THE JOURNAL OF COMPARATIVE NEUROLOGY 394:520–536 (1998)

Neocortex in the Hippocampus: An Anatomical and Functional Study of CA1 Heterotopias After Prenatal Treatment With Methylazoxymethanol in Rats

N. CHEVASSUS-AU-LOUIS,* A. RAFIKI, I. JORQUERA, Y. BEN-ARI, AND A. REPRESA
INSERM Unité 29, Université Paris 5 René Descartes, 75 674 Paris Cedex 14, France

ABSTRACT

Migration disorders cause neurons to differentiate in an abnormal heterotopic position. Although significant insights have been gained into the etiology of these disorders, very little is known about the anatomy of heterotopias. We have studied heterotopic masses arising in the hippocampal CA1 region after prenatal treatment with methylazoxymethanol (MAM) in rats. Heterotopic cells were phenotypically similar to neocortical supragranular neurons and exhibited the same temporal profile of migration and neurogenesis. However, they did not express molecules characteristic of CA1 neurons such as the limbic-associated membrane protein. Horseradish peroxidase injections in heterotopia demonstrated labeled fibers not only in the neocortex and white matter but also in the CA1 stratum radiatum and stratum lacunosum. To study the pathophysiological consequences of this connectivity, we compared the effects of neocortical and limbic seizures on the expression of Fos protein and on cell death in MAM animals. After metrazol-induced seizures, Fos-positive cells were present in CA1 heterotopias, the only hippocampal region to be activated with the neocortex. By contrast, kainic acid-induced seizures caused a prominent delayed cell death in limbic regions and in CA1 heterotopias. Together, these results suggest that neocortical heterotopias in the CA1 region are integrated in both the hippocampal and neocortical circuitry. J. Comp. Neurol. 394:520–536, 1998. © 1998 Wiley-Liss, Inc.

Indexing terms: microcephaly; cortical dysplasia; neuronal migration; limbic system; epilepsy

An important event in cortical development is the migration of young postmitotic neurons from the ventricular zone, the site of their ultimate division, to the cortical plate where they differentiate. Neuronal migration disorders lead neurons to differentiate in an abnormal heterotopic position, where they eventually accumulate to form cortical heterotopias. In humans, cortical heterotopias are encountered along the ventricle, in the white matter, or in the molecular layer (Barkovitch and Kjos, 1992). Experimental neuronal migration disorders have been induced by exposure to teratogenic agents during pregnancy (Hicks et al., 1959; Ferrer et al., 1982, 1984; Miller, 1986; Gressens et al., 1992; Sun et al., 1995) or by perinatal lesions of the developing cortex (Rosen et al., 1992; Sherman et al., 1992; Marret et al., 1996). In vitro modifications of the molecular interactions between migrating neurons and radial glia also result in neuronal migration disorders (Anton et al., 1996).

Because cortical heterotopias involve differentiation in an abnormal environment, they offer interesting clues to mechanisms underlying epigenetic influences on the determination of neuronal phenotype (Jensen and Killackey, 1984; Rakic, 1988). Moreover, studying their integration into neuronal networks offers important insights into mechanisms underlying the formation of specific synaptic connections and into the pathophysiology of associated neurological disorders. Indeed, cortical malformations including heterotopias are a major cause of drug-resistant epilepsy exhibiting seizure onset in the second decade (Dubeau et al., 1995; Eksioglu et al., 1996; Harding, 1996; Des Portes et al., 1998).

We have investigated these issues in heterotopias obtained by injection of the antimitotic agent methylazoxymethanol (MAM) in pregnant rats. In this model, MAM

Grant sponsor: INSERM; Grant sponsor: Ecole Normale Supérieure de Lyon; Grant sponsor: Association Française contre les Myopathies.

*Correspondence to: Nicolas Chevassus-au-Louis, INSERM Unité 29, Université Paris 5 René Descartes, 123 Bd. Port Royal, 75 674 Paris Cedex 14, France. E-mail: chevassus@cochin.inserm.fr

Received 11 December 1997; Revised 3 February 1998; Accepted 4 February 1998

injection at embryonic day 14 (E14) selectively kills dividing cells within 24 hours (Matsumoto et al., 1972; Cattaneo et al., 1995; reviewed by Cattabeni and Di Luca, 1997). Offspring of these mothers, termed MAM rats, are microcephalic and exhibit altered lamination in the neocortex in addition to heterotopic masses (Johnston and Coyle, 1979; Dambska et al., 1982; Yurkewicz et al., 1984). Heterotopias in the CA1 field of the hippocampus have been described by several researchers (Singh, 1977; Chen and Hillman, 1986; Collier and Ashwell, 1993), but their origin and pathophysiological consequences have proven to be enigmatic. In the present report, we show that the phenotype acquired by these cells is very similar to that of neocortical neurons of layers 2/3, leading us to propose that CA1 heterotopias constitute heterotopic supragranular neocortex. After combining horseradish peroxidase (HRP) projection tracing with a functional study of the effects of seizures on Fos expression, we suggest that CA1 heterotopias are integrated in both the neocortical and the hippocampal networks.

MATERIALS AND METHODS

Animals

Wistar rats were used throughout the experiments and were treated in accordance with the INSERM guidelines for animal care. Pregnant female rats were injected intraperitoneally (i.p.) with 25 mg/kg MAM (Sigma, St. Louis, MO) in saline at E14 (first gestation day is E0) between 1 and 3 p.m. After normal delivery, litter size was reduced to eight and pups grew with free access to food and water under diurnal conditions with lights on from 0800 to 2000.

At the age of 5 weeks, animals were taken to a separate room and placed in individual cages for seizure induction. The first group of animals received an i.p. injection of 8 mg/kg kainic acid (KA; Sigma) in saline. According to previous studies (Ben-Ari, 1985; De Feo et al., 1995), KA-induced behaviors were classified as no change, mild seizure, or full limbic seizure. Animals with full limbic seizure were killed 90 minutes (when Fos immunoreactivity is first detected; Popovici et al., 1990), 3 hours (beginning of status epilepticus and peak of Fos expression; LeGal LaSalle, 1988; Popovici et al., 1990), or 72 hours (when cell degeneration is fully recognizable; Weiss et al., 1996) after KA injection.

