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Published in:
Neuroscience Letters

DOI:
10.1016/0304-3940(85)90052-7

Neuroscience Letters, 57 (1985) 137–142
Elsevier Scientific Publishers Ireland Ltd.

NSL 03341

THE PATTERN OF CORTICAL PROJECTIONS FROM THE INTERMEDIATE PARTS OF THE MAGNOCELLULAR NUCLEUS BASALIS IN THE RAT DEMONSTRATED BY TRACING WITH *PHASEOLUS VULGARIS*–LEUCOAGGLUTININ

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(Received November 22nd, 1984; Revised version received March 9th, 1985; Accepted March 11th, 1985)

Key words: basal magnocellular nucleus – acetylcholinesterase – cortical projections – fiber pattern morphology – *Phaseolus vulgaris*–leucoagglutinin – rat

The pattern and distribution of the cortical projections from intermediate parts of the cholinergic basal magnocellular nucleus were studied by anterogradely transported *Phaseolus vulgaris*–leucoagglutinin. This immunocytochemical tracing technique reveals the detailed morphology and distibution of efferents from this intermediate area in the nucleus basalis to the various areas and layers of cortex and amygdala. Major projections with a relatively high density of terminal boutons were found in layers I, II and VI of the frontal cortex, in layers V and VI of parietal and temporal areas, in the entire perirhinal and entorhinal cortices, and in the basolateral nucleus of the amygdaloid body. From the nucleus basalis area studied, few if any projections could be demonstrated to cingulate and occipital cortical regions.

The nucleus basalis of Meynert in the human basal forebrain is a major source of cholinergic innervation of the cerebral cortex, and degeneration of this nucleus has been specifically linked to Alzheimer's disease [11, 14]. The basal magnocellular nucleus (nBM) in rodents and cats is considered homologous to the human nucleus [6, 12], therefore anatomical determination of its projection pattern has assumed great importance. Previous studies have employed retrograde tracing techniques alone, or in combination with histochemical demonstration of acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) [1, 7, 15], whereas anterograde transport studies were recently carried out by Saper [9], who employed the autoradiographic method. The results of most of these investigations strongly indicate that the nBM cholinergic cell group constitutes the subcortical origin of a cholinergic cortical innervation, but do not provide detailed information on the nBM projection pattern. Accordingly, we have used the recently introduced *Phaseolus vulgaris*–leucoagglutinin (PHA-L) anterograde tracing technique [3, 10] to obtain a detailed picture of the cortical and amygdaloid projections of the nBM. Particular interest was given to the distribution of fibers and terminal varicosities within the various cortical layers. Such

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information appears to be of great importance since it was recently shown that the fiber swellings in PHA-L-labeled material give the position of presynaptic boutons [16].

In 15 male Wistar rats, PHA-L (2.5% in Tris-buffered saline; TBS) was injected by iontophoretic delivery into a part of the nucleus basalis complex at stereotactic coordinates AP, 7.7–8.0; L, 2.6–3.8; V, 3.3–2.0, of Paxinos and Watson [8]. Compared to the topographic distribution of cholinergic somata of the nucleus basalis complex, the area of injection in this study coincides with the intermediate part of longitudinally oriented nBM as described by Bigl et al. [1]. For a considerable part, the cholinergic somata in the area of injection, also defined as ventral pallidum and substantia innominata [8], are identical to cholinergic cell group Ch 4 of Mesulam et al. [7]. For purposes of comparison, several brains of animals pretreated with diisopropylfluorophosphate (DFP) were sectioned and stained for AChE activity [1, 2, 4].

Seven days after injection of PHA-L, the animals were perfused transcardially with a short prerinse of saline followed by 0.5% paraformaldehyde–2.5% glutaraldehyde–4% sucrose in phosphate buffer. Transverse frozen sections (1:3) were treated according to the peroxidase–anti-peroxidase (PAP) method of Sternberger as described in detail elsewhere [10].

All PHA-L deposits in the area of injection produced extremely small tracer uptake areas with diameters of 300–400 μm, corresponding to the area in which cholinergic somata could be demonstrated by AChE staining and that are shown to be ChAT-positive as well [7].

