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Development of Cholinergic and GABAergic Neurons in the Rat Medial Septum: Different Onset of Choline Acetyltransferase and Glutamate Decarboxylase mRNA Expression

ROLAND BENDER, MARTINA PLASCHKE, THOMAS NAUMANN, PETRA WAHLE, AND MICHAEL FROTSCHER
Institute of Anatomy, University of Freiburg, D-79001 Freiburg (R.B.,T.N.,M.P.,M.F.), and Department of Zoology and Neurobiology, Ruhr University Bochum, D-44780 Bochum (P.W.), Germany

ABSTRACT

In the present study, we have investigated the developmental expression of the transmitter-synthesizing enzymes choline acetyltransferase (ChAT) and glutamate decarboxylase (GAD) in rat medial septal neurons by using in situ hybridization histochemistry. In addition, we have employed immunostaining for ChAT and the calcium-binding protein parvalbumin, known to be contained in septohippocampal GABAergic neurons.

A large number of GAD67 mRNA-expressing neurons were already observed in the septal complex on embryonic day (E) 17, the earliest time point studied. During later developmental stages, there was mainly an increase in the intensity of labeling. Neurons expressing ChAT mRNA were first recognized at E 20, and their number slowly increased during postnatal development of the septal region. The adult pattern of ChAT mRNA-expressing neurons was observed around postnatal day (P) 16. By using a monoclonal ChAT antibody, the first immunoreactive cells were not seen before P 8. Similarly, the first weakly parvalbumin-immunoreactive neurons were seen in the septal complex by the end of the 1st postnatal week.

These results indicate that in situ hybridization histochemistry may be an adequate method to monitor the different development of transmitter biosynthesis in cholinergic and GABAergic septal neurons. Moreover, the late onset of ChAT mRNA expression would be compatible with a role of target-derived factors for the differentiation of the cholinergic phenotype.

Indexing terms: septohippocampal projection, choline acetyltransferase, GABA, in situ hybridization, parvalbumin

The factors determining the transmitter phenotype of a neuron are poorly understood. Among other factors, diffusible molecules delivered by cells in the target region may play a role. As an example, the expression of choline acetyltransferase (ChAT), the acetylcholine-synthesizing enzyme, in septohippocampal cholinergic neurons is assumed to depend on target-derived nerve growth factor (NGF; Gnahn et al., 1983; Seiler and Schwab, 1984; Korsching et al., 1985; Gage et al., 1986, 1988, 1989; Hefti, 1986; Large et al., 1986; Mobley et al., 1986; Williams et al., 1986; Armstrong et al., 1987b; Auburger et al., 1987; Kromer, 1987; Barde, 1989; Cavicchioli et al., 1989, 1991; Li et al., 1995). One approach to find out to what extent the target region may be involved in the determination of transmitter phenotype is to correlate the developmental expression of transmitter-synthesizing enzymes with the ingrowth of fiber systems into the target region. As far as the projection of neurons in the septal complex is concerned, our previous studies have shown that first septohippocampal fibers invade the hippocampus at embryonic day

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Martina Plaschke is now at Institute of Anatomy, Humboldt University (Charité), D-10098 Berlin, Germany.

Address reprint requests to Michael Frotscher, Institute of Anatomy, University of Freiburg, P.O. Box 111, D-79001 Freiburg, Germany.

(E) 19, but the majority of fibers arrive shortly after birth (Linke and Frotscher, 1993; Linke et al., 1995). Immunocytochemical and biochemical studies on the development of cholinergic septal cells, which could be correlated to the tracer studies, have so far been controversial (Schambra et al., 1989; Thal et al., 1991). Thus, Armstrong et al. (1987a), when using a polyclonal antibody against ChAT (Bruce et al., 1985), found immunoreactive neurons in the septal complex as early as E 17, whereas Sofroniew et al. (1987), who used a monoclonal antibody (Eckenstein and Thoenen, 1982), did not find immunoreactive neurons before postnatal day (P) 7. Gould et al. (1991) observed ChAT-immunoreactive neurons at P 1, but did not study embryonic stages.

As far as the GABAergic septohippocampal projection is concerned, one is faced with the problem that GABAergic projection neurons do not stain well in immunocytochemical studies with antibodies against GABA or its synthesizing enzyme glutamate decarboxylase (GAD) unless colchicine is applied (Ribak et al., 1978; Onténiente et al., 1986; Kiss et al., 1990; Tóth et al., 1993). In developmental studies, the first GABA-immunoreactive neurons in the basal forebrain were found as early as E 16 (Lauder et al., 1986).

In the present study, we have used in situ hybridization for ChAT- and GAD mRNA in order to monitor the expression of these two transmitter-synthesizing enzymes during development and to correlate these findings with known data from the literature on the development of the septohippocampal projection. These studies were supplemented by immunocytochemical experiments employing antibodies against ChAT and the calcium-binding protein parvalbumin (PARV), which is known to be contained in GABAergic septohippocampal projection neurons (Freund, 1989; Kiss et al., 1990).

MATERIALS AND METHODS

Tissue preparation for in situ hybridization

Male and female Sprague-Dawley rats were used in the present study. For prenatal stages, exactly timed pregnant females were obtained from the Zentralinstitut für Versuchstierzucht (Hannover, Germany). Pregnant dams were deeply anesthetized with a mixture of 25% Ketavet (Parke-Davis, 100 mg/ml), 6% Rompun (Bayer), and 2.5% Vetranquil (Sanofi, 13.56 mg acepromacine/ml) at a dose of 2.5 ml/kg body weight. The embryos were delivered by cesarean section and either were transcardially perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer or were decapitated and the heads fixed by immersion in the same solution. In both groups the brains were dissected out of the skull and postfixed for 4 hours in 4% PFA after the initial fixation. As no differences were observed between perfusion-fixed and immersion-fixed animals, rats of both groups were pooled. For postnatal stages, animals of our own breeding colony were anesthetized, transcardially perfused, and the brains postfixed as described above. A total of 40 animals of different age (E 17 and E 20, P 1, P 3, P 5, P 7, P 9, P 11, P 16, and P 22; n = 4 each) were used. Adult animals (P 70) were used as controls. After fixation the brains were cryoprotected overnight in 20% sucrose in phosphate-buffered saline (PBS) containing 0.1% diethylpyrocarbonate (DEPC) to inactivate RNases, then frozen in isopentane (−40°C) and stored up to 3 months at −70°C.

