

**Electron microscopic study of expression of Brain derived neurotrophic factor (BDNF), Tyrosine Kinase Receptor B (TrkB) and pro-BDNF in the central nucleus of amygdala.** *K. Agassandian and M.D. Cassell.*

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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 CS-US link occur in the lateral/basolateral nuclei (L/BL). The acquisition of (conditioned) responses is thought to be mediated by co-opting preexisting connections between L/BL and the central nucleus component of extended amygdala (CeA) [1, 2]. However, the lateral division of the CeA and the intercalated cell masses (ICM) of its surrounding shell region, all of which contain densely spinous, striatal-like neurons, also receive direct sensory input from cortex and thalamus, suggesting some associative learning might occur in these output structures as well. BDNF mRNA expression is induced in the BL 2 hr after fear conditioning, whereas levels of several other trophic factors do not change. This demonstrates that BDNF may have a key role in amygdala-dependent learning and memory [3]. We have described in rats the branching pattern of layer V corticoamygdaloid neurons in the gustatory insular (IC) and temporal (Te2/3) cortices by reconstructing axons of BDA-filled neurons [4]. To study a possible interaction between these cortical inputs and BDNF, its receptor TrkB, and pro-BDNF, we have studied the cellular localization of these components in the CeA, L/BL and ICM.

Rat brains were fixed with 0.5% glutaraldehyde and 2% paraformaldehyde by perfusion and post-fixed with the same fixative for 12 h. Then, vibratome brain sections were processed for immunocytochemistry. Adjacent sections were taken for light (LM) and electron (EM) microscopic investigation. Sections for LM were mounted, stained with neutral red, observed and photographed with a digital camera attached to the Nikon microscope. Tissue used for EM was osmicated with 1% OsO<sub>4</sub> aquatic solution, dehydrated in alcohol and acetone series and embedded in Epon-812 mixture. Ultrathin sections were counterstained with uranyl acetate and lead citrate and examined with JEOL 1230 TEM. Images were recorded on a digital camera attached to the electron microscope.

In all areas (CeA, L/BL and ICM) we saw the accumulation of BDNF and TrkB in dendritic spines, dendrites of various diameters and the perikarya of neurons. We found that, in contrast to BDNF and TrkB experiments, pro-BDNF is expressed in dendritic spines, the bodies of neurons, especially in their nuclei. The common features of BDNF, pro-BDNF and TrkB immunocytochemistry are the similar distribution of the product of reaction in CeA, ICM and L/BL, and its major concentration in all postsynaptic densities of immunopositive structures. Very often immunopositive dendrites and spines synapsed with immunopositive axon terminals. Since we found BDNF in CeA, L/BL, and ICM, we suggest that a substrate for associative learning may be the interaction of different cortical inputs with described above immunopositive neuronal structures of CeA, ICM and L/BL. Future EM investigations of the interaction between cortical inputs and BDNF, TrkB and pro-BDNF immunopositive structures in amygdala will throw light on understanding of amygdala-dependent learning and memory.

References:

- [1] Davis, M. *J Clin. Neurophysiol* 15.5 (1998): 378-87.  
[2] Fendt, M. and M. S. Fanselow. *Neurosci Biobehav. Rev.* 23.5 (1999): 743-60.  
[3] Rattiner, L. M. et al. *J Neurosci* 24.20 (2004): 4796-806.  
[4] Agassandian, K. et al., *Faseb Journal* 18.4 (2004): A343.