The second group of animals received an i.p. injection of 60 mg/kg pentylenetetrazole (PTZ; Sigma) in saline. PTZ-induced behaviors were classified, according to Ben-Ari et al. (1981) and Moshe et al. (1994), as no change, mild clonic seizure, and violent tonicoclonic seizure. Animals with clonic seizures were killed either 1 hour (the earliest time point to detect Fos; Morgan et al., 1987) or 72 hours after injection.

The last group of animals received an i.p. injection of an equal volume of isotonic saline and was killed 1, 3, or 72 hours after injections to serve as controls for stress-induced Fos expression (Sharp et al., 1991; Senba et al., 1993).

Immunohistochemical procedure

Animals were deeply anesthetized with pentobarbital (70 mg/kg) and perfused through the aorta with sodium nitrite 0.1% in saline 0.9% and then with paraformaldehyde 4%. The brains were removed, postfixed for one night in paraformaldehyde 4%, cryoprotected in sucrose 20%, cut at 40 µm with a cryostat, and processed for free-floating immunohistochemistry. Alternatively, some postnatal day 0 (P0; n = 7 and P5 n = 5) brains were dehydrated, embedded in paraffin, sectioned at 6 µm, and Nissl stained for cytoarchitectonic analysis.

After inhibition of endogenous peroxidase activity by a 1-hour incubation in ethanol 45%, H_2O_2 0.6% in saline 0.9%, sections were incubated for 1 hour at room temperature in 0.5% gelatin in phosphate buffered saline (PBS) with 0.3% Triton X-100 (PBSTG) and, then overnight in the primary antibody. We used monoclonal antibodies against calbindin (1:2,000) and parvalbumin (1:4,000), both provided by Sigma, and a polyclonal antibody against Fos (Oncogene Science, Manhasset, NY; at a dilution of 1:2,000). Nonspecific labeling was tested by incubating sections in PBSTG alone, which resulted in absence of staining. After several washes in PBS, sections were incubated for 2 hours at room temperature in a biotinylated antibody against mouse (for monoclonal antibodies) or rabbit (polyclonal antibody) immunoglobulins. Both reagents (Dako, Carpinteria, CA) were used at a dilution of 1:500 in PBSTG. After several washes in PBS, sections were processed with the ABC Elite kit (Vector Laboratories, Burlingame, CA), and peroxidase activity was demonstrated by adding H_2O_2 0.001% after a 10-minute preincubation in diaminobenzidine (DAB) 0.05% with 0.4% of nickel ammonium sulfate in Tris (50 mM, pH 7.6). After completion of the reaction, judged visually, sections were rinsed several times, mounted on gelatin-coated slides, dehydrated, cleared in xylene, and mounted with Eukitt.

5-Bromo-2′deoxyuridine (BrdU) birthdating experiments

Pregnant female rats were injected i.p. between 1 and 3 p.m. with 100 mg/kg BrdU (Sigma) in saline NaOH 0.007N, with two injections on each side of the abdomen. One control female and two MAM-treated females were injected at E14 (together with MAM injection), E16, E18, or E20. After normal delivery, pups were taken at 3 weeks of age and perfused with paraformaldehyde 4%. After cryoprotection in sucrose, 40-µm-thick cryostat sections were treated with Triton X-100 0.5% in PBS for 1 hour for tissue permeabilization and then in HCl 2 N in water for 20 minutes at 45°C. Sections were rinsed in borate buffer 0.1 M, pH 8.5, and then in PBS. Immunohistochemical revelation of BrdU (monoclonal antibody at 1:500; Dako) was then performed as described above.

Sections in which Fos- or BrdU-positive cells were to be quantified were processed together during the whole procedure. Because only estimates of ratios (cell number or density in heterotopia vs. neocortex or CA1 within the same slices) were sought, we performed profile counts without corrections (Saper, 1996). Positive profiles were counted with a microscope at a magnification of 125× in a grid composed of 100 squares of 1 μm^2 each in three nonadjacent sections of each animal. Results were averaged, expressed as mean ± standard error of density, and compared by using the two-way Student's t-test.

Golgi impregnations

Five adult MAM rats were perfused with 10% formaldehyde and processed for Golgi-Rio Hortega. Briefly, tissue