Nonradioactive in situ hybridization

Forty-micron-thick frontal sections of the septal region were cut on a cryotom and processed as described (Wahle and Beckh, 1992). Sections were collected in 2 × SSC (0.3 M NaCl; 0.3 M sodium citrate) and washed for 2 × 15 minutes to remove the sucrose. They were then transferred to a mixture (1:1) of 2 × SSC and hybridization solution (15 minutes) before prehybridizing with hybridization solution (50% formamide, 4 × SSC, 250 μg/ml denatured salmon sperm DNA, 100 μg/ml yeast tRNA, 5% dextrane sulfate, 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.05 M phosphate buffer) at 47°C for 1–4 hours.

For hybridization digoxigenin-labeled ChAT or GAD67 antisense as well as sense probes (diluted to ca. 0.2 ng/μl) were applied, and the sections were incubated overnight at 47°C. For all steps RNase-free (DEPC-treated) solutions and sterile six-well plates were used. After washing, hybrid molecules were detected with digoxigenin antibody conjugated to alkaline phosphatase (Boehringer, Mannheim, Germany), which resulted in blue staining of the hybrid-containing cells. The color reaction was stopped after 5 hours (GAD) or 8–10 hours (ChAT) by transferring the sections to Tris-buffered saline (0.1 M Tris-HCl, pH 7.5; 0.15 M NaCl).

Finally, the sections were immersed in a solution of 4′,6-diamidino-2-phenylindole (DAPI; 20 μg/ml) to counterstain cell nuclei by a fluorescence marker, mounted on glass slides, embedded with gelatin, and coverslipped.

Probes

The 2.3-kB cDNA for feline GAD67 was kindly provided by Dr. Allan Tobin (Kaufman et al., 1986). It was inserted into transcription vector pSP65 in sense and antisense orientation. To generate transcripts of 1 kB, the cDNA was linearized with SalI. Riboprobes were transcribed from 1 μg of template DNA in the presence of digoxigenin (DIG)-UTP according to the manufacturer's recommendation (Boehringer, Mannheim, Germany). The labeled RNA (about 5–10 μg) was dissolved in 100 μl of TE buffer (100 mM Tris, pH 7.5; 50 mM EDTA, pH 8.0; 100 μg yeast tRNA; 50 U/ml RNA guard; Pharmacia). The probes were stored in aliquots at −70°C for more than 6 months without noticeable loss in activity. To check transcription efficiency, the riboprobes were separated on an agarose gel, transferred to nylon membranes (Biodyne, Pall), and the blot was stained according to the manufacturer's protocol (Boehringer).

For the synthesis of riboprobes to detect ChAT mRNA, the polymerase chain reaction (PCR) technique was used. A pair of primers was chosen to amplify a 345-bp fragment from the rat ChAT cDNA (Brice et al., 1989). Primer sequences were complementary to bases 1790–1812 (downstream) and 2135–2113 (upstream). A second primer pair was synthesized containing the same sequences but further supplied with the T7 promoter. PCR using a combination of promoter-containing and promoterless primers resulted in the synthesis of DNA fragments, which could be used as templates to synthesize riboprobes, either in sense or antisense direction. PCR was carried out as described by Förster et al. (1993). Riboprobes were transcribed from 0.3 μg purified template, tested, and stored as described above.