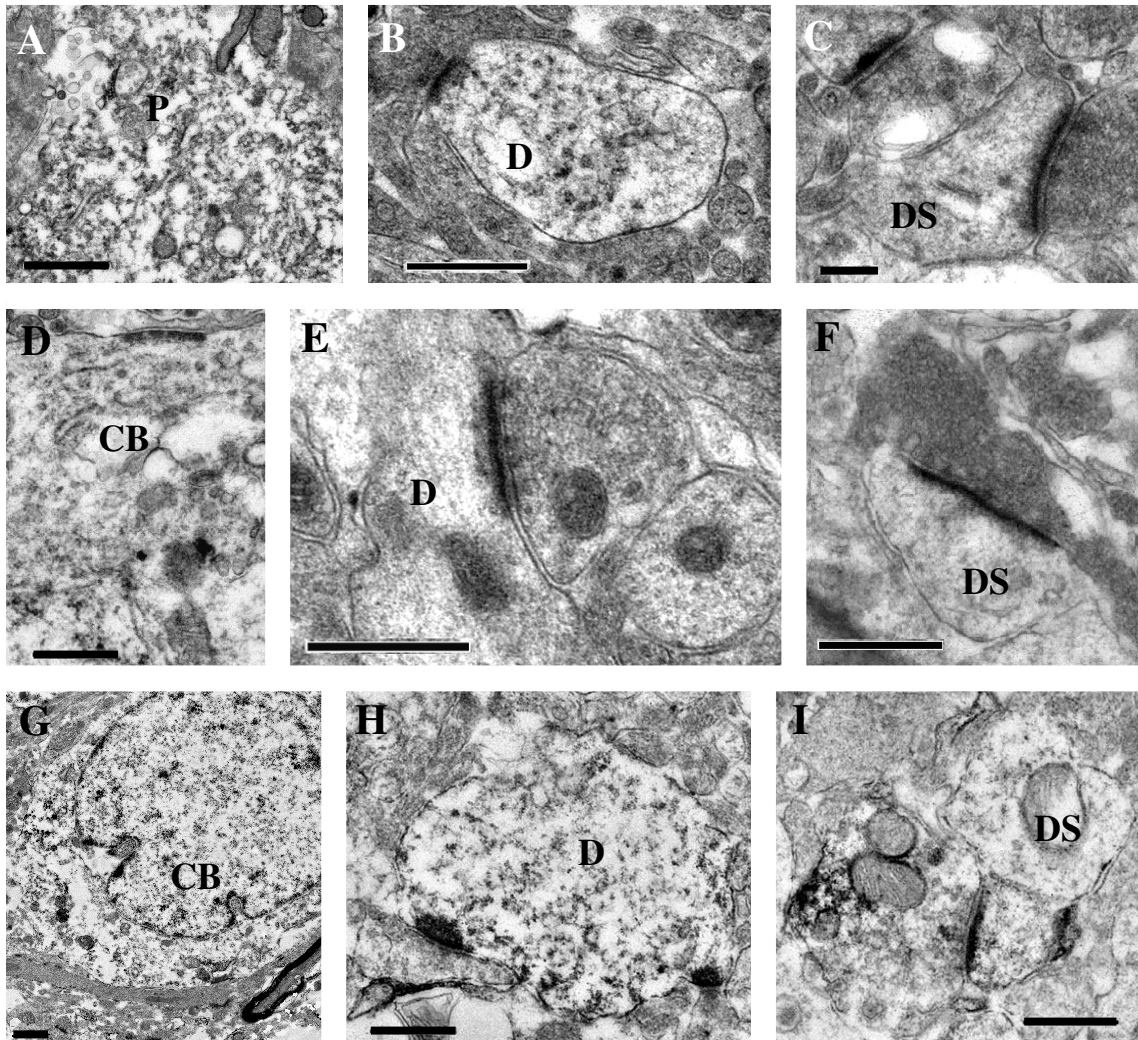


Figure 1. Central nucleus of amygdala.

BDNF-positive perikaryon of a neuron (A), dendrite (B) and dendritic spine (C);

Pro-BDNF-positive neuron (D), dendrite (E) and dendritic spine (F);

TrkB-positive neuron (G), dendrite (H) and dendritic spine (I).

D – dendrite, DS – dendritic spine, CB – cell body, P- perikaryon.

Scale bar: 1  $\mu$ m for A & G, 200 nm for C, 0.5  $\mu$ m B, D, E, F, H & I.