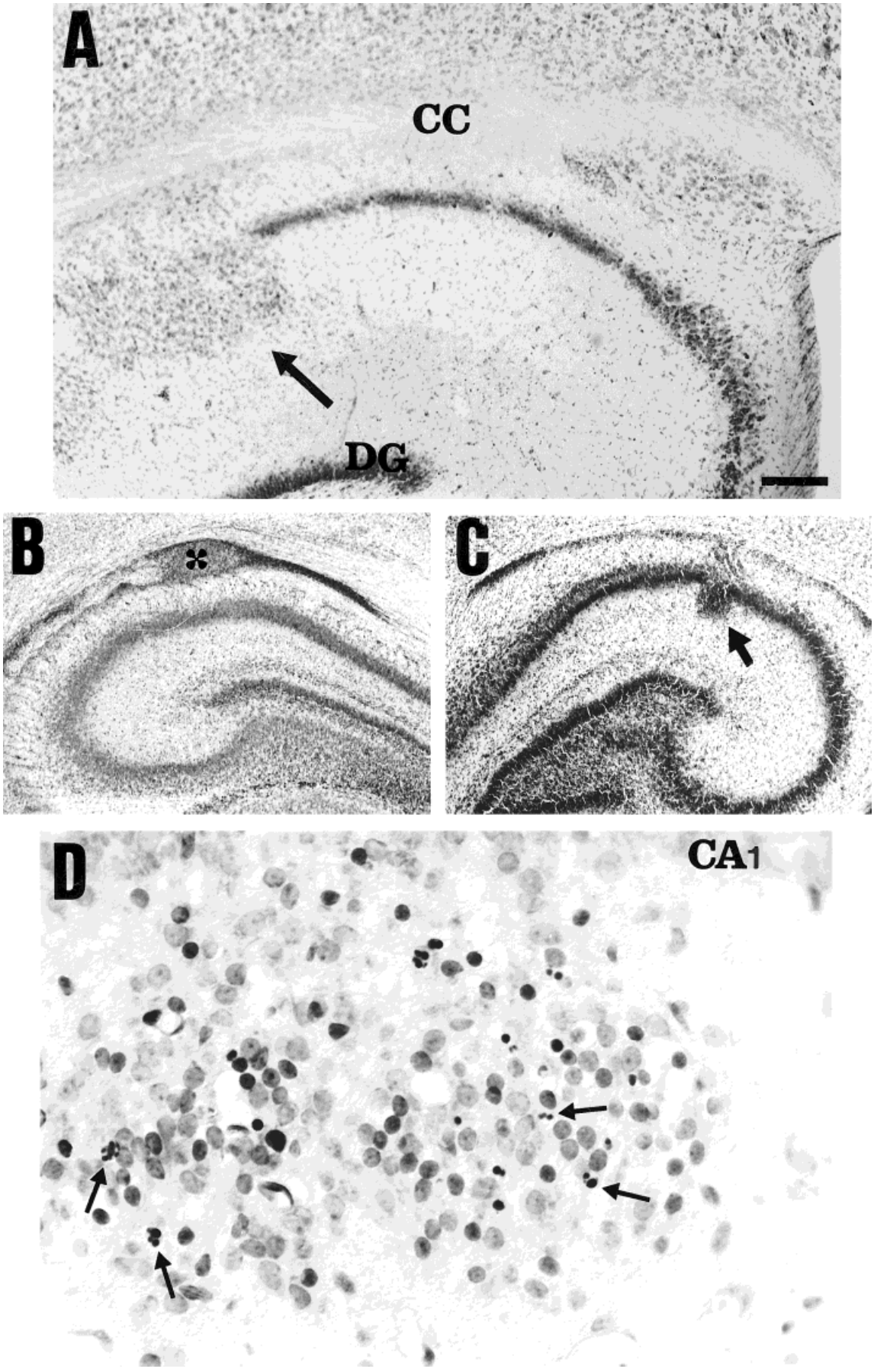

Figure 1

blocks containing the neocortex and the hippocampus were immersed for 48 hours in a solution of potassium dichromate 6%, chloral hydrate 6%, and formaldehyde 10%. After rinses in water, blocks were immersed for 24 hours in silver nitrate 1%, rinsed, sectioned at 100 μm with a Vibratome, dehydrated, cleared in xylene, and mounted between two coverslips in Permount. Impregnated representative neurons were drawn with a camera lucida at 500×.

Gallyas staining

To evaluate the long-term effects of seizures, animals were perfused with 400 ml of 4% paraformaldehyde and their brains were postfixed for 1 week in the same solution. Fifty-micron-thick sections were cut with a Vibratome, and alternate sections were stained with cresyl violet, with the silver degenerating method of Gallyas et al. (1980) modified by Nadler and Evenson (1983), or immunostained for Fos as described above.

In situ hybridization

After decapitation, 8-week-old MAM (n = 3) and control (n = 3) rat brains were frozen in isopentane (−50°C). Frontal sections (15 μm) of the whole brains were cut in a cryostat and mounted onto gelatin-coated slides and kept at −80°C. A previously described antisense oligonucleotide probe for the limbic-associated membrane protein (LAMP; Reinoso et al., 1996) was 3′ end labeled for 1 hour at 37°C in 10 μl reaction mix containing 30 pmol [α-35S] dATP (>1,000 Ci/mmole; NEN), 1 pmol oligonucleotide, and 15 U of terminal deoxynucleotide transferase (TdT; BRL, Gaithersburg, MD) in the 1× TdT buffer (BRL). Sections were brought to room temperature and fixed for 15 minutes in PBS containing 4% paraformaldehyde. After two washes in 0.1 M PBS (3 minutes each), one wash in 0.1 M PBS (15 minutes), and one wash in 0.05 M PBS (2 minutes), sections were dehydrated in ethanol and air dried. Sections were then prehybridized for 1 hour in 4× standard saline citrate (SSC) buffer containing 50% formamide, 1× Denhardt's solution, 7.5% dextran sulfate, tRNA (250 μg/ml), salmon sperm DNA (250 μg/ml), 100 mM dithiothrietol (DTT), and Poly A (250 μg/ml) and then hybridized with 1× 10^5 cpm of LAMP-specific oligoprobes at 42°C overnight in the same buffer. Sections were washed twice at room temperature in 2× and 1× SSC (with 1 mM DTT) for 15 minutes each and then in a high stringency bath of 0.5× SSC for 30 minutes at 55°C. The slices were then dehydrated, air dried, and exposed to X-Omat film (Kodak, Rochester, NY) for 6 days. Sections of MAM and control rats were incubated with the same hybridization mixtures and exposed to the same film (X-Omat, Kodak). Experiments were repeated three times. Sections were then dipped in nuclear emulsion (NTB-2, Kodak) and exposed at 4°C for 20 days. After development, sections were counterstained with cresyl violet. Autoradiograms and micrographs used to generate Figure 7 were scanned into Adobe Photoshop on a Macintosh computer, and the brightness was enhanced before printing on a Tektronix sublimation printer.

Projection tracing with HRP

Because we can know the precise stereotaxic position of heterotopias within the CA1 region in a living rat, targeted tracer injection would be extremely hazardous in vivo. Pilot experiments were conducted with carbocyanine dyes on fixed brains, but this procedure produced poor results because of the dye diffusion out of the heterotopias borders. Thus, we used extracellular injections of HRP in an vitro slice preparation (Bernardo et al., 1986; Bernardo and Woolsey, 1987), a procedure previously employed to study intracortical connections (Burkhalter, 1989).

Four adult MAM rats were deeply anesthetized with chloral hydrate and then perfused through the aorta for 1 minute with a 0–3°C oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 3.5 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 11 glucose equilibrated with 95% O_2, 5% CO_2 (pH 7.4). Coronal 400-μm-thick slices were cut with a Vibratome in chilled ACSF. After recovery at 20°C, slices were placed in a submerged recording chamber continuously superfused with ACSF and stained with a drop of methylene blue to visualize the heterotopia; 0.1–0.2 μl of a 40% solution of HRP type VI (Sigma) in ACSF was then pressure injected under visual guidance with a glass micropipette (tip diameter = 20 μm) in the heterotopia. After 4–8 hours of incubation in ACSF, slices were fixed with 1% paraformaldehyde, 2.5% glutaraldehyde. After cryoprotection with 30% sucrose, slices were cut at 60 μm with a freezing microtome. Free-floating sections were preincubated in a 0.05% solution of DAB with 0.2% nickel sulfate, to which 0.001% H_2O_2 was added after 30 minutes. After a 1-hour reaction at room temperature, sections were rinsed, mounted, dehydrated, cleared, and coverslipped with Eukitt. Microscopic examination was performed under bright-field illumination, and labeled fibers were drawn with a camera lucida at 250×.

