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Immunohistochemical study of the anatomical organization of the basal forebrain cholinergic system in the mouse brain

JULIO VILLALOBOS and VLADIMIR SALDARRIAGA

Departamento de Morfología, Facultad de Salud, Universidad del Valle, A.A. 25360 Cali, Colombia

The anatomical organization of the basal forebrain cholinergic system with emphasis on the basal magnocellular nucleus, was studied by means of the choline acetyltransferase immunohistochemistry. The basal forebrain cholinergic system extends from the medial septum to the caudal part of the globus pallidus, and is organized as a continuous valley of cholinergic neurons. The arrangement of choline acetyltransferase immunopositive neurons, allows to distinguish four regions in the basal magnocellular nucleus: an anterior part from the horizontal limb of the diagonal band of Broca to the anterior commissure, an intermediate part between the anterior commissure and the globus pallidus, subdivided into dorsal and ventral regions, and a posterior part is found in the caudal globus pallidus, where cholinergic neurons are exclusively dorsal.

Key words: *anatomic organization, basal forebrain cholinergic system, basal magnocellular nucleus, ChAT immunohistochemistry, mice*

GLOSSARY OF ABBREVIATIONS

ac: anterior commissure.
acb: nucleus accumbens.
BFCS: basal forebrain cholinergic system.
ChAT: choline acetyltransferase.
f: fornix.
GP: globus pallidus.
HBDB: horizontal limb of diagonal band of Broca.
ic: internal capsule.
LV: lateral ventricle.
MS: medial septum.
nBM: magnocellular basal nucleus.
nMPo: magnocellular preoptic nucleus.
SI: substantia innominata.
to: optic tract.
VBDB: vertical limb of diagonal band of Broca.
VP: ventral pallidum.
3V: third ventricle.

INTRODUCTION

The magnocellular basal nucleus (nBM) was first described in 1872 by Meynert, who called it the nucleus of the ansa peduncularis, because it was found to be crossed over by this pallidofugal fasciculus and for being composed of magnocellular neurons (Gorry, 1963). In recent years, it has been the center of attention of many studies, because of its involvement mainly in Alzheimer's disease (Whitehouse *et al*, 1982) and in the functional modulation of memory processes (Bartus *et al*, 1982; Jaffard and Micheau, 1994).

Correspondence to: Julio Villalobos, Departamento de Morfología, Facultad de Salud, Universidad del Valle, A.A. 25.360, Cali, Colombia. Phone: (57-2) 554-2492. Fax: (57-2) 554-2484. E-mail: jvt@mafalda.univalle.edu.co

The nBM is composed of a population of fusiform, triangular and multipolar neurons (Dinopoulos *et al*, 1988), which are spread throughout the basal telencephalon from the septal region to the caudal part of the globus pallidus (GP). The majority of these neurons are cholinergic (Mesulam *et al*, 1983a) and have been described in rats (Kimura *et al*, 1980; Sofroniew *et al*, 1982; Satoh *et al*, 1983; Mesulam *et al*, 1983a), raccoons (Bruckner *et al*, 1992), cats (Kimura *et al*, 1981), primates (Mesulam *et al*, 1983b, 1986) and humans (Hedreen *et al*, 1984; Mesulam and Geula, 1988). The anatomical organization of these neurons varies according to the species. They appear scattered in rodents (Mesulam *et al*, 1983a; Sofroniew *et al*, 1982), whereas in higher mammals these neurons appear arranged as cellular aggregations in the entire extension of the basal telencephalon (Mesulam *et al*, 1986; Hedreen *et al*, 1984; Mesulam and Geula 1988), being more numerous in the substantia innominata (SI) region.

The most accepted nomenclature, proposed by Mesulam *et al* (1983a), describes the basal forebrain cholinergic system (BFCS) as being composed of four cholinergic groups, from CH1 to CH4. The later group includes parts of the horizontal limb of the diagonal band of Broca (HBDB), the magnocellular preoptic nucleus (nMPo), the SI, the ventral pallidum (VP) and the globus pallidus (GP). The projections of these cholinergic groups are arranged topographically (Bigl *et al*, 1982; Saper, 1984; Luiten *et al*, 1987; Eckenstein *et al*, 1988; Gaykema *et al*, 1990), the CH4 group being the one which provides the major source of cholinergic innervation to the cerebral cortex (Bigl *et al*, 1982; Eckenstein *et al*, 1988; Rye *et al*, 1984; Mesulam *et al*, 1983a,b; Mesulam and Geula, 1988).

