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Projections from the Central Amygdaloid Nucleus to the Mesencephalic Trigeminal Nucleus in the Rat

K. G. Usunoff, **, ***, N. E. Lazarov*, **, D. E. Itzev**, O. Schmitt***, A. Rolfs****, A. Wree****

**Department of Anatomy and Histology, Medical University, Sofia, Bulgaria*
***Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
****Institute of Anatomy, University of Rostock, Rostock, Germany*
*****Department of Neurology, University of Rostock, Rostock, Germany*

The efferent connections of the central amygdaloid nucleus (AmCe) to the mesencephalic trigeminal nucleus (Me5) were investigated in rats. High molecular weight (10 000 mw) biotinylated dextran amine (BDA 10k), an established anterograde axonal tracer, was stereotaxically injected in the AmCe. The AmCe axons, labelled with BDA 10k ascend from the injection focus and follow two routes. The main axonal stream runs in the stria terminalis, and a smaller fiber component builds the ventral amygdalofugal pathway. From the latter deviate the axons descending in the brainstem. After the innervation of the lateral portion of substantia nigra, the labelled axons take a dorsomedial course in the mesencephalic tegmentum and reach the periaqueductal gray, innervating also the rostral (mesencephalic) portion of Me5. The axons followed to the pons descend ventrally to the motor trigeminal nucleus and bend dorsally towards the dorsolateral pons. This bundle terminates with dense axonal arborizations in the parabrachial nuclear complex and in the caudal (pontine) portion of Me5. It appears that both the pseudounipolar and multipolar neurons receive axons from AmCe. The present data indicate that the amygdala, a key structure of the limbic system, is also strongly involved in proprioception.

Key words: anterograde axonal tracing, biotinylated dextran amine, limbic system, orofacial proprioception.

Introduction

The mesencephalic trigeminal nucleus (Me5) is a unique structure in the CNS, mainly composed of pseudounipolar primary sensory neurons. In the rostral pons Me5 neurons are located in the triangle between the locus coeruleus and the medial parabrachial nucleus, and in the mesencephalon they border laterally the periaqueductal gray [5, 13]. Mesencephalic trigeminal neurons innervate the masticatory muscles, the periodontal

ligament and a subset of the extraocular muscles [1]. Unlike the "ordinary" pseudounipolar neurons in the sensory ganglia, the mesencephalic trigeminal neurons display axosomatic synaptic contacts [5, 7, 8]. The Me5 receives afferent connections almost exclusively from the brainstem structures [reviewed in 5, 6].

We presently report an afferent projection to the Me5 from a key structure of the limbic system – the amygdaloid nuclear complex, studied by anterograde axonal tracing.

Material and Methods

Ten adult Wistar rats weighing 220-260 g were used. The animals were anesthetized with Thiopental and then mounted in David Kopf stereotaxic apparatus in the flat skull position. Stereotaxic coordinates of the central amygdaloid nucleus (AmCe) were obtained from the atlas of P a x i n o s and W a t s o n [10]. Under aseptic conditions small craniotomies were performed. In the AmCe 0.25-0.5 μl biotynilated dextran amine (BDA;10%, 10.000 mw; Molecular Probes Europe BV, Leiden, The Netherlands) dissolved in phosphate buffer (PB, 0.1M, pH 7.2) was injected with a Hamilton microsyringe (Hamilton Co, Reno Nevada, USA) using a dorsal approach. At the end of the injection, the injection canula was held in place for 15 min to insure that the injected tracer had been absorbed into the tissue. After survival time of 8-21 days, the rats were deeply re-anaesthetized and perfused transcardially with phosphate buffer saline (PBS), followed by 500 ml of 4% paraformaldehyde in PB. The removed brains were postfixed overnight in the same fixative, blocked in the coronal plane and soaked in 0.5% paraformaldehyde in PB containing 20% sucrose at 4°C. Serial sections were cut at a thickness of 40 μm on Reichert Jung freezing microtome, collected in a free-floating state in PB and then processed for tracer histochemistry. A commercial avidin-biotin-HRP complex (ABC) kit was used to visualize BDA (Vectastain ABC Kit, Vector Laboratories Inc., Burlingame, USA). Briefly, the sections were preincubated in PB containing 0.1% bovine albumin (fraction V; Sigma Chemical Co, St. Louis, USA) for 20 min, and rinsed in PB for 30 min. Subsequently they were incubated in the avidin-coupled biotynilated HRP solution for 45-60 min, and rinsed again in PB for 30 min. The reaction product was developed with 0.06% 3,3'-diaminobenzidine (Sigma Chemical Co, St. Louis, USA) and 0.02% H_2O_2 in Tris buffer (0.05 M, pH 7.6) for 10-15 min in the dark. The sections were then rinsed in distilled water, mounted on chrome alum gelatin coated slides and air dried overnight. Finally, the sections were examined in Zeiss Axioplan 2 microscope and selected areas were taken with AxioCam MRc digital camera. The results from the present experiments on the amygdaloid projections to the forebrain were presented in our previous study [15].

