Subnuclear neuroarchitecture of dopaminergic afferents in the mouse central amygdala

Mirjam Richard[1], Angelika Schmitt-Böhrer[2], Philip Tovote[3], Esther Asan[1]

[1]Institute of Anatomy and Cell Biology, University of Würzburg; [2]Center of Mental Health, Department of Psychiatry, Psychosomatics and Psychotherapy, University Hospital Würzburg; [3]Defense Circuits Lab, Systems Neurobiology, Institute of Clinical Neurobiology, University Hospital Würzburg

Introduction

The central nucleus of the amygdala (Ce) is composed of subnuclei with distinct hodological and functional features. Plasticity of microcircuits formed by GABAergic neurons differentially (co-)expressing various neuropeptides underlies Ce functions in emotional behavior. Midbrain dopaminergic (DA) neurons provide a dense innervation of the Ce. Subpopulations of these DA neurons coexpress glutamatergic markers and/or peptides such as vasoactive intestinal polypeptide (VIP)[1,2], and optogenetic studies in mice suggest that DA/glutamate cotransmission mediates Ce microcircuit plasticity via differential effects on specific peptidergic, particularly somatostatin (SOM) producing neurons[3]. In order to assess the structural bases for DA-mediated effects and for possible corelease of glutamate and/or peptides, the present study aims to provide a detailed analysis of the subnuclear neuroarchitecture of DA afferents in the mouse Ce.

Results I

Heterogeneous distribution of dopaminergic afferent plexus delineates Ce subnuclear subzones

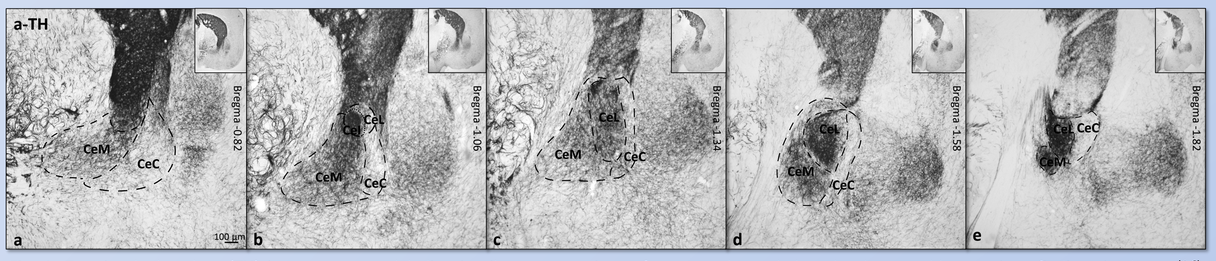

Fig. 1: Tyrosine hydroxylase (TH) immunoenzyme labeling in vibratome sections of mouse telencephalon closely resembles previous findings in the rat[(4-6)], indicating that intense TH-labeling predominantly documents DA terminal fibers. TH-immunoreactions on serial sections (from Bregma level -0.82 to -1.82) show heterogeneous distribution of DA Ce afferents within subnuclei, indicating a further subzonation particularly of the caudal lateral Ce (CeL). Densest plexus are found in intermediate (CeI) and medial lateral CeL at caudal levels, scarcest plexus in the capsular Ce (CeC).

Light microscopic evidence indicates differential colocalization of VIP in dopaminergic Ce afferents