Although there are several studies on the BFCS in rats, its organization remains unclear in rodents. Therefore, the present work was intended to study the anatomical organization of the BFCS in mice, with particular emphasis on the nBM (CH4), by means of choline acetyltransferase immunohistochemistry (anti-ChAT).

MATERIALS AND METHODS

Twelve adult BalbC mice were anesthetized with sodium pentobarbital (50 mg/kg), perfused intracardially first with Tyrode's buffer solution with sodium nitrite (0.1%) and heparin, then with 20 ml of paraformaldehyde (3%) in 0.1 M phosphate buffer (PB), pH 7.4. Brains were extracted and postfixed in the same fixative for 2 h and then submerged in 25% sucrose solution at 4°C overnight. Slices of 10 µm width were made in a cryostat (Leica) and mounted on gelatine coated slides. The immunohistochemical peroxidase-anti-peroxidase (PAP) method was done directly on the slides. These were incubated briefly for 30 min. in 0.4% Triton and 3% normal rabbit serum in 0.05 M phosphate buffer saline (PBS), pH 7.4, and then with the anti-ChAT (AB8 monoclonal antibody; kindly provided by B Wainer), diluted at 1:700 in PBS with 0.2% Triton, 0.02% sodium azide and 1% normal rabbit serum at room temperature for 12 h. Then, they were incubated during 2 h with rabbit anti-rat IgG (ICN) as secondary antibody, and then with rat PAP (ICN) diluted 1:200 in PBS. Between these last two steps, slices were washed with 50 mM PBS, then dehydrated and coverslipped with Permount.

RESULTS

The more rostral immunopositive neurons (ChAT+) were located in the septal region at the level of the medial septum (MS), the VBDB and HBDB (Figs 1, 2). Neurons in the MS are fusiform and those of the diagonal band are fusiform or triangular. ChAT+ neurons of HBDB extend rostrocaudally, and progressively, take a lateral position, being always organized in a scattered manner (Fig 2). Dorsally to these groups, there are ChAT+ neurons in the ventral region of the nucleus accumbens (Fig 2), with a larger number of them in its posterior part. At the level of the anterior commissure (ac), we found two groups of ChAT+ cells. One is located in the ventral region of the telencephalon, into the nMPo. These neurons tend to be located caudally in

