*Brain Research*, 334 (1985) 380–384
Elsevier

BRE 20800

# Strain-dependent variations in the number of forebrain cholinergic neurons

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(Accepted December 18th, 1984)

*Key words:* acetylcholinesterase — basal ganglia — basal nucleus of Meynert — cholinergic systems — forebrain — genetic study — morphology — septal nuclei

The morphological organization of putatively cholinergic neurons was studied in the forebrain of two inbred mouse strains (C57BL/6 and DBA/2) by means of acetylcholinesterase pharmacohistochemistry. In both strains, putatively cholinergic perikarya were seen in the caudato-putamen, medial septum, diagonal band, and basal nucleus of Meynert: in all these regions, their distribution was similar in both strains, but their density was significantly higher (from 20 to 32%) in DBA/2 mice. The present data demonstrate the existence of genetically determined differences in the organization of forebrain cholinergic systems.

The topographical and hodological organization of forebrain cholinergic neurons has been recently outlined[8,11]. Cholinergic interneurons are present in the neostriatum, where they are involved in extrapyramidal motor control[1], and rather complex cholinergic systems originate from perikarya located in the basal forebrain (basal nucleus of Meynert, nucleus of the diagonal band) and medial septum: these systems are thought to be responsible for different behavioural integrations and for memory and learning[12,25]. Behavioural studies on inbred mouse strains have demonstrated that C57BL/6 and DBA/2 mice are characterized by opposite open field activity and learning abilities[5,19]; biochemical studies have also shown significant interstrain differences in the metabolism of acetylcholine[10,13,20], which were suggested to be a correlate of the observed behavioural differences[16]. In the attempt to identify the morphological substrate of these biochemical and behavioural variations, we studied the organization of cholinergic neurons in C57BL/6 and DBA/2 mice. We report here that the number of forebrain cholinergic neurons is substantially different in the two mouse strains.

Male C57BL/6 and DBA/2 mice (Charles River Laboratories) were studied at 18 weeks of age, by means of a pharmacohistochemical procedure for acetylcholinesterase (AChE)[6], which allows a detailed visualization of AChE-containing neurons[2], and is suitable for the study of forebrain putatively cholinergic perikarya[11,15,21]. In order to compare histochemically treated specimens, in each experiment the same number of animals from both strains was processed in a parallel fashion. All the animals were injected intramuscularly with an irreversible AChE inhibitor (di-isopropylfluorophosphate, DFP) 6 h prior to their euthanasia. Different doses of DFP (from 2 to 3.5 mg/kg of body weight) were employed in different experimental groups. The mice were sacrificed under deep general anaesthesia by cardiac perfusion with 0.9% saline followed by 10% phosphate-buffered neutral formalin. The brains were postfixed in the same formalin solution for 48–72 h before being transferred to cold (4 °C) 30% sucrose for an additional 48 h period. The brains were cut coronally at 20 $\mu$m intervals according to a standard plane[22]. The resulting tissue sections from both strains were processed in parallel according to a standard protocol. They were immersed for 30 min into 30 $\mu$M N,N′-bis(1-methylethyl)pyrophosphoroamidic anhydride (iso-OMPA), to inhibit butyrylcholinesterase, and then incubated exactly 2 h at 21 °C in the AChE incubation medium[7]. Morphome-

