ELSEVIER

Brain Research 637 (1994) 181–189

BRAIN RESEARCH

Research Report

Genetically determined cholinergic deficiency in the forebrain of C57BL/6 mice

Anna Rita Bentivoglio, Maria Concetta Altavista, Roberta Granata, Alberto Albanese *

Istituto di Neurologia, Università Cattolica del Sacro Cuore, Largo A. Gemelli, 8, I-00168 Roma, Italy

(Accepted 5 October 1993)

Abstract

This study demonstrates that a deficiency of forebrain cholinergic neurons occurs in C57BL/6 (C57) mice, a strain characterized by poor learning capabilities. The brains of 21-day-old and 18-week-old C57 and DBA/2 (DBA) mice were studied by means of acetylcholinesterase (AChE) histochemistry and of choline acetyltransferase (ChAT) immunocytochemistry. Computer-assisted image analysis was performed on sections through the medial septum, the diagonal band of Broca, the basal nucleus of Meynert and the neostriatum. As compared to the DBA strain, C57 mice had a reduced number of forebrain cholinergic neurons. This feature was present at the age of 21 days and persisted to 18 weeks. Between-strain variations in the density of neurons were more obvious in ChAT-stained material than in AChE-stained sections. These data show that C57 mice can be regarded as a genetic mutant, whose phenotype is characterized by a reduced number of forebrain cholinergic neurons and by cognitive abnormalities. C57 mice represent a valuable model for studying the influence of genetic factors on central nervous system cholinergic mechanisms and the effects of genetically determined cholinergic deficiency on behavior and learning.

Key words: Acetylcholine; Basal nucleus of Meynert; Dementia; Diagonal band; Inbred strains; Substantia innominata; Septum; Neostriatum

1. Introduction

The relationship between central cholinergic systems and behavior has been the object of several analyses. Studies on different animal species, including rodents, have shown that learning abnormalities occur after a pharmacologic manipulation or a direct lesion of forebrain cholinergic neurons [16,30,36]. Grafting of cholinergic neurons or pharmacologic substitution therapy may partly reverse such abnormalities [37,46]. Behavioral studies of adult inbred mouse strains showed that C57BL/6 (C57) mice possess poor learning abilities and excessive open field activity [6,14]. Since inbred strains have a homozygotic genome, the behavioral abnormality of C57 mice has been interpreted as a direct aftermath of their genetic make-up [32].

Recent data from our and from other laboratories showed that aging in rodents is associated with a loss of forebrain cholinergic neurons, which directly correlates with age-dependent cognitive impairment [4,18]. Based on this evidence, we addressed the problem as to whether the behavioral deficits of C57 mice may be associated with a genetically dependent alteration of forebrain cholinergic systems [2,22]. The aim of the present study was to evaluate whether: (a) C57 mice suffer from a genetically dependent impairment of forebrain cholinergic neurons and, if so, whether (b) cholinergic neurons are lacking since early in development or degenerate precociously instead.

2. Materials and methods

2.1. Subjects

Male C57BL/6 and DBA/2 mice were studied at 3 and 18 weeks of age. Each home cage contained six animals of the same age and

* Corresponding author. Fax: (39) (6) 305 1343; E-Mail: albanese@itcaspur.caspur.it.

Abbreviations: AChE, acetylcholinesterase; C57, C57BL/6; ChAT, choline acetyltransferase; DBA, DBA/2; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; QNB, quinuclidinyl benzilate.

SSDI 0006-8993(93)E1377-F

strain. The cages were kept at a constant temperature of 21°C, on a natural daylight cycle. Access to food and water was free in the home cage. In order to compare histochemically treated specimens, in each experiment the same number of animals of a single age group were chosen from both strains; therefore couples of two mice (one from each strain) were processed exactly in a parallel fashion.

2.2. AChE histochemistry

Eight C57 (four 3-week-old and four 18-week-old) and eight DBA (four 3-week-old and four 18-week-old) mice were studied by means of Butcher's [7] histochemical technique for acetylcholinesterase (AChE), which allows a detailed visualization of AChE-containing neurons, and is suitable for the study of putative cholinergic perikarya (see [9]). Eight hours prior to perfusion, the animals were injected with di-isopropylfluorophosphate (DFP). A short survival time after DFP was chosen in order to minimize the staining of non-cholinergic, light, AChE-containing neurons, which are located in a wide number of brain regions [7]. DFP was injected i.m. as a 0.15% peanut oil solution at a dose of 3.5 mg/kg (body weight). Then, under deep general anaesthesia with sodium pentobarbital (40 mg/kg i.p.), the animals were perfused through the heart with 0.9% saline solution, followed by 10% formalin in phosphate buffer (pH 7.4). The brains were kept in the same formalin solution for 48–72 h before being transferred to cold (4°C) 30% sucrose for an additional 48-h period. Coronal sections were cut at 30 μm intervals by means of a freezing microtome, according to a standard plane [43]. All the consecutive brain sections were studied: every other tissue section was stained using Nissl's technique, while the alternate series was processed according to the following protocol. Sections were immersed for 30 min in 30 μM *N,N'*-bis(1-methylethyl)pyrophosphoroamidic anhydride (iso-OMPA), to inhibit butyrylcholinesterase, and then incubated in the AChE incubation medium, as reported previously [1].

2.3. ChAT immunocytochemistry

Four C57 (two 3-week-old and two 18-week-old) and four DBA (two 3-week-old and two 18-week-old) mice were studied using choline acetyltransferase immunocytochemistry. Mice were deeply anesthetized with chloral hydrate (400 mg/kg) and perfused through the heart with physiologic saline followed immediately by a freshly prepared solution of 4% (w/v) paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed, soaked overnight in cold (4°C) 30% sucrose in phosphate buffer, and then sectioned at 40 μm on a freezing microtome. Sections were stored overnight at 4°C in saline solution with phosphate buffer prior to staining. All the consecutive brain sections were studied: every other tissue section was stained using Nissl's technique, while the alternate series was processed according to the following protocol.