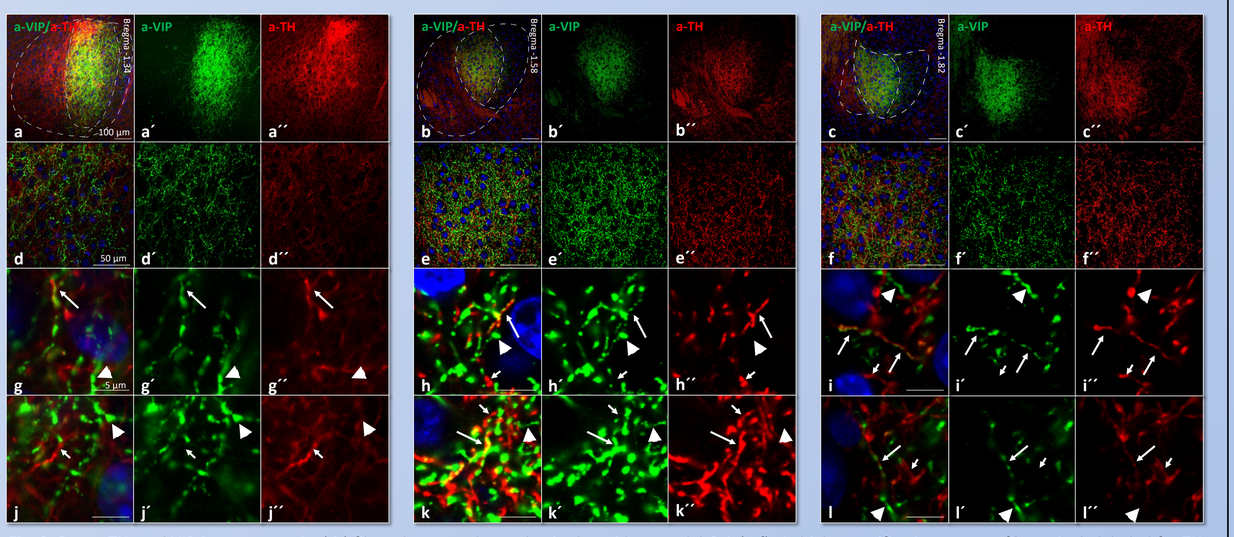

Fig. 2: Dense TH- and VIP-immunoreactive(-ir) fiber plexus partly overlap in the mid-to-caudal CeL (a-f). At high magnification, narrow fibers single labeled for TH (small arrows), varicose VIP-ir fibers (arrowheads), and fibers colocalizing TH- and VIP-immunoreactivity (arrows) are found (g-l). The proportion of dual labeled fibers increases from rostral to caudal.

Dopaminergic terminal plexus partially overlap with dense clusters of vGlut2-immunoreactive boutons

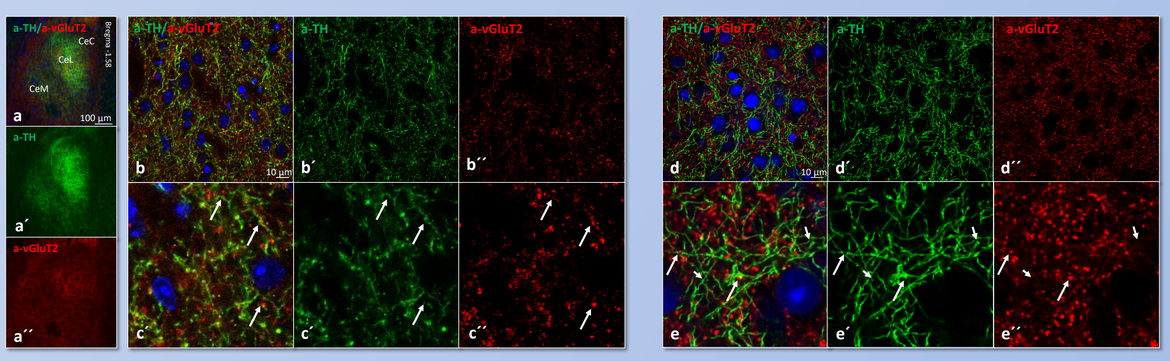

Fig. 3: Immunolabeling for TH and vesicular glutamate transporter 2 (vGluT2) shows vGluT2-ir boutons in close association with TH-ir fibers (large arrows in c,e) in the central CeL in analyses of sections reacted without antigen retrieval (a-c), although comparatively low signal-to-noise ratio renders detection of fibers and boutons difficult. Antigen retrieval treatment (d,e) leads to enhanced detectability of TH- and vGluT2-ir fibers and boutons, respectively. Associations are also seen (e, large arrows), however, numerous stretches of narrow TH-ir fibers lack associated vGlut2-boutons (e, small arrows).

Conclusions and Outlook I

- **Dopaminergic afferents differentially innervate Ce subnuclei along the rostrocaudal axis, innervation patterns provide evidence for a further subzonation of the mouse Ce**
- **Confocal analysis of TH/VIP dual immunolabeling indicates that a subpopulation of DA afferents particularly in the mid-to-caudal medial CeL derives from midbrain DA neurons expressing VIP**
- **Observation of an occasional association of vGluT2-ir boutons with CeL TH-ir axons indicates possible DA/glutamate cotransmission in a subpopulation of DA afferents similar to what has been reported for the Ncl. accumbens[7]**

➢ **Mapping and tracing studies have to be done to further assess (co)localization patterns and to elucidate specific CeL subnuclear distribution/targets and origins, respectively, of different DA fiber types**

Methods

Experiments were carried out on serial vibratome sections from perfusion-fixed brains of male adult C57BL/6 mice. Single/dual immunolabeling for light and fluorescence/confocal microscopy, immunoenzyme and immunogold labeling for electron microscopy were done using commercially available, well characterized antibodies and analysed using established methods[4-6,8]. For confocal analysis, antigen retrieval was carried out by incubating sections in citrate buffer (pH = 8,45) at 90°C for 30 minutes. Controls and cross controls (for dual labeling) without primary antibodies did not show any specific staining.