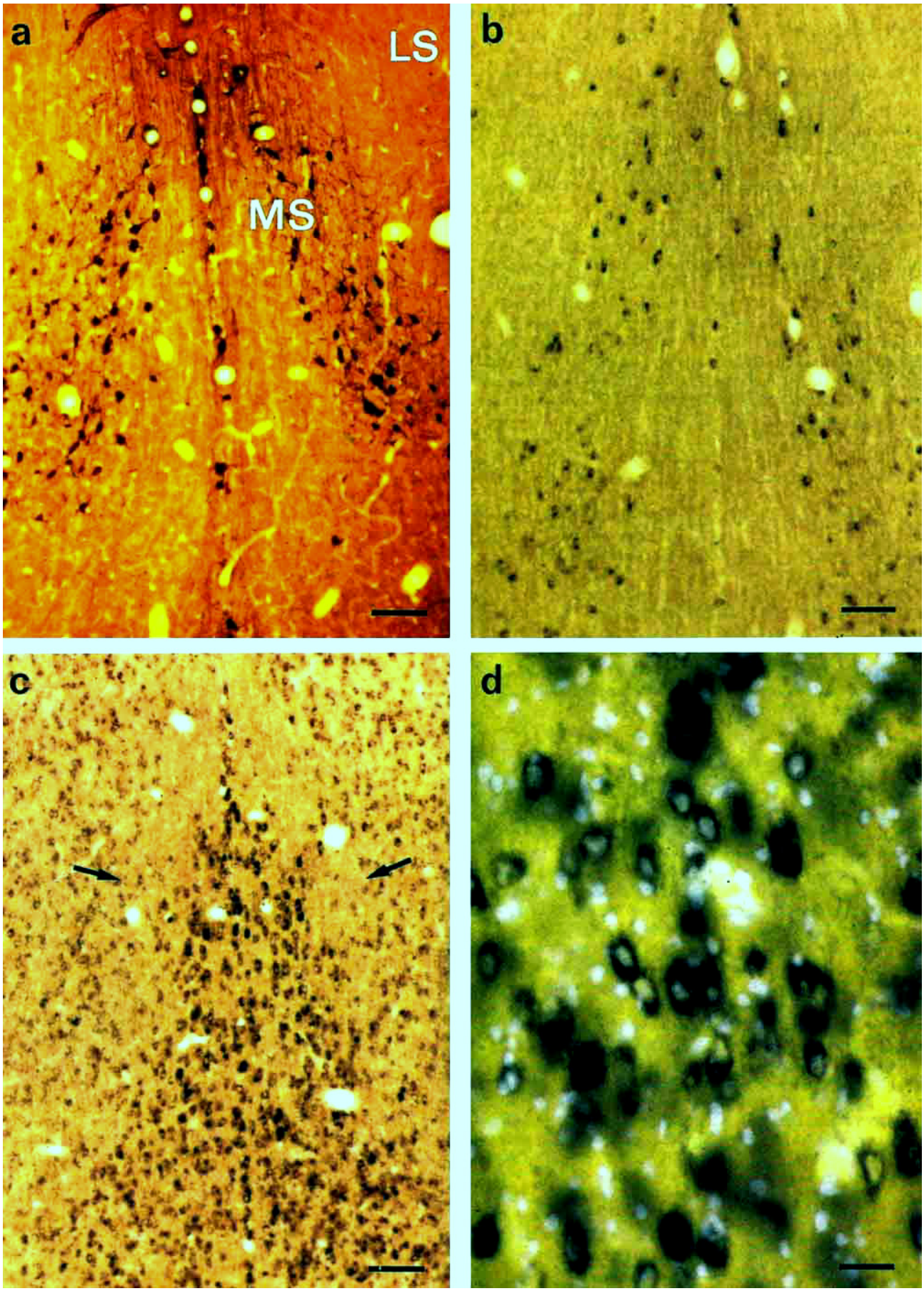

Figure 1

Tissue preparation for immunocytochemistry

Twenty-seven animals of different ages (P 5, P 7, P 8, P 10, P 12, P 15, P 16, P 17, P 20; n = 3 each) were used. The animals were anesthetized as described above and were transcardially perfused with a solution of 4% PFA, 0.1% glutaraldehyde, and 15% saturated picric acid in 0.1 M phosphate buffer (PB, pH 7.35). Brains were removed from the skull and postfixed in 4% PFA for 2 hours.

Frontal serial sections (100 μm) of the septal region were cut on a vibratome, collected in 0.1 M PB, pretreated in graded dimethylsulfoxide solutions (DMSO; 5%, 10%, 20%, 20 minutes each), and further processed either for ChAT or PARV immunocytochemistry.

Immunocytochemistry for ChAT

A monoclonal antibody from a rat–mouse hybridoma cell line (type I; Boehringer, Mannheim, Germany; see Eckenstein and Thoenen, 1982) was used. It was diluted 1:9 in 0.1 M PB containing 1% normal rabbit serum and 0.1% NaN_3. The sections were incubated for 48 hours at 4°C. After washing the sections in 0.1 M PB (5 × 10 minutes), they were incubated with the secondary antibody (biotinylated anti-rat IgG; Vectastain ABC Kit; Vector Laboratories, Burlingame, CA; diluted 1:250 in 0.1 M PB) for 2.5 hours at room temperature. The immunoreaction was visualized by an ABC reaction (Vectastain, ABC Kit, Hsu et al., 1981) with 3,3′-diaminobenzidine (DAB; 0.07% and 0.002% hydrogen peroxide in 0.1 M PB for 15 minutes). Finally, the sections were mounted on glass slides, dehydrated in graded ethanol, and coverslipped using Histokit (Shandon, Pittsburgh, PA).

Immunocytochemistry for PARV

A monoclonal antibody against parvalbumin (Celio et al., 1988) was diluted 1:5,000 in 0.1 M PB containing 1% normal horse serum and 0.1% NaN_3. Sections were incubated for 48 hours at 4°C, followed by incubation with a secondary antibody (biotinylated anti-mouse IgG, diluted 1:250) for 2.5 hours at room temperature. Detection of antibody binding was carried out as described above.

Controls

For a control of immunostaining, some sections were incubated with all but the primary antibodies against ChAT and PARV. No immunostaining occurred under these conditions. Probes transcribed in sense orientation and hybridized to control sections gave no signals.

Quantitative analysis

ChAT mRNA-expressing neurons. For our quantitative analysis we used 40-μm frontal cryostat sections through the septal complex. Between the anterior-posterior levels of the septal complex (see Fig. 1 in Naumann et al., 1994), all cells were counted in each section of the stages E 17, E 20, and P 1. In all other stages, each second section was counted, and the data were extrapolated. Cells were counted using a Zeiss Axioskop (objective lens ×25). All sections were mounted on the slides with their rostral surface upside. All ChAT mRNA-expressing cells in the medial septal nucleus (MS) on both sides were counted independent of their diameter; no corrections for developmental differences in cell size were made. Labeled cells in the vertical limb of the diagonal band of Broca (vDB) were not counted. The MS was demarcated against the vDB by a horizontal line paralleling the anterior commissure. Statistical analysis was based on the total number of ChAT mRNA-expressing cells in each animal and included the mean and standard deviation.

In addition to the cell counts, the volume of the septal region in the various developmental stages was estimated. This was done by taking into account the number of sections between the most anterior and most posterior levels of the septal complex (see Fig. 1 in Naumann et al., 1994) obtained in the various developmental stages, the dorso-ventral extensions of the septal complex (dorsal boundary: corpus callosum; ventral boundary: base of brain), and the lateral extension of the septal region between the two lateral ventricles.