A double-bridge staining procedure was performed on free-floating sections with agitation at 21°C, and consisted of the following sequential incubations: (1) 0.5% Triton X-100 in saline with phosphate buffer for 1 h; (2) 0.1% Triton X-100, 3% normal rabbit serum in saline with phosphate buffer for 1 h; (3) monoclonal rat anti-ChAT antibody (Boehringer, Mannheim, Germany) overnight, diluted 1:600; (4) rabbit anti-rat IgG diluted 1:100 for 1 h; (5) rat peroxidase anti-peroxidase diluted 1:75 for 1 h; (6) visualization of the peroxidase by 10 min preincubation with 0.05% diaminobenzidine in 0.05 M Tris-HCl (pH 7.6) at room temperature, followed by incubation in the same medium for another 6–8 min in the presence of 0.01% hydrogen peroxide present. Between each of the steps, the sections were rinsed several times in saline with phosphate buffer. Following the last step, sections were rinsed, mounted onto chrome-alum gel-coated slides, air-dried, dehydrated and cover-slipped.

2.4. Morphometry

Every AChE- or ChAT-stained section through the medial septum, diagonal band and basal nucleus, and every other section through the neostriatum, were charted on a drawing microscope, and then analyzed by means of a semiautomatic image analyzer. The brains of each couple of C57 and DBA mice, processed during a single staining session, were charted blindly by the same observer. The boundaries of areas rich in cholinergic neurons were identified by comparing alternate AChE and Nissl stained sections. This method has already been successfully employed to analyze the rodent forebrain [2,4]. Cell number, cell density (measured as the number of perikarya per mm^2), and the regional area were computed in each age group of C57 and DBA mice. These morphometric data were compared by means of two-sample Student's *t*-test. The number of AChE-stained sections considered for this morphometric analysis was as follows: (1) basal nucleus, 10 sections of each 3-week-old mouse, 14 sections of each 18-week-old mouse; (2) diagonal band, 12 sections of each 3-week-old mouse, 18 sections of each 18-week-old mouse; (3) medial septum, 6 sections of each 3-week-old mouse, 8 sections of each 18-week-old mouse; (4) neostriatum, 26 sections of each 3-week-old mouse, 20 sections of each 18-week-old mouse. The number of ChAT-stained sections considered for this morphometric analysis was as follows: basal nucleus, 16 sections of each 3-week-old mouse, 20 sections of each 18-week-old mouse; neostriatum, 54 sections of each 3-week-old mouse, 42 sections of each 18-week-old mouse.

The rostral–caudal distribution of AChE-containing cell bodies was also statistically analyzed by smoothing the series of data obtained from adjacent coronal sections. In each region, the distribution of neural density was studied by means of frequency analysis. Morphometric analysis was not performed in ChAT-stained sections through the medial septum and diagonal band, which were quantitatively analyzed only in AChE-stained material. Morphometric evaluation of the medial septal region was quite laborious also in AChE-stained sections, due to the difficulty to draw reliable boundaries even when adjacent Nissl-stained specimens were used for reference.

In 18-week-old mice, measurements of the size of individual AChE-containing neurons were performed at random throughout the basal nucleus and the neostriatum. Only neurons containing a well-defined unstained nucleus were individually chosen from any segment of the observed region; they were also measured semi-automatically by means of a computer-assisted image analyzer. Their cross-sectional areas were computed in the C57 and DBA groups; the resulting figures were compared by means of a two-sample Student's *t*-test. Since rodents have no capsula interna, we sought to verify whether differences in the size of fibers-of passage crossing the neostriatum could have affected the computation of cell density in this forebrain nucleus. In each 18-week-old mouse stained for AChE six standard sections through the neostriatum were chosen. The overall cross-sectional size of the neostriatum was compared to the total size of the unstained fibers-of-passage; data collected from C57 and DBA mice were compared by means of two-sample Student's *t*-test.

3. Results

3.1. AChE staining

The overall distribution and the appearance of AChE-containing forebrain neurons were similar in both strains. Large, intensely stained, russet, AChE-containing neurons were seen in the neostriatum, the

medial septum, the nuclei of the diagonal band, the magnocellular preoptic nucleus, and the basal nucleus. In the same regions they were intermingled with moderately stained perikarya. By contrast, lightly stained cell bodies, which are located in several other brain regions [9], could be barely seen in either group. In our comparative study, intensely and moderately AChE-stained perikarya were charted together in the Ch1–Ch4 nuclei. Cell bodies were distributed in the neostriatum with a typical patchy arrangement, matching an anatomical and biochemical mosaic, which is a feature of this territory [20]. In the medial septal nucleus, AChE-rich neurons were aggregated along the midline and sparsely distributed on each side; they merged ventrally with neurons of the vertical limb. At more caudal levels, cell bodies located in the vertical and horizontal limb nuclei abutted the ventral surface of the brain. Thus, in the Ch1–Ch3 nuclei, AChE-containing neurons constituted a continuous collection of cells, so that boundaries between different anatomical structures were sometimes difficult to draw. This, in particular, was the case for the medial septal nucleus and the nucleus of the vertical limb, which were analyzed together. As observed in coronal sections, AChE-containing cell bodies located in the basal nucleus constituted a well-demarcated grouping of large perikarya displaying a typical triangular or lentiform shape (Figs. 1 and 2).