Results

In all examined cases the injection site involved the AmCe (Fig. 1**A**). By the five cases, in which a minute injection of 0.25 μl BDA was performed, the injection foci were completely selective, e.g. there was no spillage of the tracer upon surrounding structures: intercalated cell masses of the amygdala (ventrally), basolateral and lateral nuclei of the amygdala (laterally), medial nucleus of the amygdala and optic tract (medially), and neostriatum (dorsally). By the five animals, in which a larger quantity of BDA was delivered (0.5 μl), in three cases selective injection foci were present (Fig. 1**A**), and in the remaining two cases there was a minimal spillage of the tracer along the most ventral part of the cannula track, upon the most ventral portion of the amygdalostriatal transition area and globus pallidus. Despite the minimal contamination, these two cases were excluded from systematic examination.

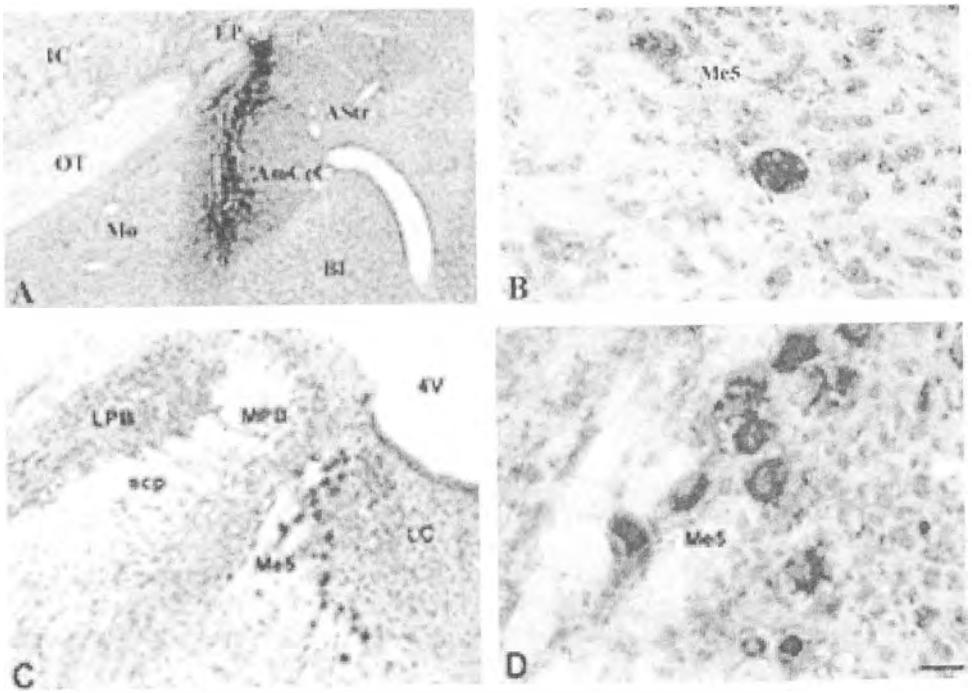

Fig. 1. **A** – A selective injection focus in the central amygdaloid nucleus (AmCe); **B** – BDA-labeled fibers and terminals in the rostral portion of Me5; **C** – Low-power view of the dorsolateral pons at the level of Me5; **D** – shows the dense field of BDA-labelled terminals in Me5. Scale bars = 100 μm (**A, C**); 50 μm (**B, D**)

