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Transient increase in the number of cholinergic neurons in the developing rat dentate gyrus

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Using a monoclonal antibody against choline acetyltransferase (ChAT), we have examined the distribution of cholinergic neurons in the rat dentate gyrus during development ChAT-positive neurons were occasionally detected in the hilus on postnatal day 2 (P2) There was a transient abrupt increase in the number and density of ChAT-positive neurons between P15 and P20 and then a decline to the adult level with few ChAT-immunoreactive neurons. A few ChAT-positive varicose fibers and punctae were first seen at P5 They increased in number and density until P20 when they reached the adult level and distribution These observations suggest the occurrence of a transient expression of cholinergic markers in the hippocampus

The distribution of cholinergic neurons during development has been studied by means of the acetylcholinesterase (AChE) stain. AChE, however, may be present in both cholinergic and non-cholinergic neurons [7]. Choline acetyltransferase (ChAT), the synthesizing enzyme for acetylcholine, is presently accepted as the best marker of cholinergic neurons [13]. In the present study we have used a monoclonal antibody against ChAT and immunocytochemical staining to study the distribution of ChAT-positive neurons in the dentate gyrus of the rat during development.

Sherman rats were used in this study. At least 3 brains were examined on each of the following postnatal days (P): P1, P2, P5, P15, P20, P28 and adult. All animals were killed by perfusion under sodium pentobarbitone anesthesia (20 mg/kg, i.p.) They were perfused through the heart with ice-cold normal saline followed by a fixative containing 3% paraformaldehyde and 0.01% glutaraldehyde in 0.05 M phosphate buffer (pH 7.4, 100 ml/100 g), then they were perfused with the same solution without glutaraldehyde (50 ml/100 g) and finally perfused with a solution containing 10% sucrose in 0.1 M phosphate buffer (25 ml/100 g). The brains were removed and immersed

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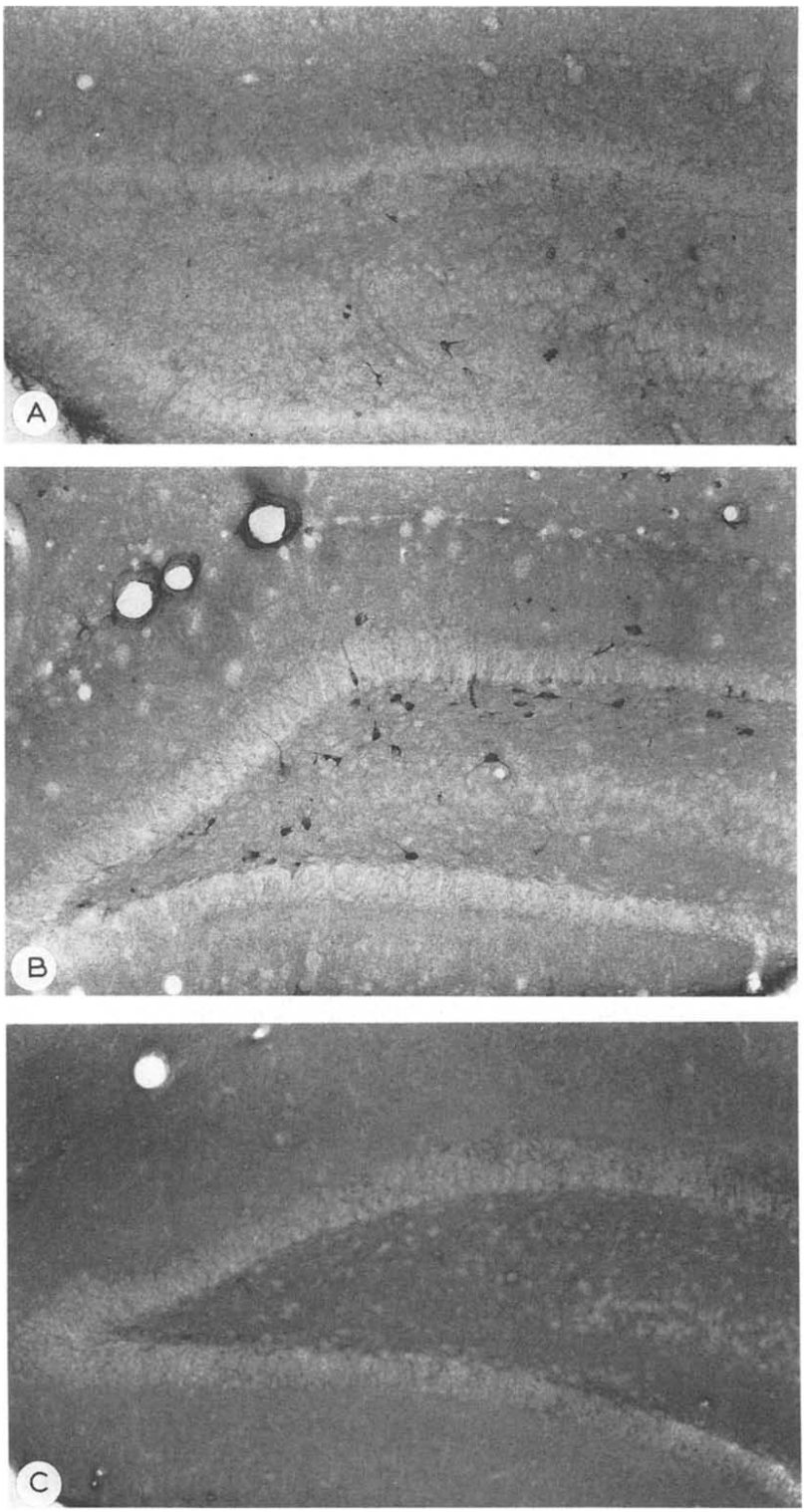
A
B
C