Upon examination of AChE-stained material, some differences between the brains of DBA and C57 mice were altogether clear. Independent observers, who had no knowledge of which mouse strain they were looking

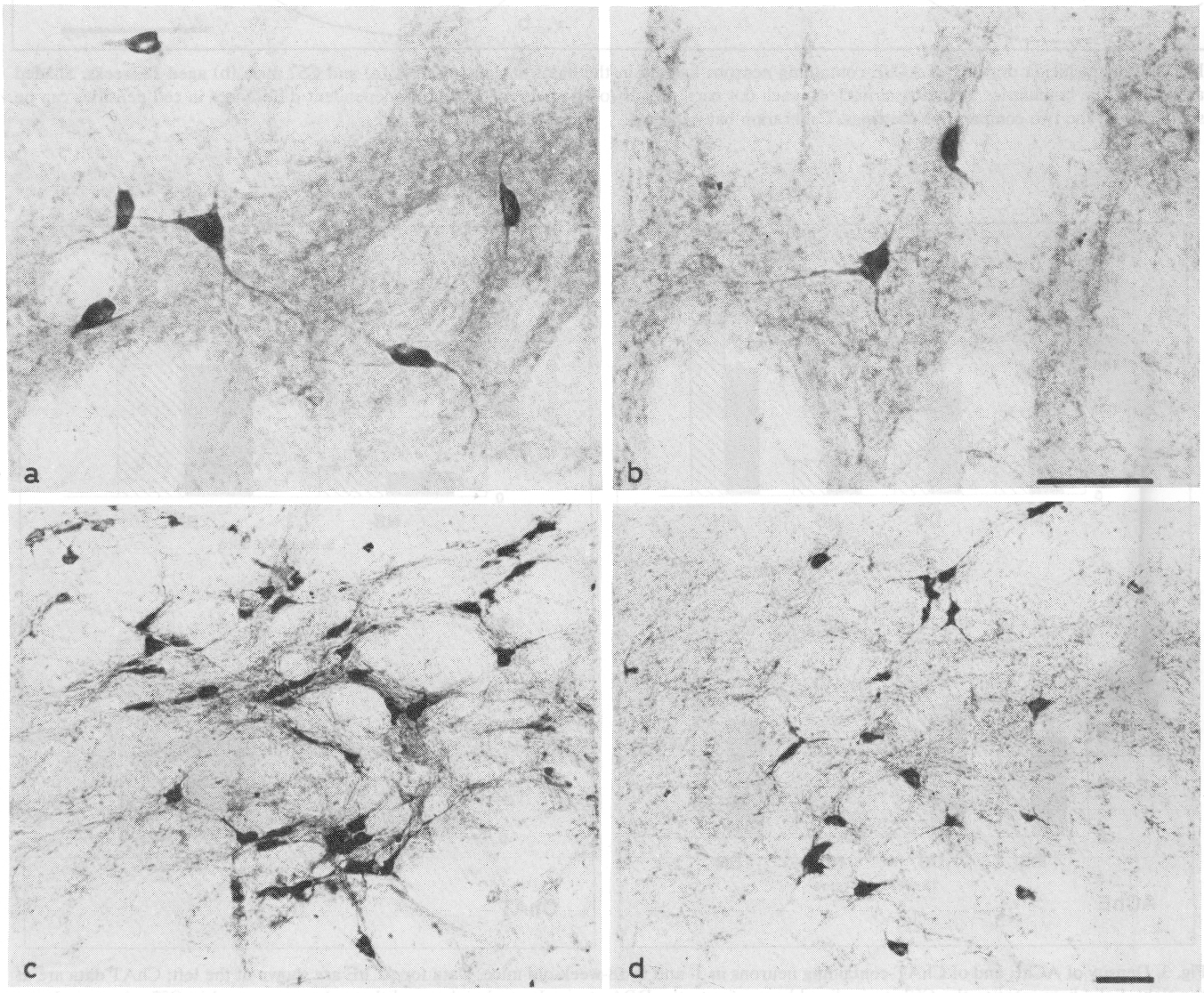

Fig. 1. ChAT-containing neurons located in the neostriatum (a,b; 36×) and in the basal nucleus (c,d; 18×) of 18-week-old mice. The DBA strain is shown at the left, the C57 strain at the right. The density of putatively cholinergic neurons is reduced in the neostriatum and in the basal nucleus of C57 mice. Calibration bars = 50 μm.

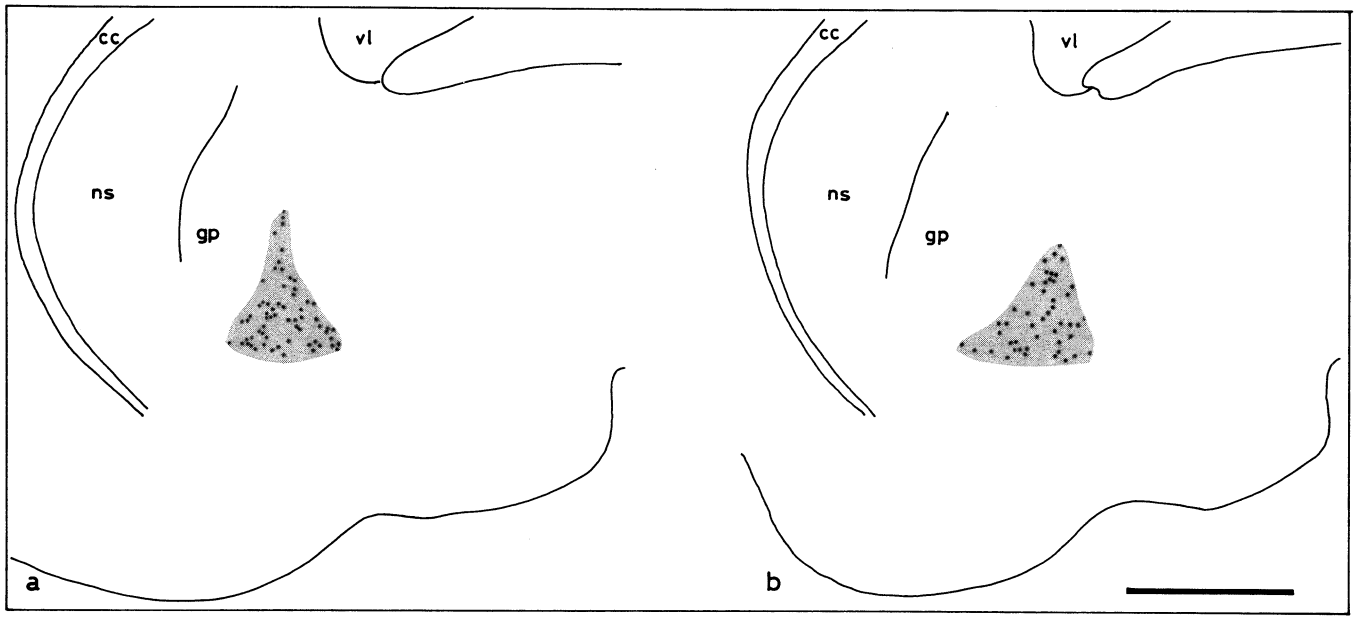

Fig. 2. Camera lucida drawing of AChE-containing neurons located in the basal nucleus of DBA (a) and C57 mice (b) aged 18 weeks. Shaded areas show the boundaries of the basal nucleus; each dot corresponds to one neuron. The strain-dependent differences in cell densities can be spotted from the two comparative drawings. Calibration bar = 1 mm.