The labelled efferent axons of the AmCe ascend from the injection site. Most fibers run in the major efferent bundle of the amygdala, the stria terminalis. A smaller number of labelled axons bend medially above the optic tract, a component of the ventral amygdalofugal pathway. The most caudally located labelled axons in the ventral amygdalofugal pathway descend to brainstem structures, and the first target is the lateral portion of substantia nigra. Afterwards, the labelled axons run dorsolaterally in the mesencephalic tegmentum towards the periaqueductal gray. Entering this area, the labelled axons run among the pseudounipolar neurons of the rostral portion of Me5 (Fig. 1**B**) that are located at the lateral border of the periaqueductal gray and innervate also the small multipolar neurons in the Me5. Further caudally the labelled axons proceed in the pontine tegmentum. They descend along the ventral border of the motor trigeminal nucleus and bend in dorsal direction towards the dorsolateral pons. The fibers run through the motor trigeminal nucleus, medially to it in the peritrigeminal nucleus, and laterally to it in the intertrigeminal nucleus. In the motor trigeminal nucleus most, if not all, labelled axons represent passing fibers, whereas in the peritrigeminal and intertrigeminal nucleus also discrete terminal bursts are present. Such are seen also in the supratrigeminal nucleus. In the dorsolateral pons dense terminal fields are present in the medial and lateral parabrachial nuclei and in the Me5, whilst no labelled axons enter the locus ceruleus (Fig. 1**C**). In the Me5 dense pericellular baskets surround the large pseudounipolar neurons (Fig. 1**D**), and the small multipolar neurons in this region are also contacted by amygdalofugal axons.

Discussion

The amygdala is relatively voluminous gray substance, located in the depth of the ventromedial temporal lobe, ventral to the caudolateral striatum and to the pallidum. It is a very complicated structure and consists of several nuclei, divided on the basis of cytoarchitectonic, hodological, histochemical and immunohistochemical studies [reviewed in 3]. The amygdala is involved in the modulation of neuroendocrine functions, visceral effector mechanisms, and in complex patterns of behavior: learning and memory, aggression and defense, pain modulation, reproduction, food intake, etc. [reviewed in 16]. The classical hodological studies, carried out by silver impregnation of degenerating axons described projections to certain basal telencephalic and hypothalamic structures but not to the brainstem [2, 9]. The introduction of modern, more sensitive techniques for tracing axonal connections led to the description of a much more extensive subcortical distribution of amygdaloid fibers [reviewed in 11]. H o p k i n s and H o l s t e g e [4] followed amygdaloid tracts to the caudal brainstem but did not describe a projection to the trigeminal nuclear complex. A projection to the caudal (pontine) portion of Me5 was noticed in the autoradiographic experiments of P o s t and M a i [12] and of P r i c e and A m a r a l [13]. The use of the most effective modern anterograde tracer enabled us to describe an unexpectedly strong projection from AmCe to the entire rostrocaudal extent of the Me5. The projection is so massive that the density of BDA labelled terminals in the Me5 rivals the density in the generally appreciated strong projection of the amygdala to the parabrachial nuclear complex (see Fig. 1**C**). On the other hand, we were unable to confirm the finding of P r i c e and A m a r a l [13] on a connection to the ventral part of locus ceruleus. Species differences (monkey versus rat) might explain this discrepancy. Our findings suggest that both neuronal types in Me5 receive an amygdaloid input. The projection to the pseudounipolar neurons is especially evident in the pontine part of the Me5, where the densely arranged mesencephalic trigeminal perikarya are surrounded by numerous labelled endings. The amygdaloid axons are also in contact with the small multipolar neurons located in close vicinity. The affiliation of latter neurons to Me5 is a matter of debate, but L a z a r o v [5, 6] provided firm evidence that the small multipolar neurons belong to the Me5, and represent GABAergic interneurons.

There is growing evidence that the amygdala is unexpectedly important subcortical nociceptive centre [16, 17]. The input of pain sensation is conducted by the spino (trigemino) – parabrachial – amygdaloid pathway, and we recently demonstrated that the amygdala receives a monosynaptic input from the dorsal horn of the spinal cord and from the spinal trigeminal nucleus [16, 17]. The present data suggest a further sensory involvement of the amygdala, e.g. a very strong monosynaptic influence over both the primary proprioceptive neurons of the Me5 and their interneurons.

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