RESULTS

Heterotopic neurons achieve migration between P0 and P5

In agreement with previous investigators (Johnston and Coyle, 1979; Dambska et al., 1982; Yurkewicz et al., 1984; Rafiki et al., 1998), we observed cortical malformations in MAM rats consisting of microcephaly with alteration of cortical lamination mainly in the external layers. The extent of this malformation was consistent among rats of the same litter. Two types of heterotopias were observed (Fig. 1A): periventricular heterotopias (PVH; that may eventually be inserted in the corpus callosum) that have been previously described as heterotopic neocortex (Ferrer et al., 1984; Yurkewicz et al., 1984; Zhang et al., 1995) and hippocampal CA1 heterotopias. The hippocampal heterotopias formed longitudinal clusters that usually extended into the stratum oriens, pyramidale, and radiatum, breaking the continuity of the CA1 pyramidal cell layer. However, heterotopias restricted to stratum oriens were sometimes observed, usually associated with conservation of

Fig. 1. Postnatal development of CA1 heterotopias. Cresyl-violet-stained sections. **A:** In an adult rat treated with methylazoxymethanol (MAM), heterotopias are observed in the hippocampal CA1 field (arrows). **B:** By contrast, at postnatal day (P) 0, the hippocampus is intact, but periventricular heterotopias (PVH) (star) interrupting the germinative zone are observed. **C:** Heterotopias are observed in CA1 at P5 (arrow). **D:** Higher magnification of a CA1 heterotopia in a cresyl-violet-stained paraffin-embedded section at P5 shows the presence of immature neurons and of pyknotic profiles (arrows), mostly in the ventral part of the heterotopia. CC, corpus callosum; DG, dentate gyrus. Scale bar = 200 μm in A–C, 20 μm in D.

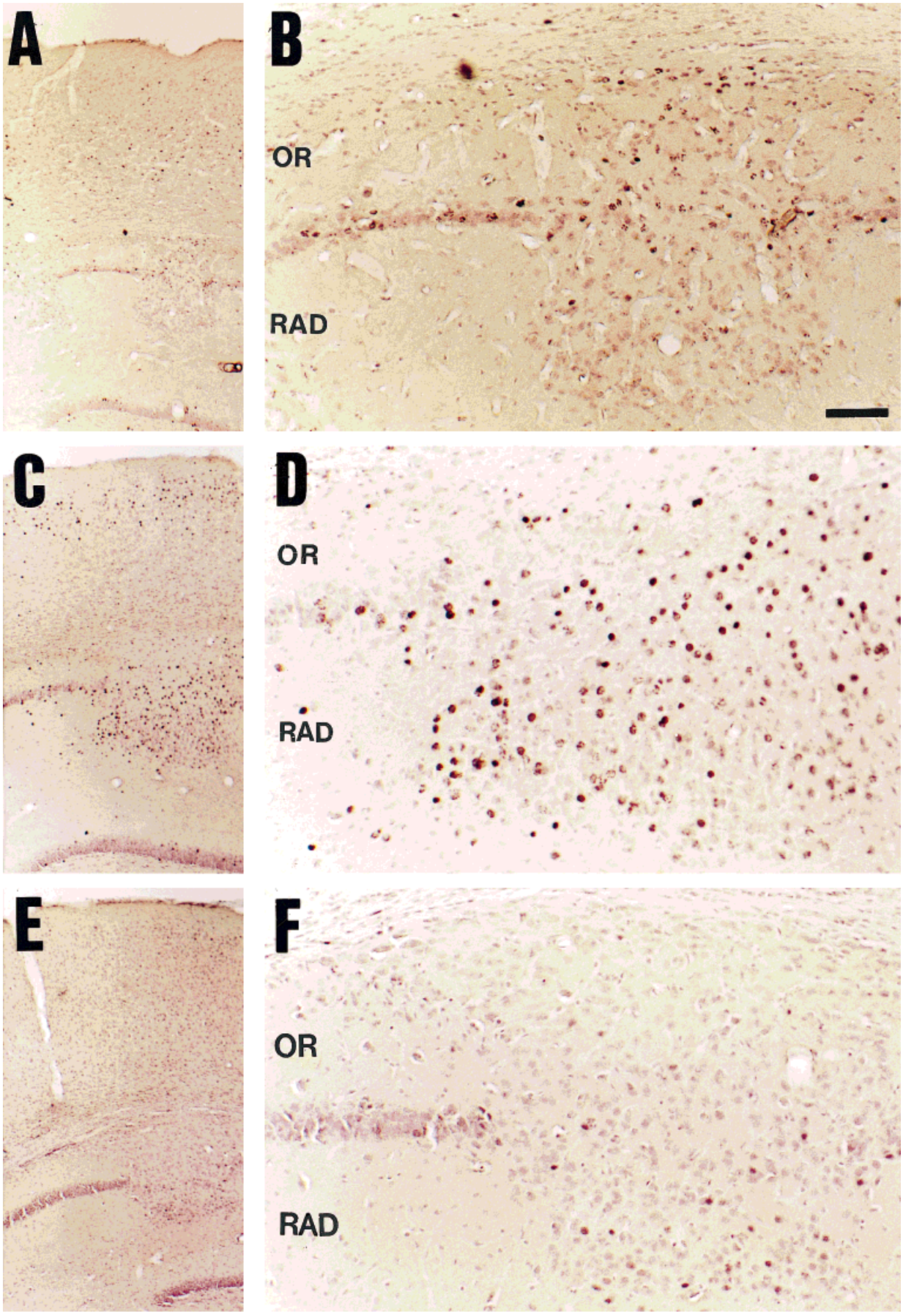

Figure 2

the continuity of the CA1 layer. CA1 heterotopias were only found in the septal pole of the hippocampus.

An important feature of CA1 heterotopias in MAM rats was that they were absent at P0 even in rats from litters with extensive malformations as adults. By contrast, PVH were readily distinguished at this age (Fig. 1B) as large masses of cells interrupting the continuity of the subventricular germinative zone. At P5, CA1 heterotopias became visible (Fig. 1C) and were composed of young undifferentiated neurons. Another prominent feature of heterotopias at this age was the frequency with which we observed pycnotic profiles (Fig. 1D), especially in the ventral portion.