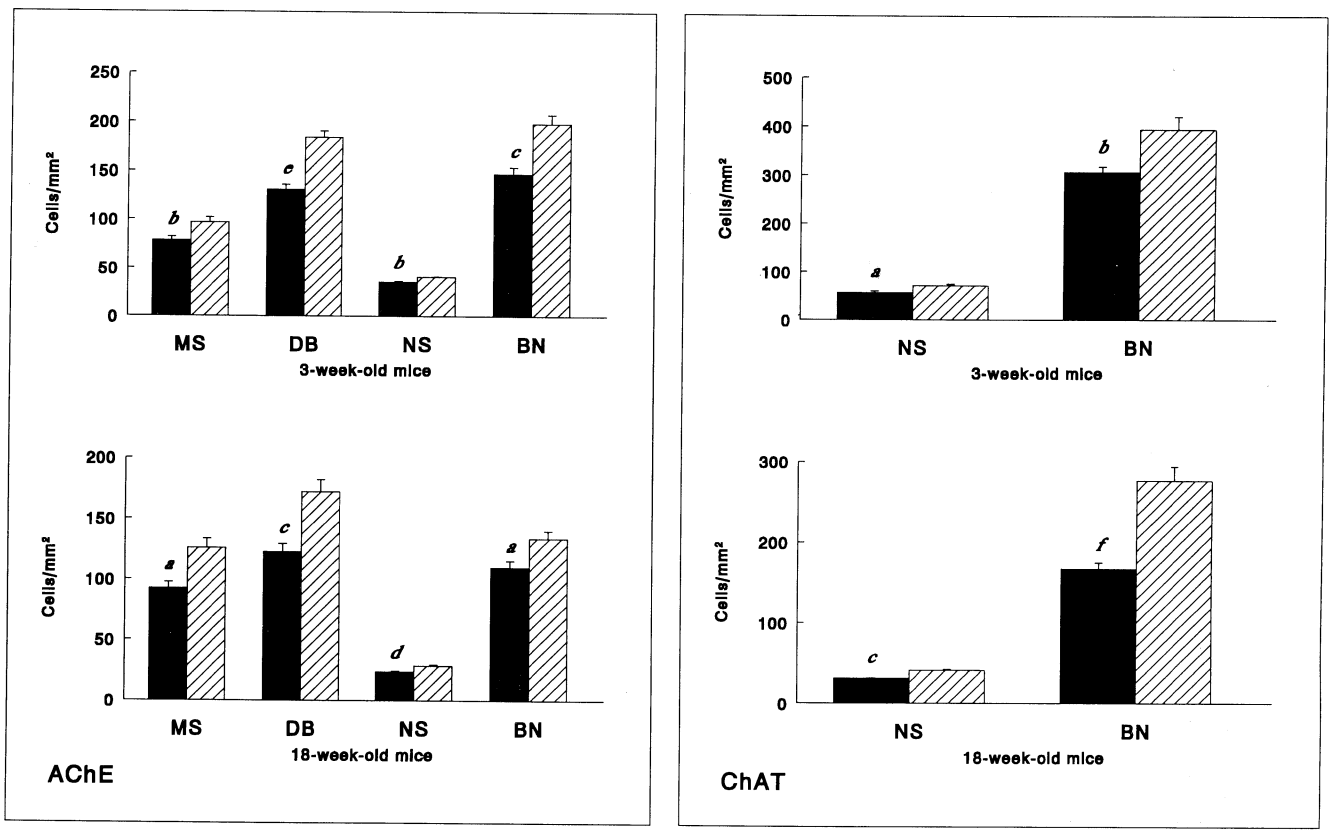

Fig. 3. Density of AChE and of ChAT-containing neurons in 3- and in 18-week-old mice. Data for AChE are shown at the left; ChAT data are at the right. Solid bars refer to the C57 strain, lined bars refer to the DBA strain. It can be observed that values measured in C57 mice are always lower than the corresponding values measured in DBA mice. The sample sizes are reported in the materials and methods section. Abbreviations: BN, basal nucleus; DB, diagonal band of Broca; MS, medial septum; NS, neostriatum. Statistical significance: *a*, $P < 0.001$; *b*, $P < 0.0001$; *c*, $P < 0.00001$; *d*, $P < 0.1 \cdot 10^{-5}$; *e*, $P < 0.1 \cdot 10^{-6}$; *f*, $P < 0.1 \cdot 10^{-10}$.

at, could consistently identify the brains of C57 mice by observing a reduced cell number. This was confirmed by morphometric analysis, which revealed clear-cut strain-dependent differences in all the forebrain cholinergic nuclei. With the only exception of a 19.23% reduction in the size of the basal nucleus in 18-week-old C57 mice, the mean area of cholinergic nuclei was of comparable size in both strains. As compared to DBA mice, in the C57 strain the overall number of AChE-containing neurons was reduced by an average 2.65% at 3 weeks of age and by an average 23.52% at 18 weeks. As a consequence, the overall strain-dependent decrease in the density of AChE-stained cells averaged 21.72% in 3-week-old mice and 22.49% in 18-week-old mice (Table 3). In 3-week-old C57 mice, cell density was reduced by 23.08% in the medial septum ($P < 0.0001$), by 28.94 in the diagonal band ($P < 0.1 \cdot 10^{-7}$), by 12.68% in the neostriatum ($P < 0.0001$), by 26.05% in the basal nucleus ($P < 0.00001$). In 18-week-old C57 mice, cell density was reduced by 26.25% in the medial septum ($P < 0.001$), by 28.87% in the diagonal band ($P < 0.00001$), by 17.09% in the neostriatum ($P < 0.1 \cdot 10^{-5}$), by 17.76% in the basal nucleus ($P < 0.001$) (Fig. 3).