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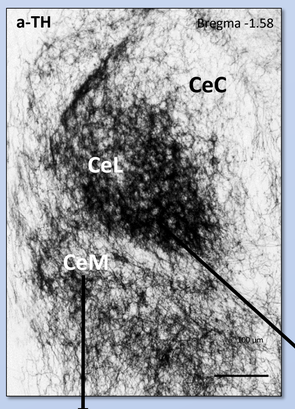
Results II

Ce neurons possess distinct ultrastructural features in different Ce subnuclei



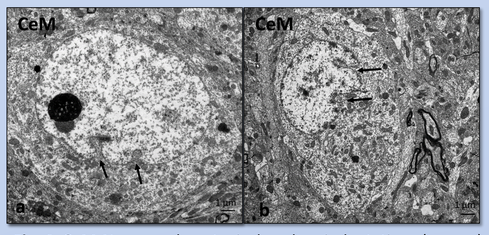


Fig. 4: CeM-Neurons show typical nuclear indentations (arrows)

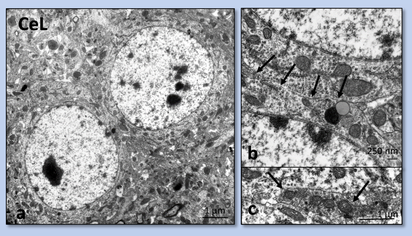


Fig. 5: CeL-Neurons with typical round nuclei (a), extensive somatic contacts and perisomatic boutons (b, c, arrows)

Dopaminergic terminal axons form multiple contacts on a subpopulation of neuronal cell bodies particularly in the CeL

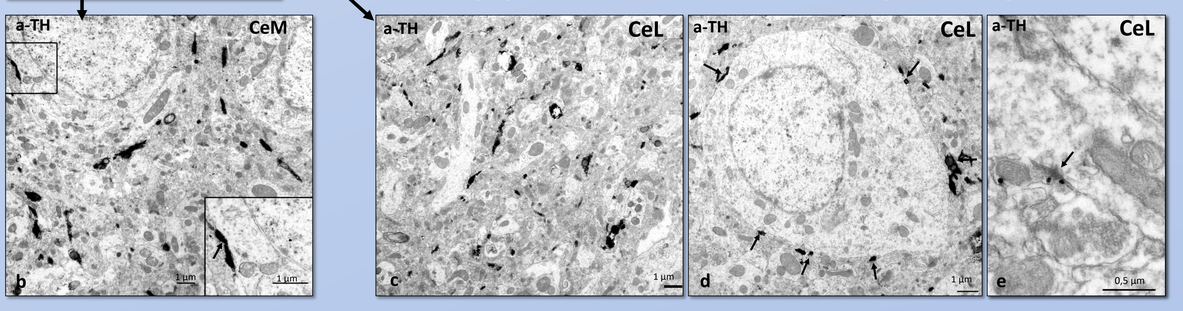


Fig. 6: Preembedding immunoenzyme (a-d) and immunogold electronmicroscopic (e) labelings show numerous narrow TH-ir dopaminergic axons and terminals in CeM (a,b) and, particularly, in the densely innervated CeL (a, c-e), forming multiple perisomatic contacts (d, arrows) with small symmetric synapses (e, arrow) on select cell bodies.

Dopaminergic afferents form symmetric and asymmetric synapses and contain dense core vesicles

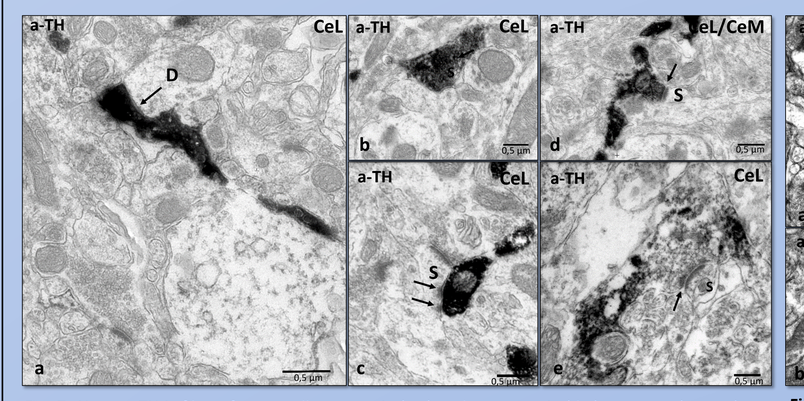


Fig. 7: Narrow TH-ir fibers form small symmetric (a-c) and asymmetric (d,e) synapses (arrows) on spines (S) and dendrites (D) in the CeL and CeM. At asymmetric synapses , TH-immunoreaction product occasionally appears excluded from the presynaptic bouton (e), indicating axonal subcompartimentation.