CA1 heterotopic cells are labeled by BrdU injection between E16 and E20 and are arranged in a laminar fashion

In agreement with previous data (Angevine and Sidman, 1961; Berry et al., 1964), we observed an inside-out gradient of neocortical neurogenesis, which took place between E14 and E20. A similar pattern and time course of neurogenesis was observed in the neocortex of MAM rats despite the severe microcephaly (Fig. 2A,C,E).

Following BrdU injection at E14, labeled cells were never observed in CA1 heterotopias. After BrdU injection at E16 (Fig. 2B) and E18 (Fig. 2D), labeled cells were observed in periventricular and CA1 heterotopias and in the CA1 layer. Following BrdU injection at E20, labeled cells were observed in the PVH and mostly in the ventral portion of the CA1 heterotopias (Fig. 2F) but were very rare in the CA1 layer. Quantification in three animals with CA1 heterotopias of comparable size demonstrated that the average number of BrdU-labeled profiles per slice was 4.5 ± 1.2 in the CA1 layer versus 52.8 ± 3.0 ($P = 0.0001$) in heterotopias. This result indicates that a significant portion of CA1 heterotopic cells had their last division at E20 and that CA1 neurogenesis was nearly achieved at this age. The number of BrdU-labeled profiles per slice of heterotopias was 10.2 ± 1.3 in the dorsal part (strata oriens and pyramidale) versus 42.6 ± 1.7 ($P = 0.0001$) in the ventral part (stratum radiatum). This result is fairly suggestive of a neurogenetic gradient than can be referred to as an inside-first outside-last gradient relative to the germinative zone. We conclude that heterotopic neurons are generated between E16 and E20, the latter formed assuming the most ventral position.

CA1 heterotopias contain small distorted pyramidal neurons and different nonpyramidal neurons

Golgi impregnations demonstrated the presence of both pyramidal and nonpyramidal neurons in the CA1 heterotopias of MAM rats (Fig. 3). Pyramidal neurons were spiny and had smaller cell bodies and dendritic trees than did adjacent CA1 pyramidal neurons. They had long and bent apical dendrites that were not paralleled. Reversed orientation of heterotopic apical dendrites (directed toward the neocortex) was occasionally observed. Terminal dendrites of both CA1 and heterotopic neurons extended to the most ventral extent of the stratum lacunosum. By contrast, dendrites of CA1 pyramidal neurons never penetrated the heterotopia. Axons were not consistently silver-impregnated in our preparations. Several types of nonpyramidal neurons, including aspiny and sparsely spiny multipolar and aspiny and sparsely spiny bipolar, were also observed. Bipolar neurons were particularly prominent at the lateral and dorsal borders of the heterotopias. Multipolar, also described as stellate, nonpyramidal neurons have never been described in the CA1 region of the hippocampus (Freund and Buzsaki, 1996).

Ontogenesis of calbindin expression is similar in CA1 heterotopias and supragranular neocortex

In adult MAM rats, the pattern of calbindin immunoreactivity (Calb-IR) in CA1 heterotopias was similar to that of the supragranular neocortex of control and MAM rats (Fig. 4A,B). It consisted of a few intensely stained nonpyramidal neurons and a large number of moderately stained small pyramidal cell bodies within a dense plexus (Fig. 4C). In the CA1 layer, we observed Calb-IR in the somata of a subpopulation of CA1 pyramidal cells.

In younger animals, there was no Calb-IR in the CA1 layer (Fig. 4D,G; see also Alcantara et al., 1993), whereas Calb-IR was already present in the neocortex in cell bodies and neurites at P10 (Fig. 4G–I; see also Sanchez et al., 1992) with a fully mature pattern at P15 (Fig. 4D–F). In CA1 heterotopias, Calb-IR was observed in nonpyramidal neuron somata and neurites at P10 and innonpyramidal neurons, small pyramidal somata, and fiber plexus at P15. This observation indicates that both the mature pattern and the ontogenesis of calbindin expression are similar in CA1 heterotopias and supragranular neocortex.

Ontogenesis of parvalbumin expression is similar in CA1 heterotopias and in the neocortex

In adult MAM rats, the pattern of parvalbumin immunoreactivity (Parv-IR) in CA1 heterotopias was similar to that of the CA1 layer and the neocortex (Fig. 5A,B). It consisted of a very dense plexus of fibers with intensely stained nonpyramidal cell bodies.

In contrast at P15 (Fig. 5C,D; in agreement with Nitsch et al., 1990; Soriano et al., 1992), Parv-IR exhibited the adult pattern in CA1 and in the deep cortical layers but was only present in cell bodies in supragranular layer. In CA1 heterotopias at P15, Parv-IR was only found in a few cell bodies with short neurites whose fibers were not stained. This observation indicates that both the mature pattern and the ontogenesis of parvalbumin expression are similar in CA1 heterotopias and supragranular neocortex.

Unlike adjacent pyramidal neurons, heterotopic neurons do not express the mRNA encoding for the LAMP

To test whether cells in the CA1 heterotopias expressed molecules specific to their environment, we performed in situ hybridization for LAMP, a well-described molecule

Fig. 2. **A–F:** Cortical neurogenesis in methylazoxymethanol (MAM) rats determined by 5-bromo-2′deoxyuridine (BrdU) injections. BrdU immunostaining with hematoxylin counterstaining in MAM rats injected with BrdU at embryonic day (E) 16 (A,B), E18 (C,D), and E20 (E,F). B, D, and F show higher magnifications of the heterotopia. Heterotopic neurons are labeled by BrdU injection from E16 to E20, with E20-generated neurons concentrated in the most ventral portion. OR, stratum oriens; RAD, stratum radiatum. Scale bar = 200 μm in A,C,E, 40 μm in B,D,F.

