

Estratto da: New Trends Funct. Neurol. - N° 3 - July-September 1987

functional neurology

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CIC EDIZIONI INTERNAZIONALI

A GENETIC STUDY OF NEOSTRIATAL CHOLINERGIC NEURONES IN C57BL/6 AND DBA/2 MICE

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SUMMARY

In the present study Butcher's pharmacohistochemical technique for acetylcholinesterase has been employed for a morphometrical analysis of striatal cholinergic neurones in crossbred C57BL/6 x DBA/2 F₁ mice. The general organization of neostriatal cholinergic systems in hybrid mice was similar to that of parent strains. However, as shown by morphometry, the size of neostriatum in hybrids was larger than that of both parental strains, and the density of striatal cholinergic neurones was significantly lower than that of DBA/2 mice, being close to that of the C57BL/6 strain. The present data indicate that a reduced number of striatal cholinergic neurones is inherited as a dominant trait by these hybrid mice.

KEY WORDS

Acetylcholinesterase, animal strains, basal ganglia, cholinergic neurones, genetic study.

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INTRODUCTION

In a previous series of studies on C57BL/6 and DBA/2 mice, two inbred strains which are characterized by opposite open field activities and learning abilities (1), we reported that DBA/2 mice possess a higher number of forebrain cholinergic neurones than C57BL/6 (2,3). This indicated that morphological differences in the organization of cholinergic neural systems are dependent on the genetic make-up, and, in addition, that a reduced forebrain cholinergic population may determine

the worse avoidance scores obtained by C57BL/6 mice (4). Behavioral patterns have also been studied in the F_1 C57BL/6 x DBA/2 offspring. Avoidance scores showed by these hybrid mice are similar to those of the C57BL/6 parental strain, and differ significantly from the ones of DBA/2 mice (4,5). Therefore, the question arises as to whether the morphological variations, which are present in parental strains, can be transmitted to the F_1 offspring, and, in the affirmative, whether the morphological organization of cholinergic neurones in F_1 hybrids may be responsible for their learning capabilities.

Until the availability of reliable immunocytochemical techniques for choline acetyltransferase (CAT), Butcher's pharmacohistochemical procedure for acetylcholinesterase (AChE) (6) has been effectively used to stain putative cholinergic neurones in the brain. Later studies comparing the staining patterns obtained by using this technique and CAT immunocytochemistry have shown that Butcher's procedure reliably stains neostriatal cholinergic neurones (see refs. 7,8). Therefore, this technique has been used in the present study as a means to analyze the morphometric arrangement of cholinergic neurones in the neostriatum of C57BL/6 x DBA/2 F_1 offspring.

METHODS

In the present study, male F_1 C57BL/6 x DBA/2 hybrids (hereafter referred to as F_1 hybrids) were studied at 18 weeks of age. In each experiment four C57BL/6 σ x DBA/2 σ (hereafter called B6xD2) and four DBA/2 σ x C57BL/6 σ (D2xB6) were processed in a parallel fashion. Six h prior to perfusion, the animals were poisoned with di-isopropylfluorophosphate (DFP); this irreversible AChE inhibitor was injected intramuscularly as 0.15% arachid oil solution in the dose of 3.5 mg/kg. After deep general anaesthesia with sodium pentobarbital (40 mg/kg i.p.), the animals were perfused intra-cardially with 0.9% saline, followed by 10% phosphate buffered formalin (pH=7.2). The brains were post-fixed in the same formalin solution for 48-72 h before being transferred to cold (4° C) 30% sucrose for an additional 48 h period. Forebrains were cut coronally at 30 μ m intervals according to a standard plane (9). The resulting tissue sections from both F_1 populations (i.e., B6xD2 and D2xB6) were processed in a parallel fashion according to a standard protocol. They were immersed for 30 min into 30 μ M N,N'-bis(1-methyl-ethyl)pyrophosphoramidic anhydride (iso-OMPA) to inhibit butyryl-cholinesterase, and then incubated for 2 h at 21° C in the AChE incubation medium (10). Morphometry was performed by means of a custom-made image analyzer, based on an Apple II microcomputer, on camera lucida drawings of 12 standard forebrain coronal sections in each animal. Only intensely stained AChE-positive neurones, which are known to be cholinergic (8), were recorded on camera lucida charts. Data from the two populations of the F_1 offspring were compared with each other and with data previously obtained from parental strains (2), by means of analysis of variance.

RESULTS

The overall distribution and the morphology of putatively cholinergic neurones in neostriatum paralleled that observed by means of histochemical and immunocytochemical techniques in rodents (11). Thus, large, darkly stained, russet, AChE-containing neurones, which are believed to represent forebrain cholinergic perikarya, were seen in the caudato-putamen, medial septum, nuclei of the diagonal band, and basal nucleus of Meynert in both strains. The comparative study between F_1 hybrids of different parental origin (i.e., B6xD2 and D2xB6, respectively) did not reveal significant differences: therefore all animals were considered as belonging to a homogeneous population (Table I).