In the neostriatum of 18-week-old mice no between-strain variations in the size of the fibers-of-passage were detected. As observed in coronal sections, fibers-of-passage accounted for 21.1% of the total cross-sectional area of the neostriatum in C57 mice, and for 22.23% in the DBA strain (Table 1).

In each animal, a comparison of morphometric data obtained in the right and left hemispheres was performed in all regions, but the medial septum and the vertical limb. No right–left asymmetries were observed, and, therefore, measurements of the right and left side were considered as belonging to a homogeneous population. Analysis of the distribution of cell densities in C57 and DBA mice revealed a rostral–caudal decrease of values measured in the basal nucleus of each strain in either age group. No rostral–caudal variations were observed in the neostriatum. Statistical analysis of rostral–caudal series confirmed the occurrence of a rostral–caudal gradient in the basal nucleus, but revealed no between-strain differences. The size of individual cell bodies was measured in the neostriatum and in the basal nucleus of C57 and DBA mice at 18 weeks of age. In each structure AChE-containing neurons were of the same size in either strain (Table 2).

Table 1
Relative cross-sectional area of fibers-of-passage in the neostriatum of 18-week-old mice (as percentage of the total area of neostriatum)
The data refer to six rostral-caudal section in each mouse.

Neostriatum	C57	DBA	P
Fibers-of-passage (%)	21.10 ± 1.36	22.23 ± 2.22	NS

Table 2
Cross-sectional area of AChE-containing perikarya in 18-week-old mice (μm^2)
The data refer to 100 sampled neurons per region in each mouse

Brain region	C57	DBA	P
Neostriatum	221.33 ± 2.38	219.71 ± 2.87	NS
Basal nucleus	306.57 ± 4.35	309.97 ± 3.66	NS

Age-related variations in cell density were computed by comparing in each strain the values measured at 3 and 18 weeks of age. In all cholinergic nuclei, but the medial septum, an age-dependent decrease in cell density occurred. The overall age-dependent decrease occurring in C57 mice was more severe than that occurring in DBA mice. In the diagonal band, a cell decrement of 6.41% (P = NS) and of 6.51% (P = NS) occurred in C57 and DBA mice, respectively; in the neostriatum cell decrement was 27.32% ($P < 0.1 \cdot 10^{-13}$) and 20.74% ($P < 0.1 \cdot 10^{-14}$); in the basal nucleus it reached 50.19% in C57 mice ($P < 0.00001$), and it was 19.98% ($P < 0.1 \cdot 10^{-6}$) in the DBA strain.

3.2. ChAT staining

The overall distribution of ChAT-containing neurons matched that of AChE-stained cells in many regions of the forebrain. ChAT-containing perikarya were observed in the cerebral cortex, in the medial septum, in the diagonal band, in the basal nucleus, and in the neostriatum, where AChE-stained cell bodies also occurred.

Morphometric analysis was performed in the neostriatum and in the basal nucleus of 3- and 18-week-old mice. No significant differences in the size of the basal nucleus were detected in either age group. The size of the neostriatum did not show between-strain differences at 3 weeks of age; but, at 18 weeks of age, the neostriatum of C57 mice was 12.41% smaller than that of the DBA strain. As compared to DBA mice, the density of ChAT-containing perikarya was significantly reduced in the neostriatum and in the basal nucleus of C57 mice of either age. Between-strain decrease in the density of cholinergic neurons located in the neostriatum was 20.54% at 3 weeks of age ($P < 0.001$), and dropped to 24.46% at 18 weeks ($P < 0.1 \cdot 10^{-10}$). In the basal nucleus, between-strain decrease in cell density was 22.26% at 3 weeks ($P < 0.0001$) and dropped to 39.56% at 18 weeks ($P < 0.00001$) (Fig. 3).

Age-related variations in cell density were computed by comparing in each strain the values measured at 3 and 18 weeks of age. An age-dependent decrease in cell density occurred in all cholinergic nuclei, and was always more severe in C57 than in DBA mice. Cell density declined in the neostriatum by 44.96% ($P < 0.1 \cdot 10^{-10}$) and by 42.11% ($P < 0.1 \cdot 10^{-10}$), respectively.

Table 3
Percent density of AChE- and ChAT-containing neurons in C57 mice, as compared to age-matched DBA mice

Brain region	3-week-old	18-week-old
AChE		
Medial septum	−23.08%	−26.25%
Diagonal band	−28.94%	−28.87%
Neostriatum	−12.68%	−17.09%
Basal nucleus	−26.05%	−17.76%
ChAT		
Neostriatum	−20.54%	−24.46%
Basal nucleus	−22.26%	−39.56%

In the basal nucleus cell density was reduced by 45.39% in C57 mice ($P < 0.1 \cdot 10^{-6}$), and by 29.80% in the DBA strain ($P < 0.1 \cdot 10^{-6}$) (Table 3).

4. Discussion

This morphological study shows that C57 mice have a reduced number of forebrain cholinergic neurons, a feature that is already present at the age of 21 days. This morphological alteration is very likely to account for the reduced activity of enzymes related to cholinergic metabolism, which has been reported in earlier biochemical studies. Thus, the present data extend earlier biochemical observations that a reduction of either AChE or ChAT occurs in the cerebral cortex of C57 mice [17,25,34,41,45].