GAD67 mRNA-expressing neurons. In the present study the percentage of GAD mRNA-expressing cells was evaluated in defined regions of the septal complex by double-labeling the sections with DAPI (which stains all nuclei) in addition to labeling the GAD67 mRNA-expressing cells by in situ hybridization (Fig. 1d). In five representative sections from each animal, the percentage of GAD mRNA-expressing cells was determined for defined regions of the lateral septum (LS), MS, and vDB.

RESULTS

In situ hybridization for ChAT mRNA

Nonradioactive in situ hybridization for ChAT and GAD67 mRNA resulted in distinct labeling of cell bodies sparing the nuclei. No staining was observed in dendrites and axons. No labeling was observed with the sense probes. The distinct staining of cell bodies allowed us to determine the number of labeled neurons in sections of the septal complex as well as to describe the shape and size of cell bodies.

In adult rats, in situ hybridization for ChAT mRNA labeled characteristic large neurons in the medial septum and in the diagonal band of Broca as known from many immunocytochemical studies (cf. Fig. 1a,b). Thus, in the medial septum the cholinergic neurons were arranged in a typical inversed V-shaped pattern. The somata of ChAT mRNA-expressing cells in the medial septum were smaller (15–25 μm) than those of cholinergic cells in adjacent regions of the diagonal band of Broca. No labeling of cholinergic neurons was observed in the lateral septum, either with in situ hybridization or immunocytochemistry.

Fig. 1. Demonstration of ChAT protein and mRNA and GAD67 mRNA in frontal sections through the medial septum of a 70-day-old rat. **a:** Immunostaining for ChAT with a monoclonal antibody. Note typical inverse, V-shaped arrangement of cholinergic neurons. MS, medial septum; LS, lateral septum. **b:** ChAT mRNA-expressing neurons visualized by nonradioactive in situ hybridization. **c:** GAD67 mRNA-expressing neurons demonstrated by nonradioactive in situ hybridization histochemistry. Note heavy staining of cells in the central portion of the medial septum. Adjacent portions, corresponding to the V-shaped arrangement of cholinergic neurons (a,b), do not contain many labeled cells (arrows). **d:** Double-labeling of septal neurons by GAD67 mRNA in situ hybridization histochemistry and DAPI fluorescence. A large number of neurons are double-labeled. Scale bars = 100 μm in a–c, 25 μm in d.

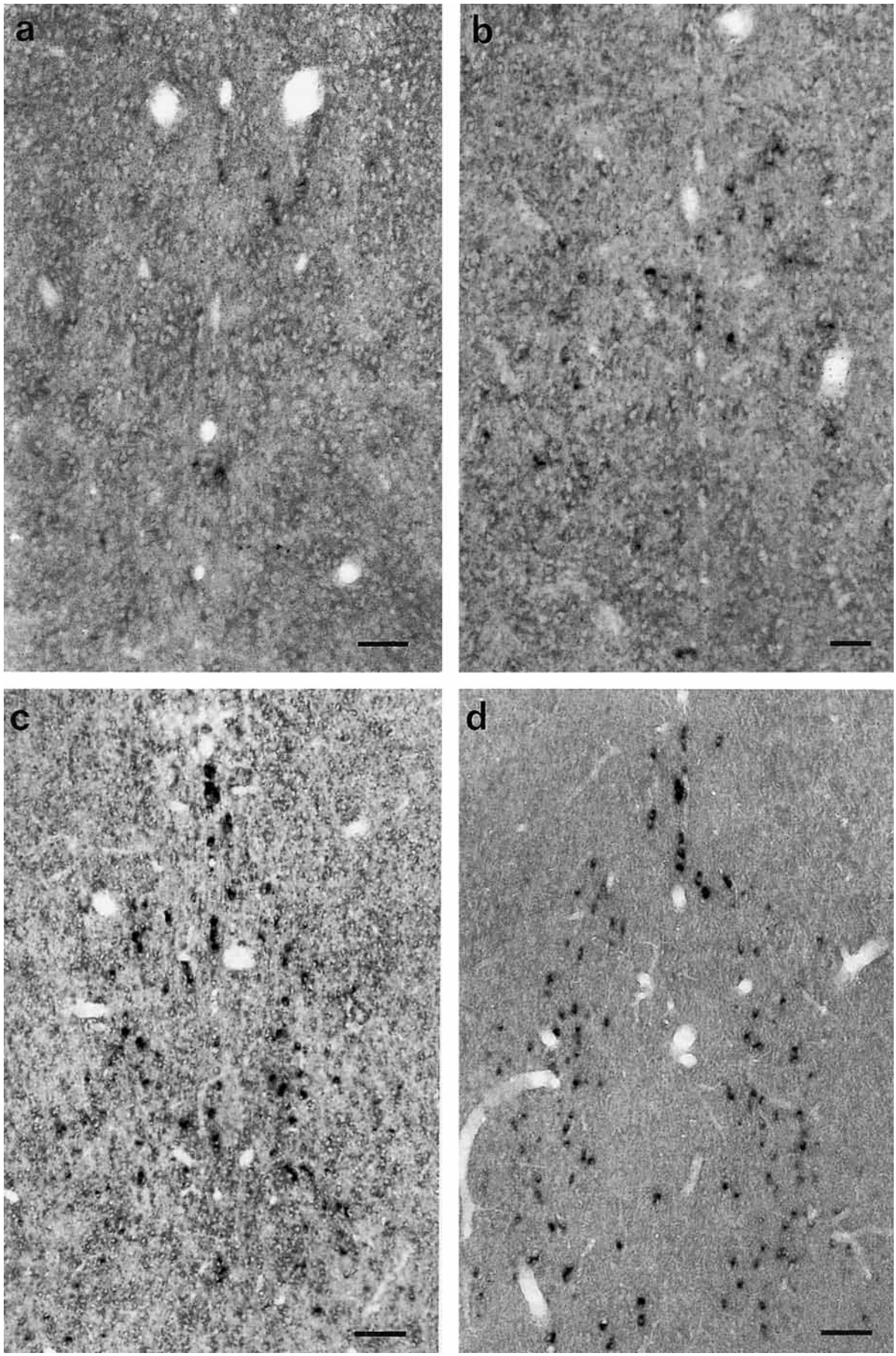

Fig. 2. Frontal cryostat sections through the medial septum stained for ChAT mRNA at different postnatal stages. **a:** Newborn rat (P 1). **b:** P 3. **c:** P 9. **d:** P 16. Note the continuous increase in the number of labeled neurons. The adult pattern is almost reached by P 16. Scale bars = 50 μm in a, 100 μm in b–d.