TABLE I

NUMBERS OF ChAT-POSITIVE NEURONS IN THE HILUS OF THE DORSAL DENTATE GYRUS, SHOWING A TRANSIENT, SIGNIFICANT INCREASE IN THE NUMBER OF ChAT-POSITIVE NEURONS DURING DEVELOPMENT, IN PARTICULAR BETWEEN P15 AND P20

Age	No of animals	No of sections	No of ChAT-positive neurons	
P1	4	12	0	
P2	3	9	0 33±0 50	
P5	3	9	1 33±1 11	*
P15	3	9	1.55±0 52	*
P20	5	15	17 1±8 38	*
P28	3	9	4.66±3 87	*
Adult	4	12	1.60±1.45	*

*$P < 0.01$ non-parametric, Mann–Withney, U-test

in 0.1 M phosphate buffer containing 30% sucrose for 1 or 2 days. Frontal sections (30 μm) were cut on a cryostat.

Immunocytochemistry was performed with a monoclonal antibody against ChAT from rat–mouse hybridoma (Type I, Boehringer Mannheim, F R.G.) [4]. The peroxidase–antiperoxidase (PAP) technique was applied. The sections were rinsed in ice-cold phosphate-buffered saline (PBS) 3 times for 10 min and incubated for 2 h at room temperature with rabbit anti-rat IgG (Miles Scientific, U.S.A.) diluted 1:250 with PBS containing 1% normal rabbit serum. The sections were again rinsed in PBS 3 times for 10 min and incubated for 4 days at 4°C with 1:20 anti ChAT in PBS. After rinsing they were incubated for 2 h at room temperature with rat PAP complex (Miles Scientific) diluted 1:100 with PBS containing 1% normal rabbit serum. After rinsing twice with PBS and once with Tris buffer (0.05 M, pH 7.6), the sections were treated for 10 min with a 0.05% solution of 3,3'-diaminobenzidine (DAB) in Tris buffer followed by the same solution of DAB containing 0.01% hydrogen peroxidase for 5 or 10 min. In control experiments, i.e. when the sections were treated in the same way but with rat serum instead of the primary antibody, immunoreactive neurons or fibers were not observed. Furthermore, sections containing the septum and diagonal band regions immunostained together with hippocampal sections revealed the typical staining pattern which has been reported in earlier studies [2, 5, 6]. Immunolabeled tissue sections were then mounted onto glass slides, air-dried, dehydrated with graded alcohols and xylene, and then covered with glass coverslips and Eukitt. The remaining sections were stained with Cresyl violet for identification of the brain regions.

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Fig 1 Photographs of ChAT-positive neurons, varicose fibers and punctae at different ages in dentate gyrus A at P5 ChAT-positive neurons are seen in the hilus Fine varicose fibers and punctae are also labeled (× 150) B. at p20. There is a remarkable increase in the number of ChAT-positive neurons and immunoreactivity of varicose fibers and punctae (× 75) C adult only 2 positive neurons are seen (× 75)

At P2, ChAT-positive neurons were occasionally observed in the hilus but fibers were not clearly detected At P5, as few ChAT-positive neurons were observed in the hilus and very fine varicose fibers and punctae were for the first time seen in this area (Fig 1A) An increase in the number of ChAT-positive neurons and immunoreactivity of varicose fibers and punctae was observed at P20 (Figs 1B, 2) In the adult, in keeping with earlier studies [5, 8, 14], only a few ChAT-immunoreactive neurons were seen, but varicose fibers and punctae were observed in all layers of this region, in particular in the supragranular layer (Fig. 1C). At all ages, ChAT-positive neurons were observed mainly in the hilus of the dentate gyrus although they were occasionally seen in the molecular and granular layers. Table I shows quantitative values obtained by counting numbers of ChAT-positive neurons from camera lucida drawings in the hilus at the dorsal level. The number of ChAT-positive neurons was significantly higher at P20 than at earlier ages ($P < 0.01$, Mann–Whitney); furthermore, there was a significant decrease in the number of ChAT-positive neurons from P20 to P28 ($P < 0.01$ Mann-Whitney) Fig. 2 shows typical ChAT-positive neurons at P20 These neurons were mainly fusiform or multipolar, and the labeled cytoplasm surrounded unstained nuclei and extended into dendritic-like processes which often oriented parallel to the granule cell layer.