In order to obtain data comparable with previous results obtained in our laboratory, in the present series of experiments two different morphological techniques were employed. AChE histochemistry was used to stain all forebrain cholinergic neurons, while ChAT immunocytochemistry was used to stain the basal nucleus and the neostriatum. This also allowed to directly compare the two staining techniques in these brain regions. Data obtained from sections stained, with ChAT were in excellent agreement with results of the AChE study. Between-strain variations in cell density were observed either in AChE- or in ChAT-stained material and were more prominent in the latter. Since no between-strain differences were found in the size of regions containing cholinergic perikarya, the observed differences in the number of cholinergic neurons were interpreted as a direct consequence of variations in cell density. in all regions, the density of putatively cholinergic neurons, as observed in ChAT-stained material, was higher than that measured in AChE-stained sections; this is in agreement with earlier observations comparing the two staining procedures in the same species [24]. Either in the neostriatum or in the basal nucleus, the number of histochemically labelled perikarya was higher in ChAT- than in AChE-stained specimens; this difference was particularly marked in the basal nucleus. A cumulative count of intensely and moderately stained AChE-containing perikarya showed that, in the neostriatum, the total number of AChE-stained cell bodies was in average 33.71% lower than that of ChAT-containing neurons; in the basal nucleus, instead, intensely and moderately stained AChE perikarya underestimated the number of ChAT-containing ones by an average 46.99%.

The present study allows to rule out the possibility that C57 mice may have a normal development of cholinergic systems, followed by an untimely loss of cholinergic perikarya. The present data indeed show that between-strain variations occur as early as 21 days postnatal, i.e. at a time when the development of cholinergic perikarya located in the forebrain is about completed [8]. This implies that C57 mice have a genetic make-up expressing a cholinergic deficient phenotype in the forebrain. The observation that surviving cholinergic perikarya are not shrunken nor atrophic (Table 2) is also in keeping with the view that the C57 phenotype does not depend on a degenerative process. This sets a clear difference with morphometric variations observed in the forebrain of aged rodents, where cell loss is associated with a significant reduction in the size of surviving cholinergic cell bodies [3,4]. Interestingly, it has been observed that age-dependent loss and shrinkage of cholinergic neurons located in the neostriatum indeed occur in C57BL/6N mice aged 2 years [29]. This indicates that age dependent shrinkage can also develop in strains which are genetically depleted of forebrain cholinergic neurons, and also explains why the density of cholinergic neurons is reduced in either strains (and to a higher extent in C57 mice) between 3 and 18 weeks of age.

The demonstration of a reduced number of cholinergic neurons in the C57 strain fits well with earlier behavioral data, showing that these mice have poor learning capabilities and excessive open-field activity [5,6,39]. Our morphologic study reveals that the cholinergic deficiency is particularly severe in those regions which are directly responsible for the observed behavioral abnormalities (e.g. in the basal nucleus). However, cholinergic cell loss also affects the neostriatum, whose cholinergic interneurons are responsible for extrapyramidal motor control, a behavior that is not apparently affected in the C57 strain. Thus, genetically dependent loss of cholinergic neurons is widespread, but not haphazard, in the forebrain of C57 mice, as it affects neural populations which are located in different forebrain regions and possess different anatomical connections. In addition, recent autoradiographic data on the striatum and the laterodorsal tegmental nucleus of C57 and DBA mice showed that QNB binding was lower in the C57 strain by 28% and 31%, respectively [42]. This is in keeping with the view that C57 mice are

a genetic model of brain cholinergic deficiency in rodents.

Recently it has been reported that ChAT, AChE, and QNB muscarinic binding increase in the cerebral cortex, in the hippocampus, and in the striatum of C57 mice from postnatal day 10 to 150 [47]. In the medial septum, instead, a gradual increase occurred until 30 days, which was followed by a progressive decline of the three markers until postnatal day 150 [47]. These observations are in general agreement with our study, and particularly so, since they report a modest variation of AChE and QNB binding from postnatal day 21 onward. The authors did not study the basal nucleus and did not specify the regions of the neostriatum from which they collected samples [47]. The occurrence of significant regional variations in the number of cholinergic perikarya located in the neostriatum [3] may have affected the analysis of their data.

The C57 model of cholinergic deficiency differs from other experimental conditions characterized by a specific impairment of cholinergic transmission in the brain, such as lesions brought about by the local injection of excitotoxins or of cholinergic neurotoxins either within forebrain cholinergic nuclei [23,26] or in their cortical target territories [33,44]. In the case of C57 mice, in fact, the cholinergic deficiency occurs early in brain development and is genetically dependent. The availability of a large number of animals with constantly reproducible features sets a clear difference with models based on brain lesions. The observation of a cholinergic deficient phenotype in C57 mice raises the question as to whether other biochemical abnormalities are also present in this mouse strain. This is probably the case, since C57 mice differ from other inbred strains in several features which are unrelated to cholinergic function [19,40]. Furthermore, C57 mice are known to be particularly sensitive to MPTP, a susceptibility which depends on the genetic make-up and varies considerably in different strains of mice [21]. Taking into account all these considerations, C57 mice can be regarded as genetic mutants with a phenotype characterized by a reduced number of forebrain cholinergic neurons and by variations in other classes of neurotransmitters.

Present knowledge does not allow to clarify whether the cholinergic deficiency of C57 mice is brought about by a single genetic product or by the effect of multiple genes. Cholinergic deficiency may be caused directly by a gene product (e.g. lack of a neurotrophic factor) [10,12,31] or may be secondary to other biochemical abnormalities (e.g. galanin hyper-innervation of the basal forebrain) [11]. The first possibility seem more likely, as it would also account for an early occurrence of cholinergic abnormalities during development of C57 mice. In order to test this hypothesis, the possibility that growth factors (such as nerve growth factor, which is specific to cholinergic neurons [15]) may allow a better development of forebrain cholinergic neurons could be evaluated.

Search for the genetic product affecting the cholinergic deficient phenotype of C57 mice may be relevant for understanding human diseases related to cholinergic deficiency. It is known that Alzheimer's disease may be transmitted genetically in some specific pedigrees. The disease is associated with shrinkage and loss of cholinergic neurons located in the basal forebrain [13,28,35,38,48]. The abnormal genetic product implicated in familial cases of Alzheimer's disease is still unknown; nevertheless, therapeutic approaches based on the administration of cholinergic drugs or of neurotrophic factors specific to cholinergic neurons have been already undertaken [27]. To this respect, the study of C57 mice may provide a new valuable model for testing such therapeutic tools.