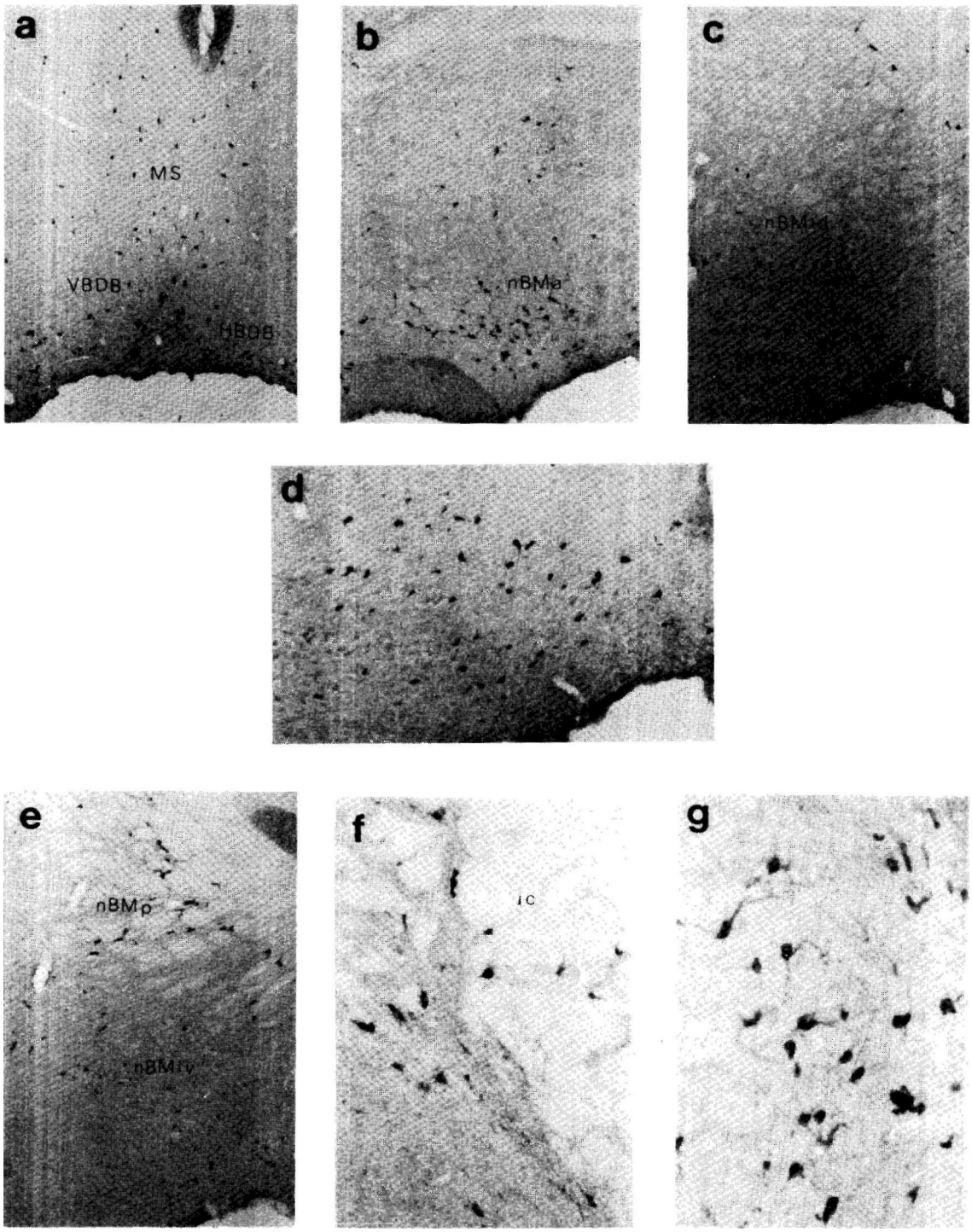

Fig 1. Rostro-caudal evolution (a-g) of immunohistochemical ChAT+ neurons in basal forebrain of mice. **a**. In the septal region, ChAT+ neurons are located in medial septum (MS) and in diagonal band of Broca, vertical (VBDB) and horizontal (HBDB) limbs. **b**. Distribution of neurons in anterior part of the magnocellular basal nucleus (nBMa) at the level of anterior commissure, ac. **c**. In Intermediate part of the nBM, neurons are located in a diagonal orientation, with ventral (nBMiv) and dorsal (nBMid) parts. **d**. In anterior part of the nBM (nBMa) ChAT+ neurons are located into the magnocellular preoptic nucleus. **e**. In rostral portion of posterior part of nBM (nBMp), lateral to the internal capsule (ic) into the globus pallidus (GP), few neurons of nBMid. **f**. In caudal part of the nBMp, ChAT+ neurons are located just lateral to the internal capsule (ic). A few neurons appear into the ic. **g**. CHAT+ neurons in NBMp.

dorsal position, but ventral to the GP (Figs 1, 2). The other group is located just ventral to the ac, at the level of the anterior part of the SI. These neurons have oval and multipolar forms. In posterior regions of the ac, we found scattered cholinergic neurons which