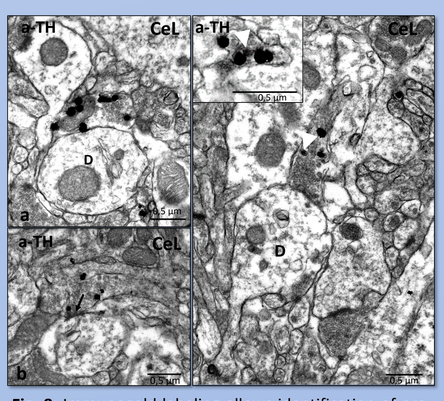


Fig. 8: Immunogold labeling allows identification of pre-synaptic vesicle pools (arrow in b). Dense core vesicles arrowheads) indicate presence of peptides in some labeled DA axons.

Results III

Ce dopaminergic afferents differentially target subpopulations of peptidergic neurons

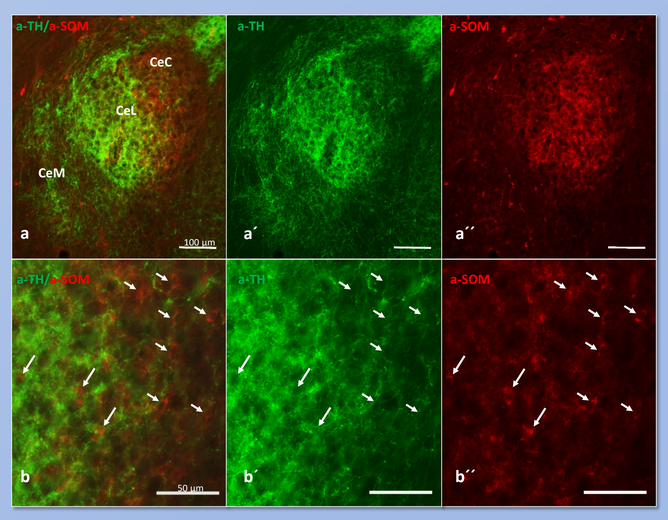


Fig. 9: Dense TH-ir fiber plexus only partly overlap with SOM-ir neuron clusters in the medial CeL (a). SOM-ir cell bodies are detectable at higher magnification in the central CeL (b). While some are localized among the dense DA-afferent plexus and appear to be contacted by small TH-ir boutons (large arrows), many SOM-ir cell bodies are found in the lateral CeL and lack significant perisomatic contacts (small arrows).

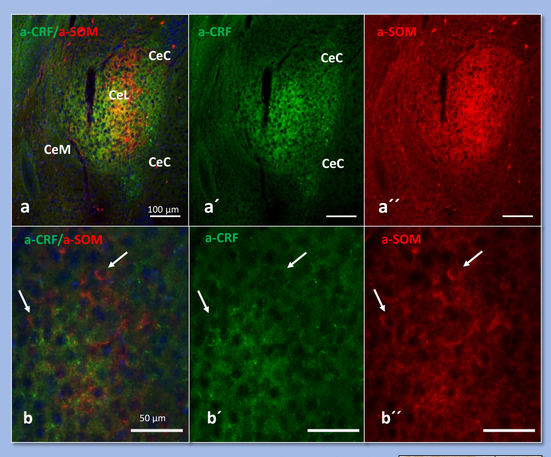
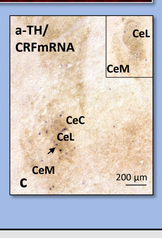


Fig. 10: Corticotropin releasing factor (CRF)-ir elements partly overlap with SOM-ir cell clusters in the medial CeL (a,b), coinciding with TH-ir fiber plexus in this localization (cf. 2b, 9a), confirming previous findings in dual in situ hybridization/ immunolabelings (c)[6]. CRF-ir cell bodies, in contrast to SOM-ir ones (arrows in b), cannot be easily discerned in immunolabelings.



Conclusions and Outlook II

- **DA afferents form symmetric and asymmetric synapses on CeL and CeM neurons displaying characteristic ultrastructural features**
- **Presence of dense core vesicles, formation of asymmetric synapses and evidence for axonal subcompartments support LM and functional findings of peptide content and glutamate cotransmission in CeL DA afferents**
- **SOM- and CRF neuron clusters partly overlap in the mid-to caudal CeL; densest plexus of DA afferents coincide with CRF-neuron clusters whereas SOM-neurons appear differentially contacted.**

➢ **Dual immunoelectron microscopy is needed to confirm peptide/vGlut2-presence in DA afferents and to analyse contacts between different types of DA afferents and identified target neurons**

Literature

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Contact: mirjam.richard@stud-mail.uni-wuerzburg.de