Most ascending fibers originating from this part of the nBM reached the cortex via the ventral striatum, running in the external capsule and corpus callosum and penetrating the cortex from deep to superficial layers. The highest density of labeled fibers and terminal boutons were observed in the frontal cortex. Lesser, but still dense, innervation was seen in the perirhinal and entorhinal cortices (Fig. 1). From anterior to posterior, the rich innervation of the frontal cortex becomes gradually less abundant in the frontoparietal areas and is extremely scarce in the temporal and occipital cortices. The entire cingulate cortex also at prefrontal levels is devoid of labeling in the present injection cases. A reverse anterior–posterior pattern can be observed in the olfactory cortical areas. In the anterior primary olfactory (piriform) cortex, terminal labeling is sparse or even absent. More caudally, at the level of the amygdala, fibers are present in the piriform lobe and more posteriorly develop in a rich pattern of terminations in the entorhinal cortex. In the perirhinal cortex parallel to the rhinal fissure, the projection pattern is regular and present over its entire longitudinal extent.

Apart from the distribution over the entire cortex there also appears to be a pattern in fiber morphology and distribution of terminals over the different layers of the various cortical areas. Although anterograde labeling of both fibers and terminal boutons was found in all layers of the frontal cortex, a certain pattern of projection was nonetheless evident. In the deep multiform layer VI, fibers coursed in all directions but preponderantly parallel to the corpus callosum (Fig. 2). Terminal structures in