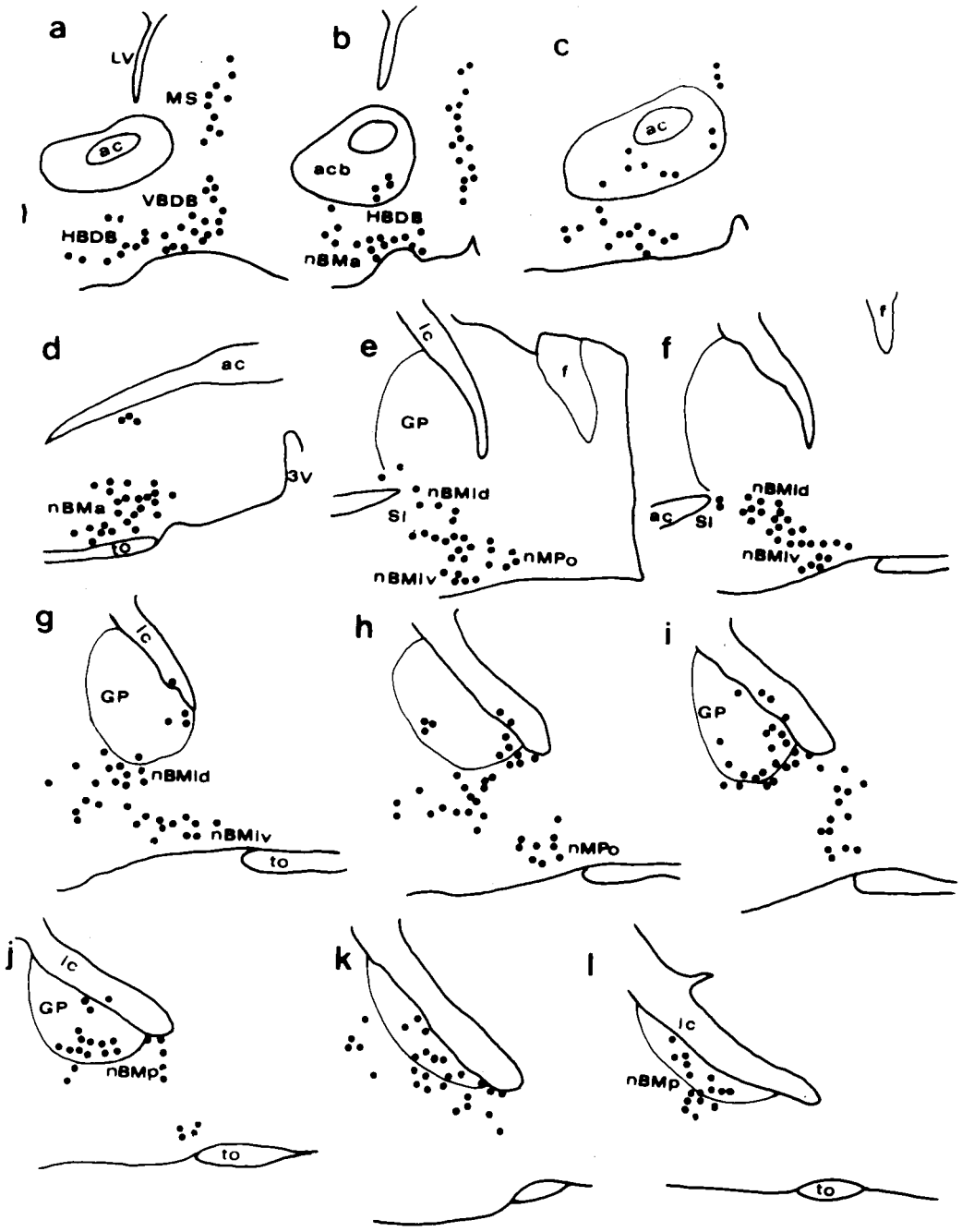

Fig 2. Schematic drawing (from camera lucida observations) of rostro-caudal organization (a-l) of basal forebrain cholinergic system (BFCS). ChAT+ neurons represented by black circles. **a.** Septal region. *ac*, anterior commissure; *HBDB*, horizontal limb of diagonal band of Broca; *LV*, lateral ventricle; *MS*, medial septum; *VBDB*, vertical limb of diagonal band of Broca. **b,c,d.** Anterior part of magnocellular basal nucleus (nBMa), where neurons are located in HBDB and in the magnocellular preoptic nucleus (nMPo), with another group located in nucleus accumbens (*acb*). *to*, optic tract; *3V*, third ventricle. **e,f,g,h.** Intermediate part of magnocellular basal nucleus, with dorsal (nBMid) and ventral (nBMiv) regions. This part remains expanded to i, located in nMPo. *f*, fornix; *GP*, globus pallidus; *ic*, internal commissure; *SI*, substantia innominata. **g,h**. More rostral part of posterior magnocellular basal nucleus (nBMp), located in GP. **i,j,k,l**. More caudal part of magnocellular basal nucleus, where ChAT+ neurons are located lateral to ic within GP.

extend diagonally and laterally from the ventral region, at the level of the nMPo, toward the SI and the VP (Figs 1, 2). At the level of the internal capsule (IC) we found a relatively high number of scattered ChAT+ neurons which extended into the SI and the VP, and some neurons into the GP. According to the disperse organization of these neurons, three groups can be differentiated: a ventral group which would appear to be the nMPo residue, another group between the VP and the SI, and a third group into the GP, where neurons group together in the lateral region of the IC (Fig 1). In the most posterior levels of the GP we found an immunopositive neuronal population along the lateral and ventral regions of the IC (Figs 1, 2).