Acknowledgements. The present study was supported in part by CNR (Consiglio Nazionale delle Ricerche) Grant 91.00346.04. The authors thank Dr. E. Dell'Anna for helpful suggestions and comments.

5. References

[1] Albanese, A. and Butcher, L.L., Acetylcholinesterase and catecholamine distribution in the locus ceruleus of the rat, *Brain Res. Bull.*, 5 (1980) 127–134.

[2] Albanese, A., Gozzo, S., Iacopino, C. and Altavista, M.C., Strain-dependent variations in the number of forebrain cholinergic neurons, *Brain Res.*, 334 (1985) 380–384.

[3] Altavista, M.C., Bentivoglio, A.R., Crociani, P., Rossi, P. and Albanese, A., Age-dependent loss of cholinergic neurones in basal ganglia of rats, *Brain Res.*, 455 (1988) 177–181.

[4] Altavista, M.C., Rossi, P., Bentivoglio, A.R., Crociani, P. and Albanese, A., Aging is associated with a diffuse impairment of forebrain cholinergic neurons, *Brain Res.*, 508 (1990) 51–59.

[5] Ammassari-Teule, M. and Caprioli, A., Spatial learning and memory, maze running strategies and cholinergic mechanisms in two inbred strains of mice, *Behav. Brain Res.*, 17 (1985) 9–16.

[6] Bovet, D., Bovet-Nitti, F. and Oliverio, A., Genetic aspects of learning and memory in mice. The study of differences among strains and individual subjects is a most promising topic in psychobiology, *Science*, 163 (1969) 139–149.

[7] Butcher, L.L., Acetylcholinesterase histochemistry. In A. Björklund and T. Hökfelt (Eds.), *Handbook of Chemical Neuroanatomy. 1. Methods in Chemical Neuroanatomy*, Elsevier, Amsterdam, 1983, pp. 1–49.

[8] Butcher, L.L. and Hodge, G.K., Postnatal development of acetylcholinesterase in the caudate-putamen nucleus and substantia nigra of rats, *Brain Res.*, 106 (1976) 223–240.

[9] Butcher, L.L. and Woolf, N.J., Histochemical distribution of acetylcholinesterase in the central nervous system: clues to the localization of cholinergic neurons. In A. Björklund, T. Hökfelt and M.J. Kuhar (Eds.), *Handbook of Chemical Neuroanatomy. 3. Classical Transmitters and Transmitter Receptors in the CNS, Part 2*, Elsevier, Amsterdam, 1984, pp. 1–50.

[10] Cavicchioli, L., Flanigan, T.P., Dickson, J.G., Vantini, G., Daltoso, R., Fusco, M., Walsh, F.S. and Leon, A., Choline acetyl-

transferase messenger RNA expression in developing and adult rat brain: regulation by nerve growth factor, *Mol. Brain Res.*, 9 (1991) 319–325.

[11] Chan-Palay, V., Galanin hyperinnervates surviving neurons of the human basal nucleus of Meynert in dementias of Alzheimer's and Parkinson's disease: a hypothesis for the role of galanin in accentuating cholinergic dysfunction in dementia, *J. Comp. Neurol.*, 273 (1988) 543–557.

[12] Corsi, P. and Coyle, J.T., Nerve growth factor corrects developmental impairments of basal forebrain cholinergic neurons in the trisomy 16 mouse, *Proc. Natl. Acad. Sci. USA*, 88 (1991) 1793–1797.

[13] Coyle, J.T., Price, D.L. and DeLong, M.R., Alzheimer's disease: a disorder of cortical cholinergic innervation, *Science*, 219 (1983) 1184–1190.

[14] Crusio, W.E., Schwegler, H. and Vanabeelen, J.H.F., Behavioural and neuroanatomical divergence between two sublines of C57BL/6J inbred mice, *Behav. Brain Res.*, 42 (1991) 93–97.

[15] Dreyfus, C.F., Effects of nerve growth factor on cholinergic brain neurons, *Trends Pharmacol. Sci.*, 10 (1989) 145–149.

[16] Dunnett, S.B., Rogers, D.C. and Jones, G.H., Effects of nucleus basalis magnocellularis lesions in rats on delayed matching and non-matching to position tasks. Disruption of conditional discrimination learning but not of short-term memory, *Eur. J. Neurosci.*, 1 (1989) 395–406.

[17] Ebel, A., Hermetet, J.C. and Mandel, P., Comparative study of acetylcholinesterase and choline acetyltransferase enzyme activity in brain of DBA and C57 mice, *Nature New Biol.*, 242 (1973) 56–58.

[18] Fischer, W., Gage, F.H. and Björklund, A., Degenerative changes in forebrain cholinergic nuclei correlate with cognitive impairments in aged rats, *Eur. J. Neurosci.*, 1 (1989) 34–45.

[19] Gozzo, S., Renzi, P. and D'Udine, B., Morphological differences in cerebral cortex and corpus callosum are genetically determined in two different strains of mice, *Int. J. Neurosci.*, 9 (1979) 91–96.

[20] Herkenham, M. and Pert, C.B., Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum, *Nature*, 291 (1981) 415–418.

[21] Hoskins, J.A. and Davis, L.J., The acute effect on levels of catecholamines and metabolites in brain, of a single dose of MPTP in 8 strains of mice, *Neuropharmacology*, 28 (1989) 1389–1397.

[22] Iacopino, C., Altavista, M.C., Gozzo, S. and Albanese, A., Quantitative pharmacohistochemistry of acetylcholinesterase in neostriatum of inbred strains of mice, *Brain Res.*, 374 (1986) 402–408.

[23] Johnston, M.V., McKinney, M. and Coyle, J.T., Evidence for a cholinergic projection to neocortex from neurons in basal forebrain, *Proc. Natl. Acad. Sci. USA*, 76 (1979) 5392–5396.

[24] Levey, A.I., Wainer, B.H., Mufson, E.J. and Mesulam, M.-M., Co-localization of acetylcholinesterase and choline acetyltransferase in the rat cerebrum, *Neuroscience*, 9 (1983) 9–22.