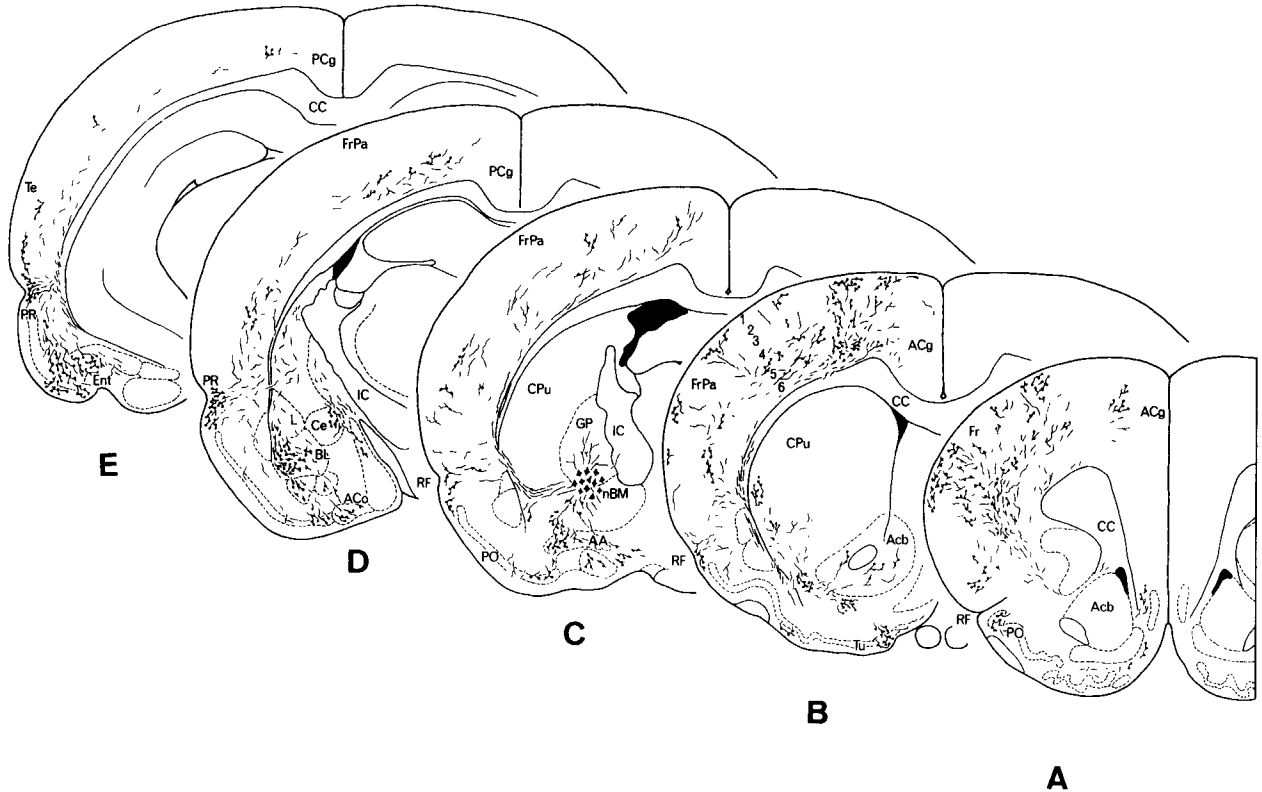

Fig. 1. Coronal sections through the brain from anterior (A) to posterior (E) in which are indicated the anterogradely labeled fibers and terminal boutons following a *Phaseolus vulgaris* lectin injection in the magnocellular nucleus basalis (C). Note that only the projections to the cortex and amygdala are depicted. AA, anterior amygdaloid area; Acb, nucleus accumbens; ACg, anterior cingulate cortex; ACo, anterior cortical amygdaloid nucleus; BL, basolateral amygdaloid nucleus; CC, corpus callosum; Ce, central amygdaloid nucleus; CPu, caudate-putamen; Fr, frontal cortex; FrPa, fronto-parietal cortex; GP, globus pallidus; IC, internal capsule; L, lateral amygdaloid nucleus; PCg, posterior cingulate cortex; PO, primary olfactory cortex; PR, perirhinal cortex; RF, rhinal fissure; Te, temporal cortex; Tu, olfactory tubercle.