DISCUSSION

The basal forebrain cholinergic system (BFCS) extends from the septal region to the caudal part of the GP in mice. This system includes the MS, VDBB, HDBB and the nBM. This later nucleus includes the HDBB, nMPo, SI, VP and medial parts of GP. The boundaries between these cholinergic groups are not clear and the BFCS appears to be a continuous and spread band of cholinergic neurons. Our results are in agreement with other reports in rats (Bigl *et al*, 1982; Satoh *et al*, 1983; Mesulam *et al*, 1983a).

The CH1 to CH4 nomenclature proposed by Mesulam *et al* (1983a) for the BFCS is based on the organization of the topographic projections of this system in rats and primates (Mesulam *et al*, 1986). However, the organization of these projections appears to be complex. The CH1/CH2 complex projects to the hippocampus (Mesulam *et al*, 1983a; Gaykema *et al*, 1990) and we have also described projections to the cerebral cortex (Lamour *et al*, 1982). The medial part of the CH3 group projects to the cerebral cortex (Bigl *et al*, 1982; Villalobos *et al*, 1990, 1996), and its lateral part, to the olfactory bulb (De Olmos *et al*, 1978). These cortical afferents are not arranged in a gradient manner. The CH3 group projects to different regions, as the anterior pole of the medial frontal cortex (Villalobos *et al*, 1996) and the medial regions of the primary visual cortex (Carey and Riek, 1987). Concerning the CH4 group or nBM, its projection pattern to the cerebral cortex is even more complex, with one restricted region of this nucleus projecting to different areas of the cerebral cortex (Saper, 1984; Bigl *et al*, 1982; Rye *et al*, 1984; Luiten *et al*, 1987). Topographical projections from the BFCS to specific cortical areas, such as visual (Carey and Riek, 1987), somatosensory (Baskerville *et al*, 1993) or frontal cortices (Villalobos *et al*, 1996), have also been described. Additionally, it projects to other regions, like the thalamus (Hreib *et al*, 1988; Parent *et al*, 1988; Steriade *et al*, 1987), hypothalamus (Bina *et al*, 1993), cerebral amygdala (Koliatsos *et al*, 1988) and brainstem (Divac, 1975; Parent *et al*, 1988). The complexity of the organization of the SI/VP projections is also known (Grove, 1988). For this reason, and in agreement with Bigl *et al* (1982), we should consider the BFCS as an anatomical entity, of which the nBM or CH4 is an important component.

Our results, do not show clear boundaries between HDBB and anterior parts of the nBM (Figs 1, 2). Rather they appear as a continuous band of cholinergic neurons, maintained to the level of the decussation of the ac. Caudally to this level, two cholinergic neuronal groups appear clearly, one dorsal and other ventral (Figs 1, 2). This later group disappears progressively at the level of the internal capsule. At this level, the cholinergic neurons appears into the GP. On the other hand, in a paper published in this issue (Villalobos *et al*, 1996), we show a topographical organization of the projections from the nBM to the frontal cortex, the anterior part projecting to the dorso-medial rostral regions, the posterior part to the dorso-lateral and caudal dorso-medial regions, and the intermediate part to the entire frontal cortex. This later result was in agreement with Luiten *et al* (1987).

Considering the above, we can divide the nBM into three cholinergic regions. An anterior region (nBMa) extends from the VBDB to the ventral level of the ac decussation (Fig 1, a-d). An intermediate region (Fig 1, e-i) extends from the nBMa to the caudal part of the GP, and can be further subdivided into a

dorsal part (nBMid), including the SI/VP complex, and a ventral part (nBMiv) in the nMPo. Finally, a posterior part (nBMp) is located in the more caudal region of the GP. The cholinergic neurons of this later part are exclusively dorsal (Fig 2, j-l).

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