TABLE I - SUMMARY OF MEAN VALUES ($\pm$ S.E.M.) IN MORPHOMETRIC ANALYSIS OF NEOSTRIATUM IN THE TWO POPULATIONS OF F_1 HYBRIDS.

	D2xB6	B6xD2	p
Cells	105.45 $\pm$ 3.79	113.85 $\pm$ 2.55	ns
Area (mm ²)	5.02 $\pm$ 0.12	5.02 $\pm$ 0.09	ns
Density (cells/mm ²)	21.21 $\pm$ 0.71	22.79 $\pm$ 0.37	ns

Between generation comparison revealed that the size of neostriatum was significantly larger in hybrids than in parental strains (19.5% larger as compared to C57BL/6 mice, $p < 0.001$; 15% larger as compared to

TABLE II - COMPARATIVE MORPHOMETRY OF NEOSTRIATUM IN C57BL/6 AND DBA/2 MICE, AND IN THEIR F_1 OFFSPRING.

	C57BL/6	DBA/2	F_1
Cells	98.17 $\pm$ 2.71 ^a	123.30 $\pm$ 3.43 ^b	109.65 $\pm$ 2.30
Area (mm ²)	4.13 $\pm$ 0.10	4.30 $\pm$ 0.09	5.02 $\pm$ 0.07 ^c
Density (cells/mm ²)	23.72 $\pm$ 0.43 ^a	28.61 $\pm$ 0.54 ^b	22.01 $\pm$ 0.41 ^d

a: significantly different from DBA/2 ($p < 0.001$)

b: significantly different from C57BL/6 ($p < 0.001$)

c: significantly different from both C57BL/6 and DBA/2 parental strains ($p < 0.001$)

d: significantly different from DBA/2 ($p < 0.001$); not different from C57BL/6.