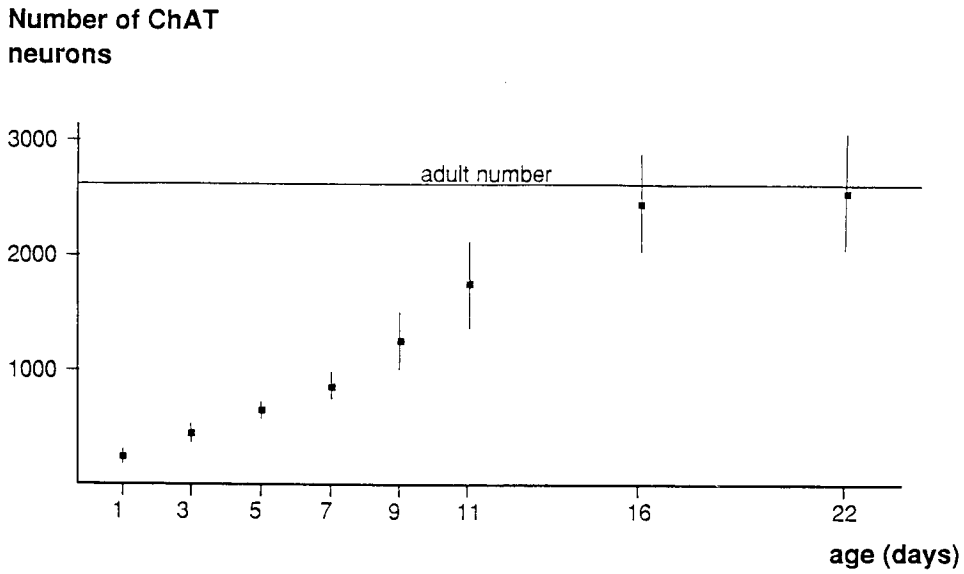

Fig. 3. Total number of ChAT mRNA-expressing neurons in the rat medial septum during postnatal development as determined from cell counts in 40-μm sections. Data expressed as mean ± standard deviation (n = 4 for each stage).

On E 17, the earliest stage analyzed, no ChAT mRNA expression in MS neurons was found yet. The first weakly stained neurons were observed with in situ hybridization at E 20. Heavily stained neurons were not found before P 1 (Fig. 2a). During the 1st postnatal week, the number of ChAT mRNA-expressing neurons increased continuously (Fig. 2b). Beginning with P 9, the characteristic arrangement of cholinergic neurons in the medial septum is recognizable (Fig. 2c). At this postnatal stage, more intensely stained neurons with larger cell bodies than in earlier stages were noticed. The adult pattern of ChAT mRNA-expressing neurons is seen at P 16 (cf. Figs. 2d, 1b). This continuous increase in the number of ChAT mRNA-expressing neurons during the first 2 postnatal weeks is confirmed by our quantitative analysis (Fig. 3).

The number of ChAT mRNA-expressing neurons increased in parallel with the volume of the septal complex in the various developmental stages, which might indicate that the differentiation of cholinergic septal neurons reflects the maturation of the septal complex as a whole. It should be kept in mind, however, that our volume measurements were not restricted to the MS where the ChAT mRNA-expressing neurons were counted.

In situ hybridization for GAD67 mRNA

In the septal complex of adult rats, a large number of GAD67 mRNA-expressing neurons are found (Fig. 1c). Roughly two types of cells can be differentiated, heavily stained cells mainly located in the medial septal nucleus, and weakly stained neurons preferentially found in the lateral septum. The numerous heavily labeled GAD67 mRNA-expressing cells in the medial septal nucleus filled in the space that was found unstained in the sections labeled for ChAT mRNA or protein (cf. Fig. 1a–c). Using counterstaining with DAPI to label the total population of septal cells, we estimated that approximately 40% of all cells in the medial septum expressed GAD67 mRNA (Fig. 1d). Our observations on GAD67 mRNA-expressing cells in adult rats are in line with previous studies on these neurons (Esclapez et al., 1993, 1994).

Our developmental study revealed a remarkable difference between the number of GAD67 mRNA- and ChAT mRNA-expressing neurons in the septal complex. GAD67 mRNA-expressing neurons were present as early as on E 17, and on E 20 the septal complex as a whole was heavily stained when compared with surrounding neocortical and striatal regions (Fig. 4a). With further development we mainly noticed an increase in the staining intensity of GAD67 mRNA-expressing cells in the medial septal nucleus and in the diagonal band (Fig. 4b,c). Figure 4b,c illustrates sections from animals perfused at P 9 and P 22, respectively. As can be seen in these figures, there is a change in the intensity of staining from lateral to medial septal regions with increasing age. An almost adult pattern is seen at P 22 (cf. Figs. 1c, 4c).

The early expression of GAD67 mRNA in the septal complex is supported by our quantitative analysis (Fig. 5). Thus, the percentage of GAD mRNA-expressing cells from all (DAPI-labeled) cells in the medial septum is high at all stages analyzed.