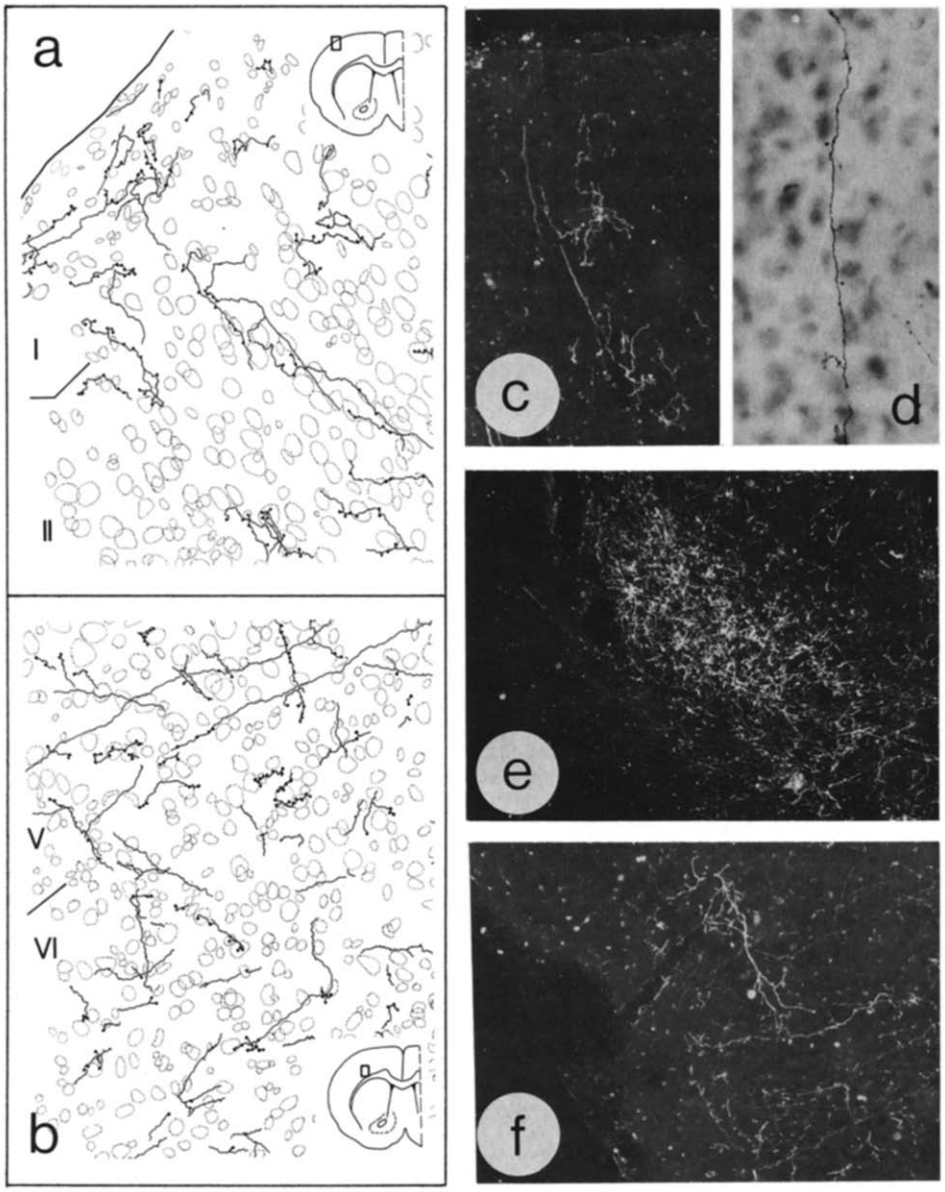

Fig. 2. a and b: camera lucida drawings of PHA-L anterogradely labeled nBM projections to layers I and II (a) and layers V and VI (b) of the fronto-parietal cortex (compare with Fig. 1B). c: dark-field photomicrograph of terminal efferent labeling in layer I and II after a PHA-L injection in the nBM. d: photomicrograph of a single PHA-L-labeled fiber in layer II to show the detail of morphology obtained with the PHA-L method. e: dark-field photomicrograph of a dense network of labeled projections in the basolateral amygdaloid nucleus after nBM injection of PHA-L. f: terminal labeling in the perirhinal cortex following nBM injections.

layer VI were seen more often in the form of closely packed clusters of boutons instead of the longer chains of boutons 'en passant'. The parallel pattern gradually changed to a radially oriented fiber pattern in layer V, where termination was slightly less abundant. In layers IV and III, fibers ran predominantly radial, perpendicular to the cortical surface. This configuration changed in layers II and I when more complex ramifications were evident. In layer I of the frontal cortex, a T-shape type of branching was obvious, resulting in a relatively large number of fibers that ran parallel to the pial surface (Fig. 2). The number of terminals in layers I and II of the frontal lobe was relatively high, especially compared to layers III and IV. This pattern of labeling for the frontal cortex did not apply to all other areas. In the fronto-parietal and temporal neocortices, labeling was generally restricted to layers V and VI, except for a conspicuous projection to the more superficial layers of the motor area of the frontal cortex (Fig. 1B, C). More posteriorly, the labeling was exclusively confined to the deeper layers.

The termination pattern in the various layers of perirhinal (PR) and entorhinal (Ent) areas was complex and less organized as compared to the neocortical projections. Fibers ran parallel and perpendicular to the surface, carrying boutons in all layers, especially in layer II. In the entorhinal cortex, however, fibers branched more abundantly, which gave the appearance of multiform, complex terminal fiber arrangements richly provided with terminal varicosities. Although the pattern of fibers in Ent appears to be very complex as compared to the perirhinal cortex, the density of terminations was highest in layer II, as well.

Apart from the projections of the nBM to the various cortical structures, a major limbic projection, to the amygdaloid body, was quite conspicious. Although all of the 'deep' amygdaloid nuclei received projections from the nBM, the basolateral nucleus was by far the major target for nBM efferents (Figs. 1D and 2).

The pattern of labeling in the various cortical fields and amygdaloid body was not identical in all cases, in spite of the fact that the injections were localized in a relatively small portion of the nBM. This confirms the data obtained with retrograde tracing methods that led to the conclusion that nBM cells close to each other may have considerably different projection patterns [1,6]. In our anterograde tracing experiments it became evident that all injections in the ventral pallidal–substantia innominata complex resulted in rich labeling of frontal cortical areas. It is clear, however, that the innervation of the amygdaloid body originates from cells in the substantia innominata, rather than from the more dorsally situated ventral pallidum. The cells of origin projecting to the entorhinal/perirhinal cortices were also found in the substantia innominata, much more than in the ventral pallidum.

The present findings on the efferent projections of the nBM to cortical and amygdaloid structures agree well with the results of the anterograde and retrograde transport, and the combined retrograde transport and (immuno-)histochemical studies cited earlier [1, 6, 12, 13, 15]. These studies also support the view that all cortical and amygdaloid projections from the nBM are cholinergic in nature, as does the finding that AChE and ChAT in the neocortex is significantly reduced after large nBM lesions [5, 13]. The projections described in the present report are therefore most likely cholinergic.

The advantage of anterograde transport techniques, and the PHA-L technique in

particular, over retrograde transport methods is the revealed detail of structure and distribution of nBM efferents and their presynaptic endings over the entire target structures. In the search for an animal model for degenerative changes that appear in pathological cases of Alzheimer's disease, such detail of information may prove to be essential for understanding (1) the functional role of the cholinergic basal nucleus–neocortical system, and (2) the consequences for target structure function following degeneration of the basal magnocellular nucleus. With respect to the latter questions, the knowledge on the position of presynaptic boutons is of great importance of correlating anatomical data on cholinergic input with data obtained in cholinergic receptor binding studies.

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