The principal results of the present study are that ChAT-positive neurons are first

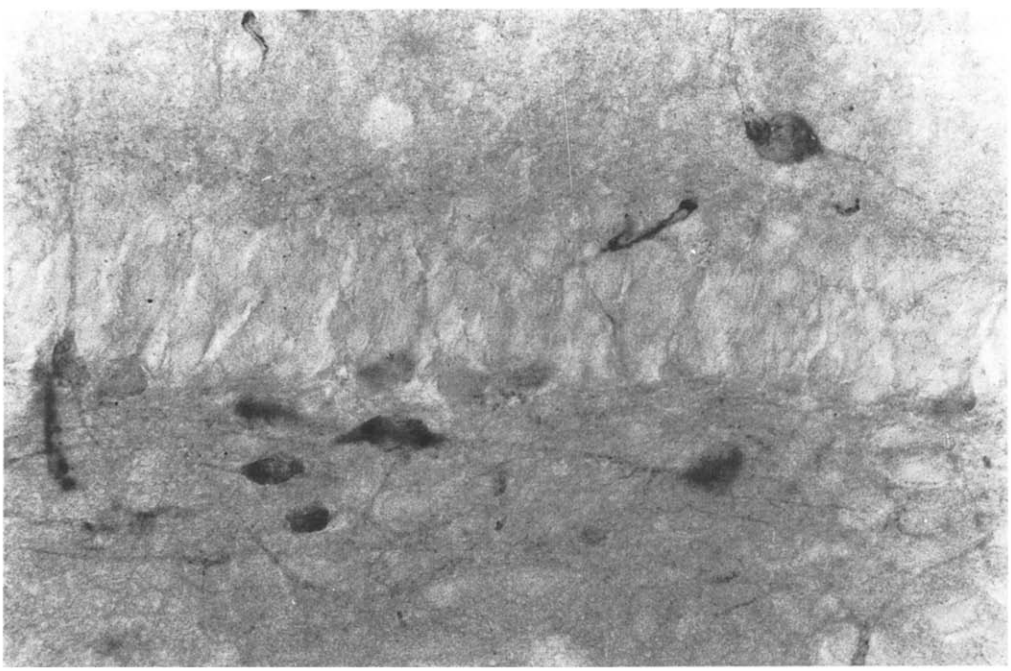

Fig 2 Photograph of ChAT-positive neurons, varicose fibers and punctae at P20 with high magnification (×400) ChAT-positive neurons are fusiform or multipolar and the labeled cytoplasm surrounds unstained nuclei and extends into dendritic-like processes which are often oriented parallel to the granule cell layer

seen between P2 and P5 in the dentate gyrus and that there is a significant increase in number at P20 and then a decline. At P20, ChAT-positive neurons are found mainly in the hilus and occasionally in the granular and molecular layers. This localization of ChAT-positive neurons is comparable to acetylcholinesterase staining during development [9]. It is of interest to note that ChAT-positive neurons are reminiscent of Amaral's multipolar or fusiform cells [1] and that their distribution is comparable to that of somatostatin-reactive cells [12, 15]. ChAT-positive varicose fibers and punctae were found at P5 and increased in density until P20 when they reached the adult level and distribution, with ChAT-positive fibers and punctae in all layers of dentate gyrus in agreement with Frotscher and Leranth [5]. The observed low number of ChAT-positive neurons in adult rat is in general agreement with earlier studies [5, 8, 14].

Our results suggests that ChAT is transiently expressed in neurons in the developing rat dentate gyrus between P15 and P28. The role of the transient increase in the number of ChAT-positive neurons is unknown. An increased production of ChAT in the medial septum and diagonal band between one and two weeks after birth, previously described both with immunocytochemical [2] and biochemical methods [11], may precede the transient expression of ChAT in the hippocampus around the 3rd week of postnatal life. This corresponds to a phase with maximal axonal growth and arborization in this region [3]. A similar transient expression of neurotransmitter markers has been observed in the developing neocortex [10]. These transient phenomena appear to involve both early and late forming neurons as well as afferent and efferent projections.

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