ChAT immunostaining

With the monoclonal ChAT antibody no immunostained neurons were observed in the septal complex before P 8 (Fig. 6a). Still only few, weakly ChAT-immunoreactive neurons were noticed on P 12 (Fig. 6b). There was a continuous increase in number and staining intensity of ChAT-immunoreactive neurons in the medial septum during the following postnatal week (data not shown). Taken together, our developmental studies on the expression of ChAT in neurons of the septal complex indicate that the mRNA for ChAT can be detected about 1 week before the first neurons can be stained immunocytochemically with the present monoclonal antibody.

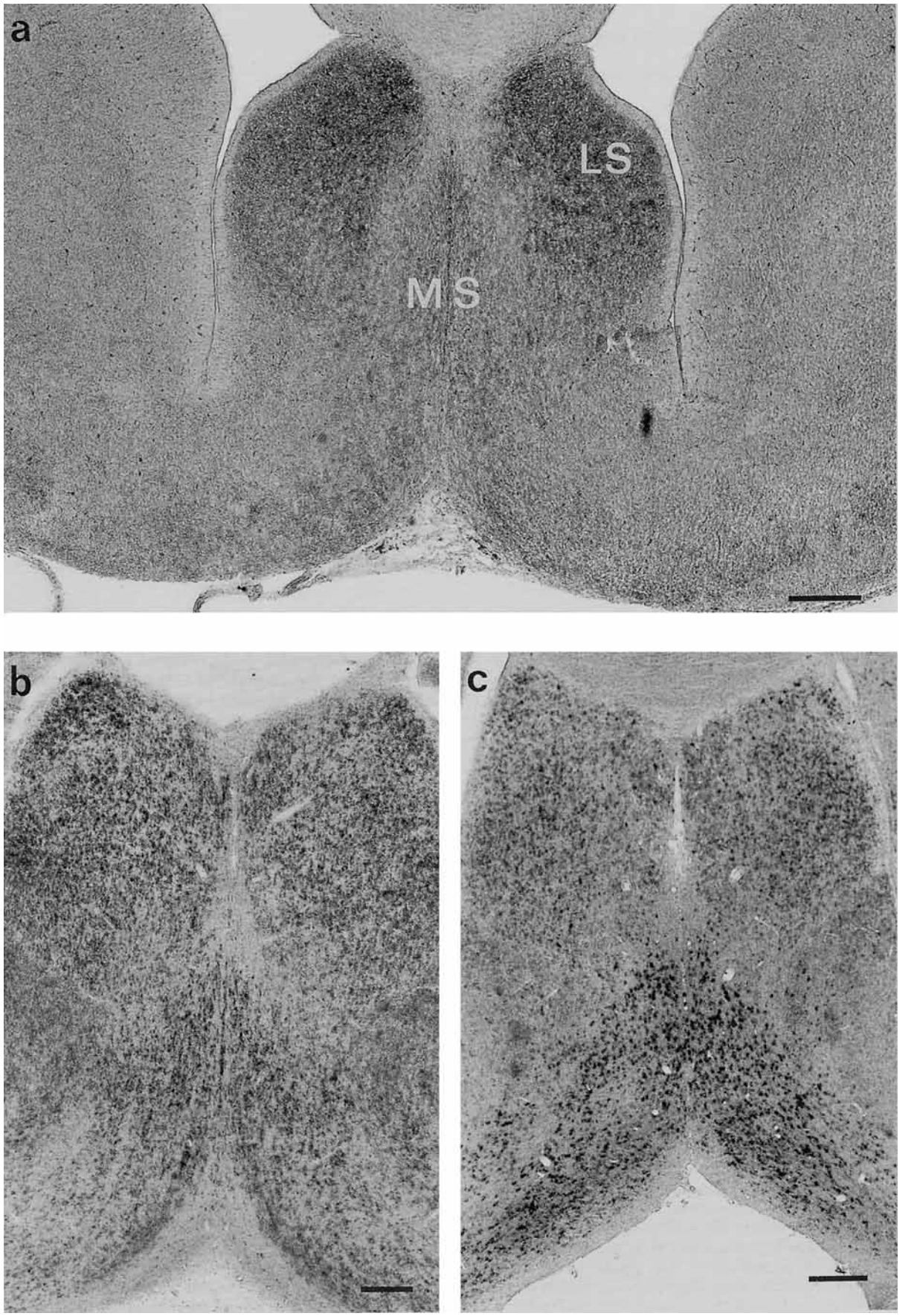

Fig. 4. Frontal cryostat sections through the septal area of embryonic and postnatal rats labeled for GAD67 mRNA. **a:** At E 20 many neurons are labeled in the septal complex, whereas adjacent regions are weakly stained. Note particularly intense staining of the lateral septum (LS). MS, medial septum. **b:** At P 9 many neurons in the lateral and medial septum are equally stained for GAD67 mRNA. **c:** Later in development (P 22), there is a shift toward a more intense staining of medial septal neurons for GAD67 mRNA. Scale bars = 250 μm.

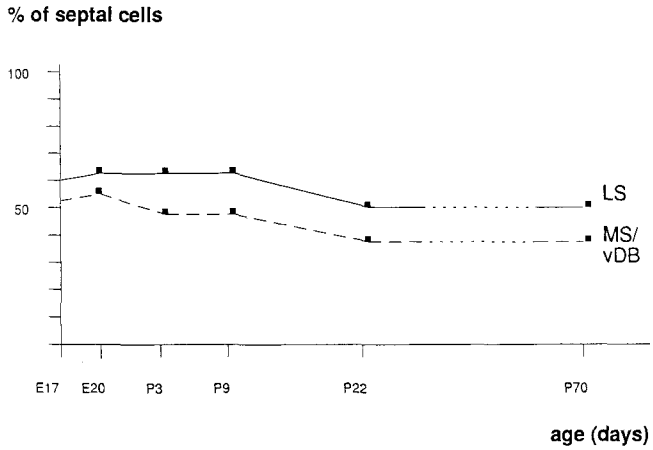

Fig. 5. Percentage of GAD67 mRNA-expressing neurons from all DAPI-labeled cells in defined septal regions (LS, lateral septum; MS/vDB, medial septum/vertical limb of the diagonal band) at various embryonic and postnatal stages ($n = 4$ for each time point). In five representative sections from each animal, GAD mRNA-expressing neurons and DAPI-labeled cells were evaluated for these regions.