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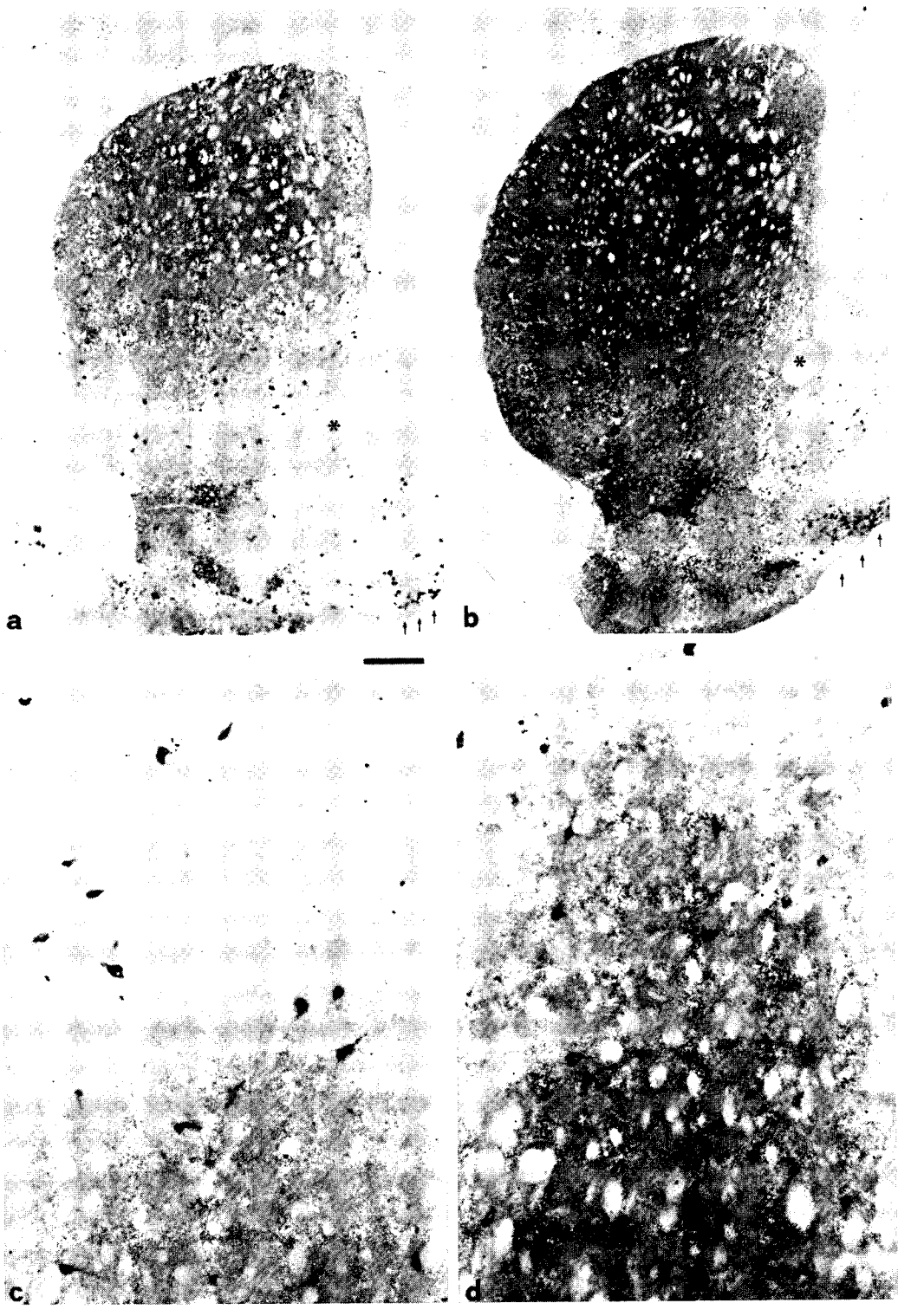


Fig. 1. AChE histochemistry performed 6 h after the administration of DFP (3.5 mg/kg) allows a clear identification of putatively cholinergic perikarya in the forebrain of both C57BL/6 (a,c) and DBA/2 mice (b,d). Two corresponding brain sections obtained during a single experimental session are shown. Low-power microphotographs (a,b) depict the entire extent of neostriatum, the anterior commissure (*), and partially the basal forebrain (arrows). It can be observed that in C57BL/6 strain (left column) AChE staining in the neuropil is less intense and that the number of AChE-containing neurons is lower. Scale bar: for a,b = 0.4 mm; for c,d = 0.1 mm.

try was performed by means of computer-assisted image analysis on camera lucida drawings of standard forebrain sections. Data from the two strains were compared by analysis of variance.

When mice were injected with lower doses of DFP (2 mg/kg), the AChE-containing cell bodies were clearly detectable in the C57BL/6, but not in the DBA/2 strain. In the latter strain AChE deposits of the striatal neuropil were so intensely stained that individual cell bodies could not be clearly visualized. When mice were injected with the highest doses of DFP (3.5 mg/kg), AChE staining in neuropil was significantly decreased in both strains, thus allowing comparative morphological studies of individual cholinergic perikarya. Under these conditions, DBA/2 mice still displayed more intense AChE staining in striatal neuropil (Fig. 1)[3].