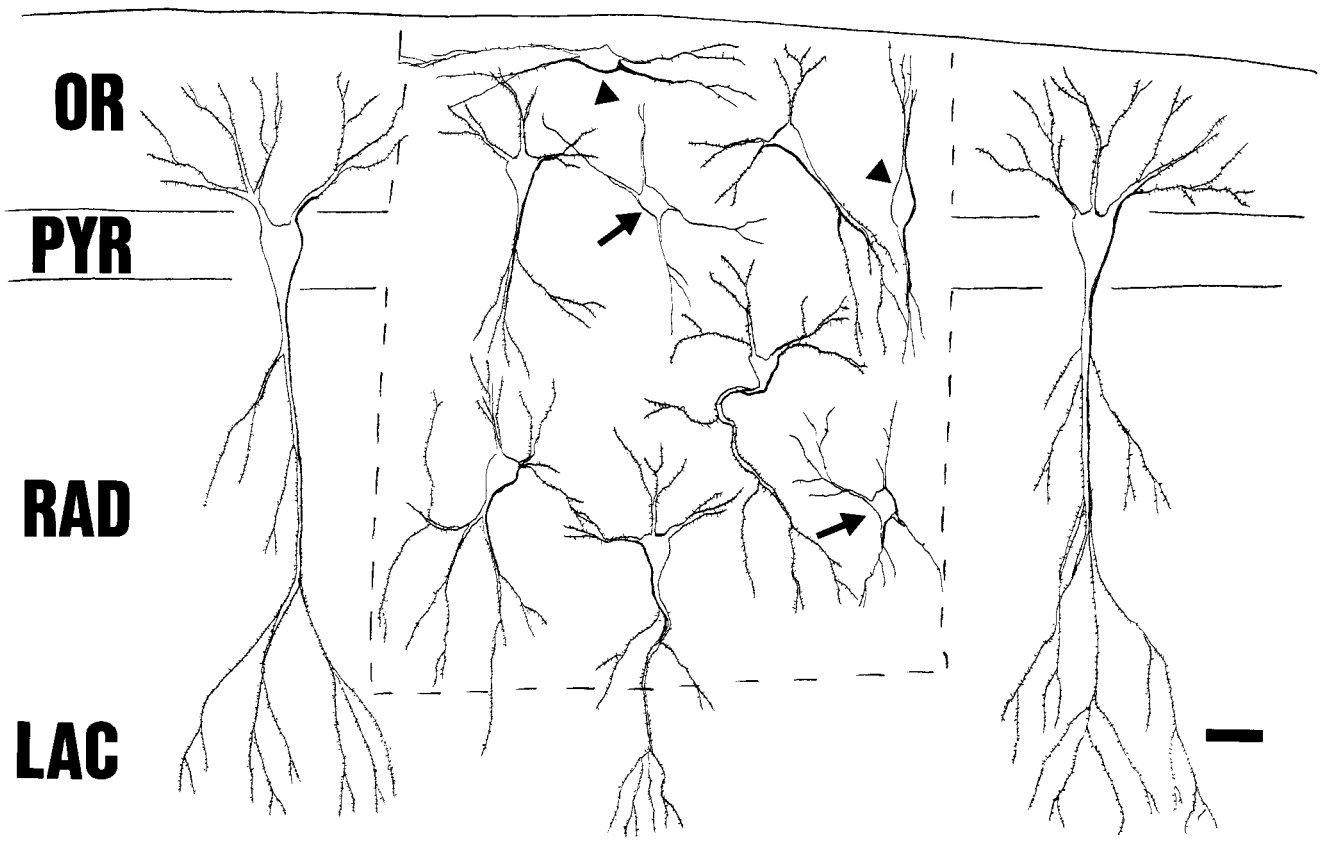

Fig. 3. Camera lucida drawing of representative Golgi-impregnated neurons in adult CA1 heterotopic areas. The small size of heterotopic pyramidal neurons contrasts with the large pyramidal neurons of the CA1 layer. Several types of nonpyramidal neurons including multipolar (arrows) and bipolar (arrowheads) are also observed. PYR, stratum pyramidale; RAD, stratum radiatum; OR, stratum oriens; LAC, stratum lacunosum. Scale bar = 20 μm.

selective to the limbic system (Levitt, 1984, 1985). LAMP is expressed in the limbic system but not in the neocortex of adult rats (Reinoso et al., 1996). Autoradiograms demonstrated a very high expression of the LAMP transcript in the whole hippocampus of both control (Fig. 6A,B) and MAM (Fig. 6C,D) animals. This finding contrasted with the low level of expression in the neocortex. LAMP was also strongly expressed in the amygdala, the entorhinal cortex, the habenula, the reticular nucleus, and the hypothalamus. These data show that MAM treatment did not alter the pattern of LAMP expression.

In CA1 heterotopias as in adjacent neocortex, the level of expression of the LAMP transcript was very low. This result was further confirmed by emulsion analysis at the cellular level, which showed a dense accumulation of silver grains in the CA1 layer (Fig. 6E) but not in the CA1 heterotopias (Fig. 6F). This finding cannot be explained by a lower cellular density in heterotopias because we also observed a dense accumulation of silver grains in the amygdala, which has a cellular density similar to heterotopias (Fig. 6G).

Heterotopic neurons have projections both in the neocortex and in the CA1 stratum radiatum and lacunosum

After pressure injection of HRP, the injection site consisted of a small dark dot surrounded by a cluster of darkly stained cell bodies with well-marked dendrites. In eight slices, the injection site was confined to the heterotopia. In all cases (Fig. 7A,B), fine HRP-labeled fibers, sometimes with varicosities, were observed in the neocortex adjacent to the heterotopia and in the subcortical white matter. In three cases where the injection site was confined to the ventral part of the heterotopia, HRP-labeled fibers were also observed laterally in CA1 stratum radiatum and medially in stratum lacunosum (Fig. 7B), corresponding to the Shaffer collateral pathway and temporoammonic pathway respectively. This staining was not due to the labeling of en passant fibers because such labeled fibers were never observed after similar injections performed in the CA1 stratum radiatum of control slices of MAM rats ($n = 2$).