Parvalbumin immunostaining

Previous studies have shown that the calcium-binding protein parvalbumin is a reliable marker for septohippocampal GABAergic neurons (Freund, 1989; Kiss et al., 1990). Indeed, our previous studies combining retrograde Fluoro-Gold tracing of septohippocampal neurons with parvalbumin immunocytochemistry revealed many double-labeled cells in the medial septum (Naumann et al., 1992). In the present developmental study, we did not observe clearly immunoreactive cells before postnatal day 8. Even at this stage, the cells were only faintly stained, although proximal dendrites could be followed for some distance (Fig. 6c). With increasing age, first the number of immunoreactive cells and then the staining intensity increased. In Figure 6d heavily immunoreactive neurons from P 15 are shown. The adult pattern of parvalbumin immunostaining is reached by P 20. These results indicate that parvalbumin immunostaining cannot be used as a method to study the early development of GABAergic septohippocampal projection neurons because this protein is expressed late in these cells, whereas the transmitter can already be demonstrated in the prenatal period.

DISCUSSION

By using nonradioactive in situ hybridization, we have shown in the present study that there is a developmental difference between cholinergic and GABAergic neurons in the medial septum–diagonal band complex. Thus, we found neurons expressing the mRNA for GAD67 as early as E 17, i.e., when most cells in the septal complex have become postmitotic (Bayer, 1979; Brady et al., 1989), whereas ChAT mRNA-expressing cells were not found before E 20. Our results also indicate that in situ hybridization may be a more reliable approach than immunocytochemistry to study the development of cholinergic and GABAergic septal neurons, because the first ChAT-immunoreactive cells were only found at P 8. Our immunocytochemical studies thus confirm the data of Sofroniew et al. (1987) who used the same monoclonal ChAT antibody.

Immunostaining of septal neurons for GAD and GABA requires the application of colchicine (Ribak et al., 1978; Onténiente et al., 1986; Kiss et al., 1990; Tóth et al., 1993), which is difficult in young animals. In the present study we have applied immunocytochemistry for parvalbumin, which is known to be a marker for GABAergic septohippocampal projection neurons in adult rats. We have shown here that the first parvalbumin-immunoreactive cells are found in the septal region by the end of the 1st postnatal week, which precludes this approach in studies on the early development of GABAergic neurons. The late appearance of parvalbumin immunoreactivity in the septal region is in line with observations in other telencephalic areas. Soriano et al. (1992) similarly observed the first parvalbumin-positive cells in the neocortex by the end of the 1st postnatal week as did Nitsch et al. (1990) in the rat hippocampus. In Triton X-100–treated frozen sections Solbach and Celio (1991) found a few PARV-immunoreactive neurons in the septal complex as early as E 21. We conclude that in situ hybridization may be the appropriate method to study the onset of the expression of transmitter-synthesizing enzymes in septal cells. Immunocytochemistry for ChAT and PARV has proven its usefulness in studies on adult animals (Naumann et al., 1992).

We regard it as the main result of the present study that GAD67 mRNA is expressed much before the mRNA for ChAT. Moreover, the early expression of GAD67 mRNA is paralleled by the presence of GABA immunoreactivity in septal cells at the same embryonic stages (Lauder et al., 1986), whereas immunoreactivity for ChAT appears much later than the expression of ChAT mRNA (present results). Several factors may account for the late appearance of ChAT immunoreactivity. One explanation would be that the ChAT protein undergoes post-translational changes resulting in changes in immunostaining.

As far as our analysis of GAD mRNA expression is concerned, we have to consider that there are at least two main isoforms of GAD mRNA, i.e., GAD65 and GAD67 (Erlander et al., 1991), both of which are expressed in GABAergic septal neurons (Esclapez et al., 1993, 1994; Feldblum et al., 1993). We have preferred the probe for GAD67 mRNA, because this was shown to be the prominent isoform in the medial septum (Feldblum et al., 1993; Esclapez et al., 1994).

Our results suggest that GABA is synthesized much earlier in septal neurons than acetylcholine. Previous studies have indicated that both transmitter substances are not coexpressed by the septal cells (Köhler et al., 1984; Brashear et al., 1986; Kosaka et al., 1988; Kiss et al., 1990; Gritti et al., 1993). It can be concluded that the septal cells start to synthesize GABA before the first septohippocampal fibers have reached the hippocampus. In previous studies employing DiI as an anterogradely transported tracer, we observed the first labeled septohippocampal fibers in the hippocampus on E 19 (Linke and Frotscher, 1993; Linke et al., 1995). The earlier onset of GAD synthesis in septal neurons seems to preclude a role of the target region in the determination of the transmitter phenotype in GABAergic septohippocampal neurons. In contrast, we cannot exclude the possibility that the late appearance of PARV and the postnatal increase in the intensity of GAD67 mRNA staining in GABAergic MS/vDB neurons are caused by the target region. The late embryonic onset of ChAT mRNA expression would be compatible with the hypothesis that the expression of ChAT in these cells is turned on and maintained by