[25] Mandel, P., Ebel, A., Hermetet, J.C., Bovet, D. and Oliverio, A., Études des enzymes du système cholinergique chez les hybrides F1 de souris se distinguant par leur aptitude au conditionnement, *C.R. Acad. Sci. (Série D)*, 276 (1973) 395–398.

[26] Mantione, C.R., Zigmond, M.J., Fisher, A. and Hanin, I., Selective presynaptic cholinergic neurotoxicity following intrahippocampal AF64A injection in rats, *J. Neurochem.*, 41 (1983) 251–255.

[27] Mayeux, R., Therapeutic strategies in Alzheimer's disease, *Neurology*, 40 (1990) 175–180.

[28] McGeer, P.L., McGeer, E.G., Suzuki, J., Dolman, C.E. and Nagai, T., Aging, Alzheimer's disease, and the cholinergic system of the basal forebrain, *Neurology*, 34 (1984) 741–745.

[29] McNeill, T.H. and Koek, L.L., Differential effects of advancing age on neurotransmitter cell loss in the substantia nigra and striatum of C57BL/6N mice, *Brain Res.*, 521 (1990) 107–117.

[30] Mitchell, S.J., Rawlins, J.N.P., Steward, O. and Olton, D.S., Medial septal area lesions disrupt theta rhythm and cholinergic staining in medial entorhinal cortex and produce impaired radial arm maze behavior in rats, *J. Neurosci.*, 2 (1982) 292–302.

[31] Mobley, W.C., Rutkowski, J.L., Tennekoon, G.I., Buchanan, K. and Johnston, M.V., Choline acetyltransferase activity in striatum of neonatal rats increased by nerve growth factor, *Science*, 229 (1985) 284–287.

[32] Oliverio, A., Castellano, C., Ebel, A. and Mandel, P., A genetic analysis of behavior: a neurochemical approach, *Adv. Biochem. Psychopharmacol.*, 11 (1974) 411–418.

[33] Pearson, R.C.A., Sofroniew, M.V. and Powell, T.P.S., Hypertrophy of immunohistochemically identified cholinergic neurons of the besal nucleus of Meynert following ablation of the contralateral cortex in the rat, *Brain Res.*, 311 (1984) 194–198.

[34] Pryor, G.T., Schlesinger, K. and Calhoun, W.H., Differences in brain enzymes among five inbred strains of mice, *Life Sci.*, 5 (1966) 2105–2111.

[35] Ransmayr, G., Cervera, P., Hirsch, E., Ruberg, M., Hersh, L.B., Duyckaerts, C., Hauw, J.J., Delumeau, C. and Agid, Y., Choline acetyltransferase-like immunoreactivity in the hippocampal formation of control subjects and patients with Alzheimer's disease, *Neuroscience*, 32 (1989) 701–714.

[36] Ridley, R.M., Aitken, D.M. and Baker, H.F., Learning about rules but not about reward is impaired following lesions of the cholinergic projection to the hippocampus, *Brain Res.*, 502 (1989) 306–318.

[37] Ridley, R.M. and Baker, H.F., Can fetal neural transplants restore function in monkeys with lesion-induced behavioural deficits?, *Trends Neurosci.*, 14 (1991) 366–370.

[38] Rossor, M.N., Svendsen, C., Hunt, S.P., Mountjoy, C.Q., Roth, M. and Iversen, L.L., The substantia innominata in Alzheimer's disease: an histochemical and biochemical study of cholinergic marker enzymes, *Neurosci. Lett.*, 28 (1982) 217–222.

[39] Samorajski, T., Delaney, C. and Durham, L., Effect of exercise on longevity, body weight, locomotor performance, and passive-avoidance memory of C57BL/6J mice, *Neurobiol. Aging*, 6 (1985) 17–24.

[40] Sansone, M., Ammassari-Teule, M., Renzi, P. and Oliverio, A., Differeneffects of apomorphine on locomotor activity in C57BL/6 and DBA/2 mice, *Pharmacol. Biochem. Behav.*, 14 (1981) 741–743.

[41] Schwab, C., Bruckner, G., Castellano, C., Oliverio, A. and Biesold, D., Different levels of acetylcholinesterase and choline acetyltransferase activities in C57Bl/6 and DBA/2 mice are not accompanied with different density of cortical acetylcholinesterase reactive fibers, *Neurochem. Res.*, 15 (1990) 1127–1133.

[42] Schwab, C., Bruckner, G., Rothe, T., Castellano, C. and Oliverio, A., Autoradiography of muscarinic cholinergic receptors in cortical and subcortical brain regions of C57BL/6 and DBA/2 mice, *Neurochem. Res.*, 17 (1992) 1057–1062.

[43] Sidman, A.L., Angevine, J.B. and Taber Pierce, E., *Atlas of the Mouse Brain and Spinal Cord*, Harvard University Press, Cambridge, USA, 1971.

[44] Sofroniew, M.V. and Pearson, R.C.A., Degeneration of cholinergic neurons in the basal nucleus following kainic or *N*-methyl-D-aspartic acid application to the cerebral cortex in the rat, *Brain Res.*, 339 (1985) 186–190.

[45] Tunnicliff, G., Wimer, C.C. and Wimer, R.E., Relationships between neurotransmitter metabolism and behaviour in seven inbred strains of mice, *Brain Res.*, 61 (1973) 428–434.

[46] Ueki, A. and Miyoshi, K., Reversal of learning impairment in ventral globus pallidus-lesioned rats by combination of continuous intracerebroventricular choline infusion and oral cholinergic drug administration, *Brain Res.*, 547 (1991) 99–109.

[47] Virgili, M., Contestabile, A. and Barnabei, O., Postnatal maturation of cholinergic markers in forebrain regions of C57BL/6 mice, *Dev. Brain Res.*, 63 (1991) 281–285.

[48] Vogels, O.J.M., Broere, C.A.J., ter Laak, H.J., ten Donkelaar, H.J., Nieuwenhuys, R. and Schulte, B.P.M., Cell loss and shrinkage in the nucleus basalis Meynert complex in Alzheimer's disease, *Neurobiol. Aging*, 11 (1990) 3–13.