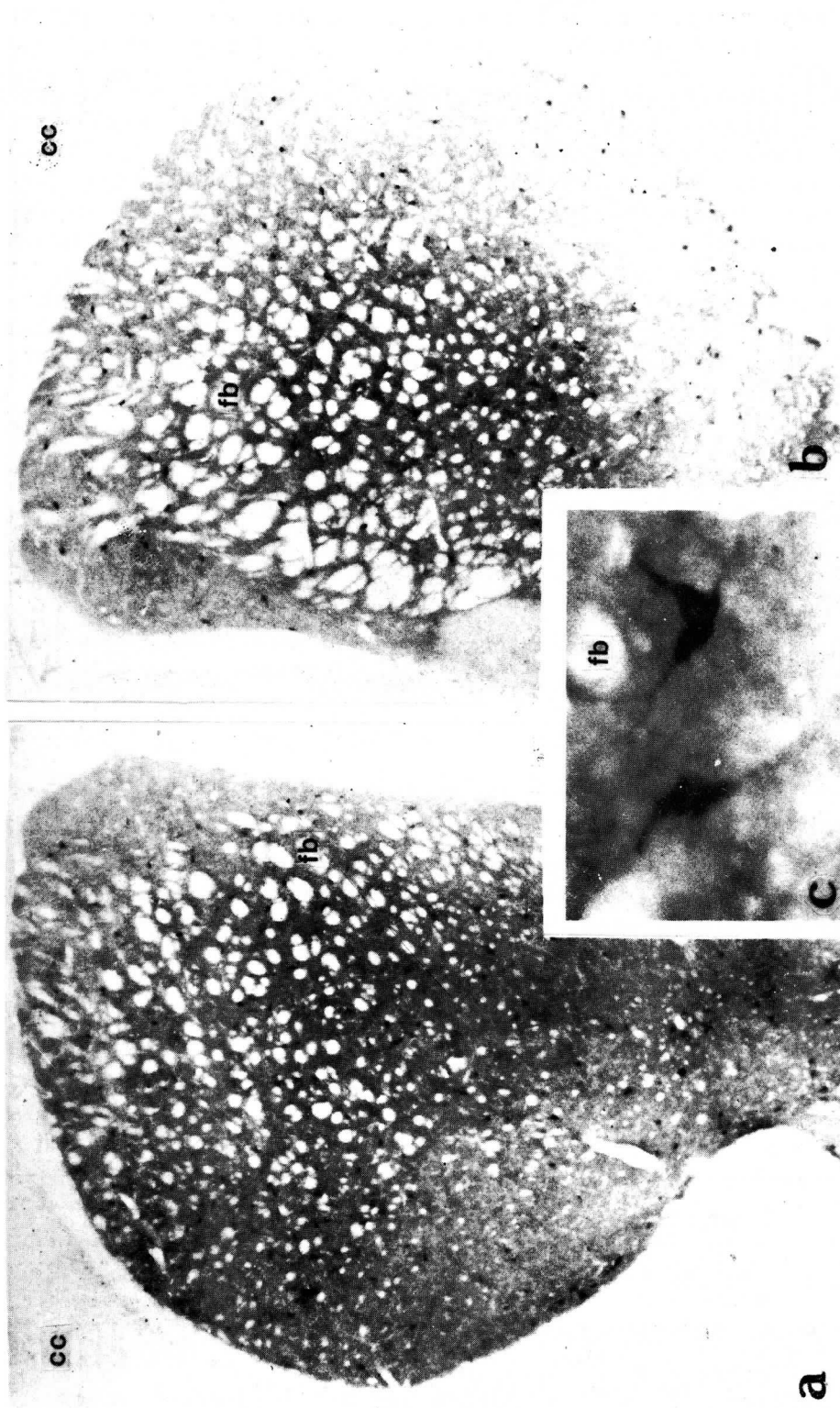


FIG. 1 - Coronal sections of the neostriatum in F_1 hybrids as seen at low (a, b) and high (c) magnifications. a and b show two different rostrocaudal levels. Abbreviations: cc: cerebral cortex; fb: fibre bundle of the internal capsule.

DBA/2, $p < 0.001$). Cell counts performed on the same standard sections showed that the mean number of striatal cholinergic neurones was in between values obtained from C57BL/6 and DBA/2 mice, but it was not significantly different from either parental strain. Neural density in the F_1 offspring was 28% lower than in DBA/2 mice, and it did not differ from that of C57BL/6 strain (Table II).

DISCUSSION

The present data indicate that the general organization of striatal cholinergic neurones in F_1 hybrids is similar to that seen in parental strains, as well as in other rodents (11) (Fig. 1). They also show that the size of neostriatum is larger in F_1 hybrids than in parents. This finding reflects a genetic phenomenon, known as heterosis or "hybrid vigour", by which the crossbred F_1 offspring from two inbred parental lines either deviate significantly from the expected midparent value or exceed the extreme parental value (see ref. 12). The present study does not allow to determine whether the larger striatal size depends on an increase of striatal cell number (either neurones or glial cells) or of the extracellular matrix. In addition, it is also possible that variations in the size of fibre bundles crossing the neostriatum may account for changes in the overall striatal size. Unfortunately, technical limitations did not allow us to evaluate the net parenchymal size of neostriatum.

Still, as shown by this study, heterosis is likely to affect the number of striatal cholinergic neurones in hybrids. In fact, cell counts performed in the neostriatum revealed that, although it does not reach statistical significance, the global number of counted neurones in F_1 hybrids is in between that of parental strains. Since the size of neostriatum in F_1 hybrids is significantly larger than that of both parental strains, neural density of striatal cholinergic neurones in F_1 hybrids turns out to be similar to that of the C57BL/6 mice (Table II). This is in keeping with earlier biochemical observations, which have shown that, in B6xD2 F_1 hybrids, the activity of enzymes related to the metabolism of acetylcholine is similar to that of parental strain C57BL/6. In fact, Van Abeelen (13) reported that, as compared to DBA/2 mice, whole brain AChE activity is reduced both in C57BL/6 and in F_1 hybrids. Similar results have also been obtained by measuring choline acetyltransferase activity in the temporal cortex of C57BL/6 and DBA/2 mice, and in their F_1 offspring (14).

Interestingly, behavioural features, which are probably related to brain cholinergic hypo-activity (14), indicate that hybrids display low learning ability scores and high scores in open field tests, as C57BL/6 mice do (5,15). Taking together morphological, biochemical and behavioural data, it is apparent that F_1 mice display a genetically determined central nervous system cholinergic hypo-activity, which is based on the presence of a reduced number of cholinergic cell bodies, and is inherited as a dominant trait.

Therefore, variations in the anatomo-functional organization of central nervous system cholinergic neurones may represent the substrate of some genetically determined behavioural variations. To this respect, however, the factors responsible for the development and viability of central nervous system cholinergic neurones are still unknown. Furthermore, when considering the complexity of functions exerted by central nervous system cholinergic pathways, it can be assumed that multiple genes are responsible for these morpho-functional variations. Still, on the grounds of present knowledge, a single-gene effect cannot be ruled out, and this is matter for future investigations. The understanding of genetic control of central nervous system cholinergic activity may have some important clinical implications. In fact, it has been suggested that in Alzheimer disease the deficiency of a neurotrophic factor specific to cholinergic neurones may occur (16); this can bring about the depletion of cholinergic perikarya as seen in these patients (17). Still, further neurobiological studies are required before thorough clinical conclusions can be drawn.

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Received: February 5, 1987

Accepted: April 7, 1987