observed in all subdivisions of the amygdala (Figs. 1, 2). The greatest density of BDNF IR was found in the lateral CE, the intercalated cell masses, and BL, whereas in the lateral part of the amygdala, the reaction product was much less dense and more dispersed (Fig. 1). BDNF IR was seen mainly in neurons in BL, but diffuse terminal-like fields were found in CE and ICM.

TrkB IR was mostly concentrated in CE and ICM. The lateral and basolateral nuclei also showed a lower density of reaction product (Fig. 2) similar to that found in the lateral part of the amygdala after BDNF processing (Fig. 1).

In contrast to previous studies (Zhou et al., 2004), the distribution of pro-BDNF did not show IR dominant in any particular subdivision of the amygdala though punctuate IR was densest in the CE, and numerous perikarya with IR mainly in the nucleus were observed in BL.

2.2. *Electron microscopy*

BDNF IR was found in cell bodies only in BL (Fig. 3A). However, the dendrites, dendritic spines of CE neurons, and axon terminals contacting dendrites and spines in both BL and CE were immunoreactive (Figs. 3B–E). Some IR terminals were observed in the vicinity of perikarya of CE neurons, but contacts were never found.

In CE, TrkB IR (Fig. 4) was concentrated in neurons (particularly the nuclei), dendrites, dendritic spines, and axon terminals. The major concentration of BDNF and TrkB IR in dendrites and dendritic spines in CE was associated with postsynaptic densities (PSD). Immunoreactive PSDs were more electron dense and larger than non-IR PSDs (Figs. 3D,

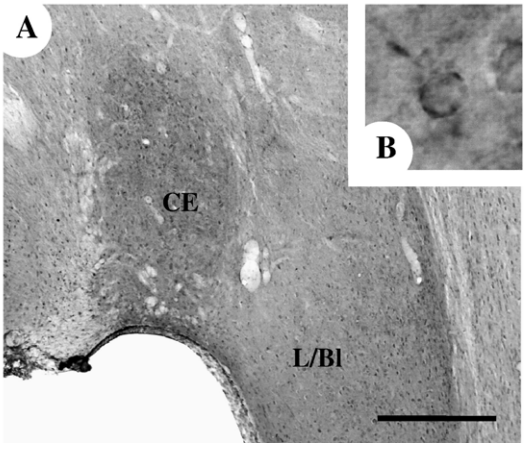

Fig. 1 – (A) Horizontal section of amygdala processed for BDNF immunohistochemistry. (B) High power view of neuronal soma surrounded with BDNF immunoreactive axon terminals in CE. L/BL—lateral and basolateral subdivisions of amygdala; CE—central extended amygdala. Scale bar: 0.5 mm.