Comparative interstrain morphological and morphometrical assessments were performed in animals injected with high doses of DFP. In both strains, the overall distribution and the morphology of putatively cholinergic forebrain neurons parallelled that observed by means of histochemical and immunocytochemical techniques[11,14]. Thus, large, darkly stained, russet, AChE-containing neurons, which are believed to represent forebrain cholinergic perikarya[21], were seen in the caudato-putamen, medial septum, nucleus of the diagonal band, and basal nucleus of Meynert of both strains. The comparative study, which was performed on standard coronal sections through the neostriatum (11 rostrocaudal levels), diagonal band and medial septum (6 levels), and basal nucleus (6 levels), did not show significant interstrain differences in the size of these structures. However, cell counts performed on the same standard sections revealed significant differences both in the number of AChE-containing neurons ($P < 0.01$ in the neostriatum; $P < 0.05$ in the diagonal band and medial septum) and in their densities ($P < 0.001$ in each case) (Fig. 2). In fact, in all the examined regions DBA/2 mice contained more cholinergic perikarya than C57BL/6. Interstrain differences in the density of forebrain cholinergic neurons were 20% in the neostriatum, 25% in the medial septum and diagonal band, and 32% in the basal nucleus.

Our morphometric analysis indicates that the forebrain of DBA/2 mice contains more cholinergic neurons than that of the C57BL/6 strain. These data are

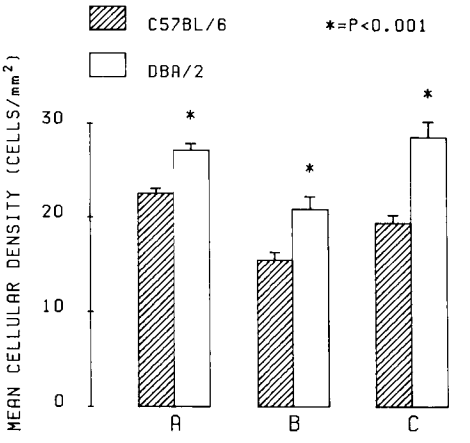


Fig. 2. Histogram of interstrain differences in the density of AChE-containing perikarya. A: neostriatum. Data refer to 6 animals (66 sections) of each strain. B: diagonal band and medial septum. Data refer to 4 animals (24 sections) of each strain. C: basal nucleus of Meynert. Data refer to 4 animals (24 sections) of each strain. Boundaries were delineated as they appear in sections counterstained for Nissl's substance. In each animal the right and left hemispheres were analyzed. As no left-right differences were found, values from right and left are shown together.

in agreement with previous biochemical studies, which have shown that AChE activity and acetylcholine turnover are significantly higher in the brain of DBA/2 than in C57BL/6 mice[9,20,24]. Taken together, morphological and biochemical data suggest that interstrain differences in the number of cholinergic forebrain neurons are directly correlated to functional activity of the forebrain cholinergic systems.

The present findings indicate that differences in brain cholinergic pathways are dependent on the genetic make-up. Present knowledge does not allow to demonstrate whether this is a primary effect or else a consequence of variations in other neurochemical classes of neurons. The first hypothesis seems, however, more likely in view of the fact that interstrain differences were seen in various forebrain nuclei, which differ significantly in their afferent and efferent hodological organization. Since the distribution and the morphological appearance of cholinergic neurons did not reveal significant interstrain differences, variations in densities of cholinergic perikarya and terminals are probably in relation to genetically determined different developmental patterns. To this regard, comparative developmental and cross-breeding studies are currently in progress in our laboratories.

Demonstration of strain-specific differences in the organization of forebrain cholinergic systems may have some important clinical implications. It has been recently suggested that Alzheimer's disease, a degenerative pathology of human brain which primarily affects memory and cognitive functions, may be correlated to a reduction in choline acetyltransferase and AChE in the forebrain[23]. Morphological studies have further demonstrated a significant depletion of cholinergic perikarya in the basal forebrain of these patients[17,26]. The etiology of Alzheimer's disease, as well as of other degenerative neurological disorders, is still unknown. A recent hypothesis is that a neurotrophic factor specific to cholinergic neurons may be deficient[4]; in addition, the existence of genetic markers specific to Alzheimer's disease has been also demonstrated[18]. Therefore, the possibility exists that genetically determined variations in the number of cholinergic neurons may be important in influencing the susceptibility to the expression of this brain disease. In this respect, the study of C57BL/6 and DBA/2 mice might constitute an animal model for the understanding of impairments in human forebrain cholinergic transmission.

The authors are greatly indebted to Prof. G. Macchi, and to Prof. A. Oliverio, for their guidance and for revising the manuscript. They also thank Dr. F. Amenta, whose laboratory facilities were used. Excellent technical assistance was given by Mr. M. Battaglia, Mr. B. Ghirotti, Mr. R. Mallucci, and Mr. G. Rosi. This work was supported in part by CNR (Consiglio Nazionale delle Ricerche) Grant 82.02105.05.

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