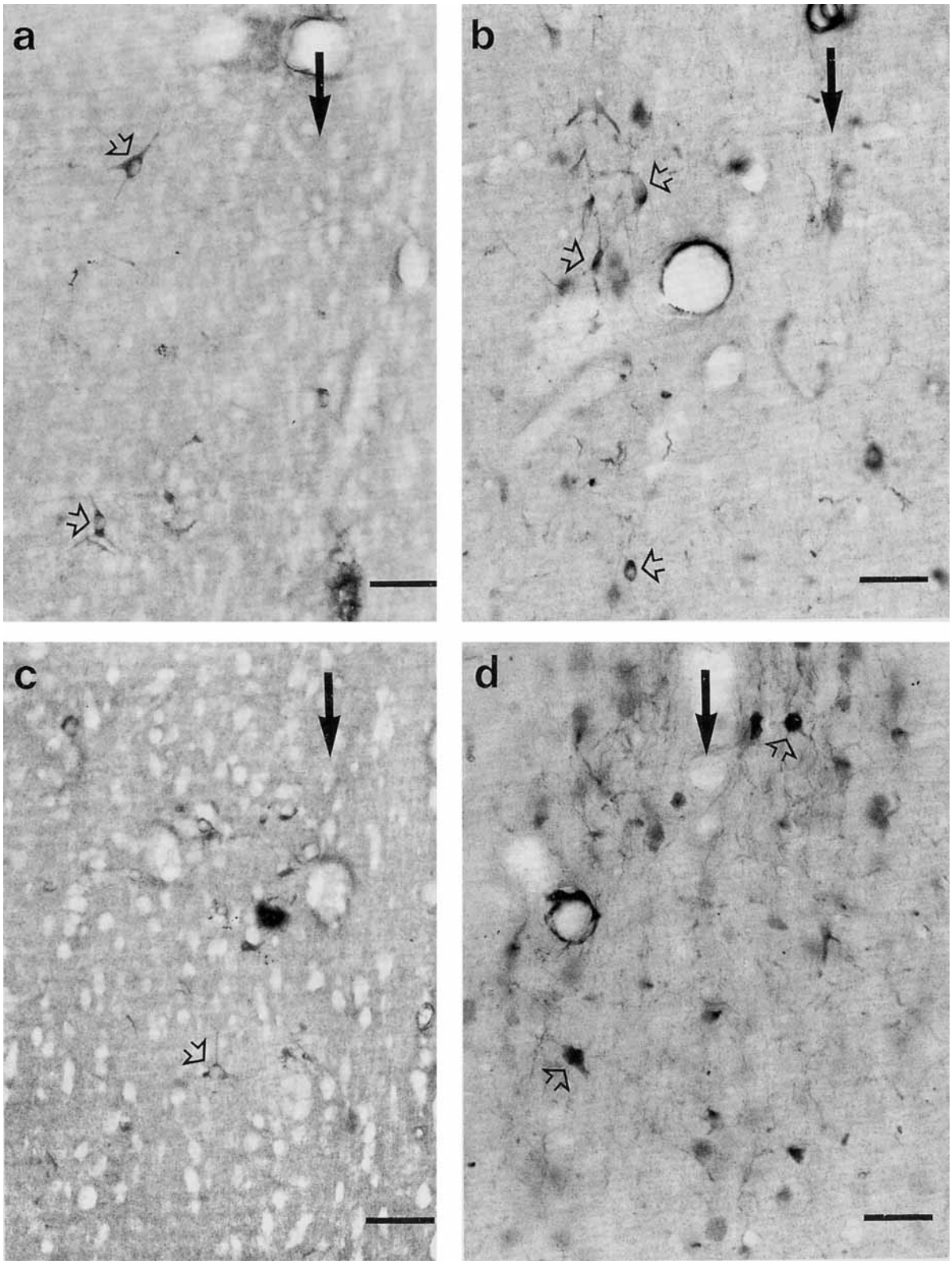

Fig. 6. Immunostaining for ChAT and PARV at early postnatal stages. **a:** Only very few, weakly ChAT-immunoreactive neurons (open arrows) are found at P 8. Arrow marks midline in all panels. **b:** At P 12, the number of ChAT-immunoreactive neurons (open arrows) has slightly increased. **c:** At P 8, only very few, slightly parvalbumin-immunoreactive neurons (open arrow) are found in the medial septum. **d:** At P 15, parvalbumin-immunoreactive neurons in the medial septum are heavily stained (open arrows) and have increased in number. Scale bar = 50 μm.

target-derived factors (Gnahn et al., 1983; Seiler and Schwab, 1984; Korsching et al., 1985; Gage et al., 1986, 1988, 1989; Hefti, 1986; Large et al., 1986; Mobley et al., 1986; Williams et al., 1986; Armstrong et al., 1987b; Auburger et al., 1987; Barde, 1987; Kromer, 1987; Cavicchioli et al., 1989, 1991; Li et al., 1995). We have observed the first ChAT mRNA-expressing neurons in the medial septum at E 20, which is in accordance with a recent study by Li et al. (1995). Moreover, the septohippocampal cholinergic neurons but not the GABAergic neurons are known to express both the low-affinity NGF receptor (p75 receptor) and the trk receptor (e.g., Springer et al., 1987; Koh and Higgins, 1991; Holtzman et al., 1992; Steininger et al., 1993; Gibbs and Pfaff, 1994; Heckers et al., 1994; Sobreviela et al., 1994; Li et al., 1995; Singh and Schweitzer, 1995). One is tempted to speculate that the transmitter expression of the two types of septohippocampal neurons is regulated in different ways. For the cholinergic component an inductive role of the target region can be assumed. This is in line with reports on the developmental expression of the NGF gene (Large et al., 1986; Auburger et al., 1987; Förster et al., 1993) and with studies employing blocking NGF antibodies during development (Vantini et al., 1989). In the presence of blocking NGF antibodies, ChAT immunoreactivity in the MS was decreased. In mice lacking the NGF gene (Crowley et al., 1994), an altered development of ChAT neurons in the basal forebrain was observed (Chen et al., 1994).

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