PTZ-induced clonic seizures induce Fos expression in the heterotopia and in the neocortex but not in CA1

To test whether the neocortex and heterotopia were functionally connected in vivo, we induced selective neocortical activation (Ben-Ari et al., 1981) in adult MAM rats by i.p. injections of PTZ. After PTZ injection, we observed different types of seizures of different degrees in both control and MAM animals (Table 1). Ninety-three percent of MAM versus 64% of control ($P = 0.004$, Chi-square test) animals displayed seizures after PTZ injection. This result

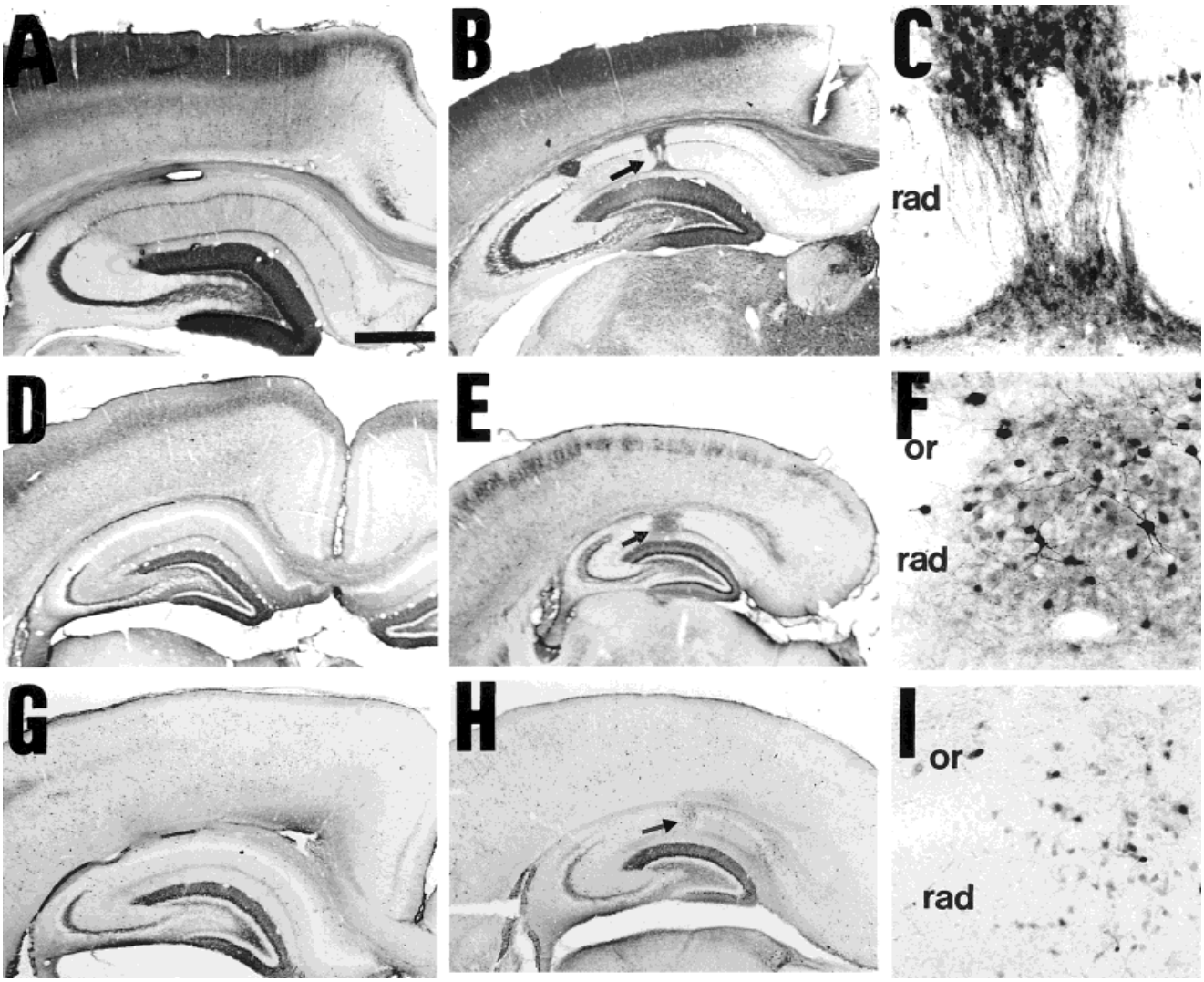

Fig. 4. **A–I:** Ontogenesis of calbindin immunostaining in control and methylazoxymethanol (MAM) rats. Calbindin immunostaining in control (A,D,G) and MAM rats (B,E,H) at P35 (adults), P15, and P10. Higher magnification of the heterotopias (arrows) are shown in C, F, and I. OR, stratum oriens; RAD, stratum radiatum. Scale bar = 250 μm in A,B,D,E,G,H, 50 μm in C,F,I.

indicates that MAM rats have an increased sensitivity to PTZ and confirms previous data with flurothyl-induced seizures (Baraban and Schwartzkroin, 1996). Only animals (both MAM and control groups) with mild clonic seizures were conserved for Fos studies.

One hour after saline injection, a moderate density of Fos-like immunoreactive (FLIR) cells could be detected in the basal hypothalamus and arcuate nucleus, in the olfactory tubercles, and in the habenula in both control and MAM rats. Sparse scattered FLIR cells were also found in the temporal and occipital neocortex and in the dorsomedial nucleus of the amygdala. The rest of the neocortex and the hippocampus were completely devoid of FLIR cells. These experiments indicate that basal and stress-induced levels of Fos expression are not modified in MAM animals. Specifically, heterotopia did not appear to be prone to stress-induced Fos expression.

One hour after PTZ-induced clonic seizures in control animals (Fig. 8A), we observed FLIR cells throughout the entire neocortex, especially in the supragranular layers, and in thalamic nuclei including the reticular and ventroparietal nuclei. No FLIR cells were found in the hippocampus. The same pattern of corticothalamic expression was found in MAM animals. The PVH also contained FLIR cells. In addition, FLIR cells were found in CA1 heterotopias, mainly in the dorsal region (Fig. 8B). In fact, CA1 heterotopias were the only hippocampal region to contain FLIR cells after PTZ-induced seizures. No cell death in any brain region could be detected by Gallyas staining 72 hours after PTZ-induced clonic seizures.

KA induces Fos expression and cell death in the heterotopias and in CA1 but seldom in the neocortex.

To test if CA1 neocortical heterotopias were integrated in the hippocampus as suggested by connectivity studies (Fig. 7B), we induced selective hippocampal activation (Ben-Ari,

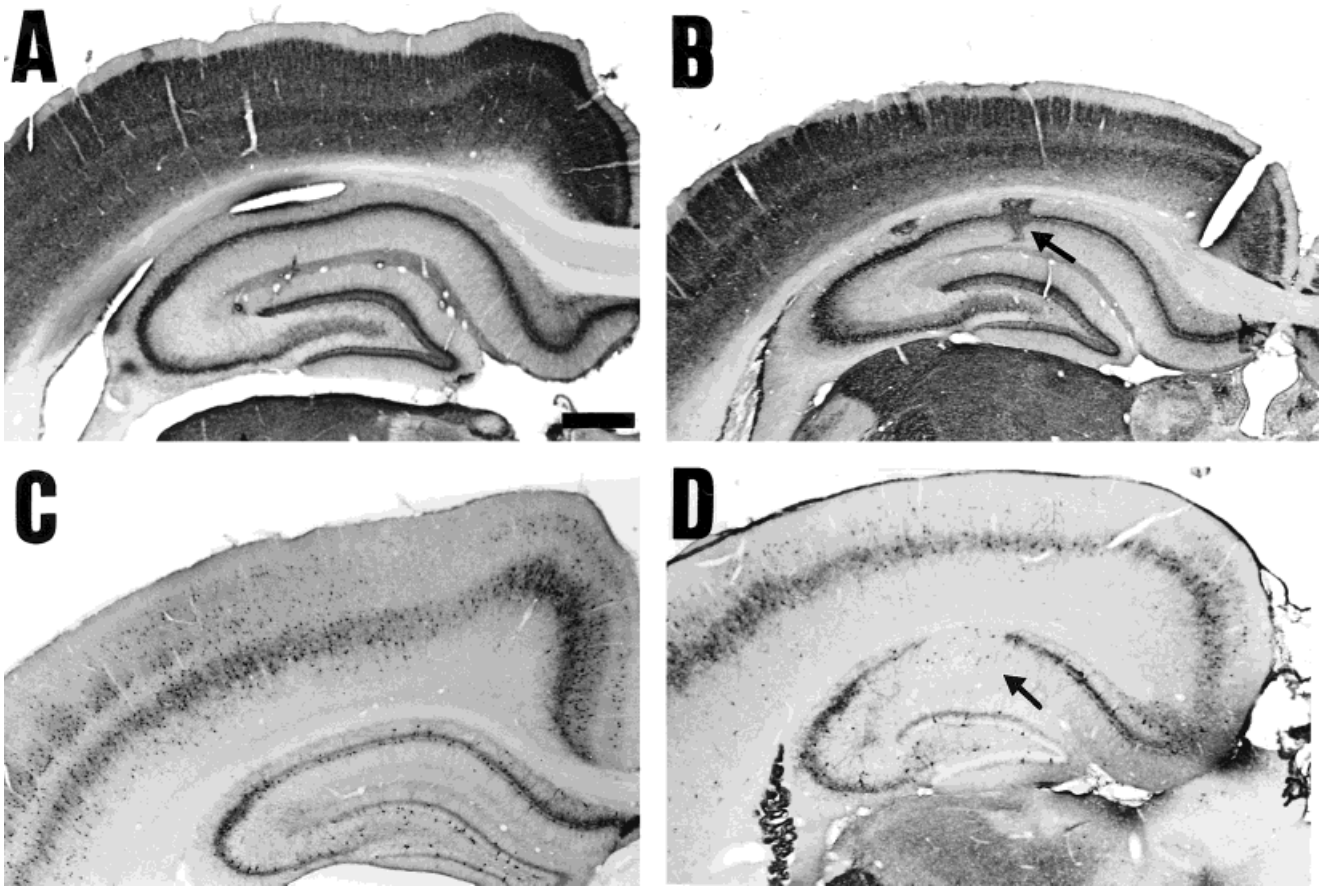

Fig. 5. **A–D:** Ontogenesis of parvalbumin immunostaining in control and methylazoxymethanol (MAM) rats. Parvalbumin immunostaining in an adult control (A) and MAM (B) rat. A dense parvalbumin immunoreactivity (Parv-IR) is observed in the heterotopia and in the neocortex and CA1 region. At P15 in MAM animals (D), CA1 heterotopias (arrow) contain Parv-IR only in cell bodies, whereas a dense axonal and dendritic Parv-IR has already developed in the adjacent CA1 pyramidal layer, as in the control (C). OR, stratum oriens; RAD, stratum radiatum. Scale bar = 250 μm in A,B, 150 μm in C,D.

1985) by i.p. injection of KA in adult MAM rats. In agreement with a previous report (De Feo et al., 1995), we found that the behavioral sensitivity to KA is similar in MAM and control adult rats (Table 1). Ninety minutes after injection, FLIR cells were found in both control and MAM animals in the dentate gyrus, the entorhinal cortex, and the amygdala, but never in the cortical heterotopias. Three hours after KA injection, we found a high density of FLIR cells in the limbic structures of MAM animals, including the hippocampus, and a low density in the neocortex (Fig. 9A) as described in normal animals (Popovici et al., 1990). In CA1 heterotopias (Fig. 9C) and in PVH (Fig. 9B), we observed a high density of FLIR cells compared with the adjacent neocortex. Quantification revealed that the density of FLIR cells was sixfold higher (25.75 ± 1.30 vs. 4.3 ± 0.80 FLIR cells/100 μm^2, $P = 0.0001$) in cortical heterotopias than in the adjacent neocortex.

Seventy-two hours after KA injection, both control and MAM rats displayed degenerating cells, mainly in the CA1 and CA4 regions of the hippocampus (Fig. 10A,B). Degenerating cells were also observed in the amygdala and entorhinal cortex. With cresyl violet staining, degenerating cells exhibited darkly stained nuclei with perinuclear clusters of condensed chromatin (Fig. 10C). In adjacent sections, these cells appeared to be highly argyrophilic. In CA1 heterotopias of MAM rats, degenerating cells, as identified with cresyl violet or Gallyas staining, were located mainly in the ventral part of the heterotopic masses and tended to form a layer (Fig. 10B). However in two of six cases, apart from the CA1 heterotopias, no degeneration was observed in the CA1 layer (Fig. 10E,F). Degenerating cells were only occasionally found in PVH and in the neocortex. No FLIR could be detected in these animals except in the nuclei of scattered cells dispersed in the CA4 field.

DISCUSSION

Mechanisms of MAM-induced hippocampal heterotopia formation

Heterotopias in the CA1 region of the hippocampus after prenatal MAM injection (Singh, 1977; Dambska et al., 1982; Chen and Hillman, 1986) or irradiation (Hicks et al., 1959; Ferrer et al., 1993) have been described by several researchers, but the developmental significance of such heterotopias have remained elusive. Singh (1977) observed that they appeared postnatally and that they contain pyramidal cells that are considerably smaller than

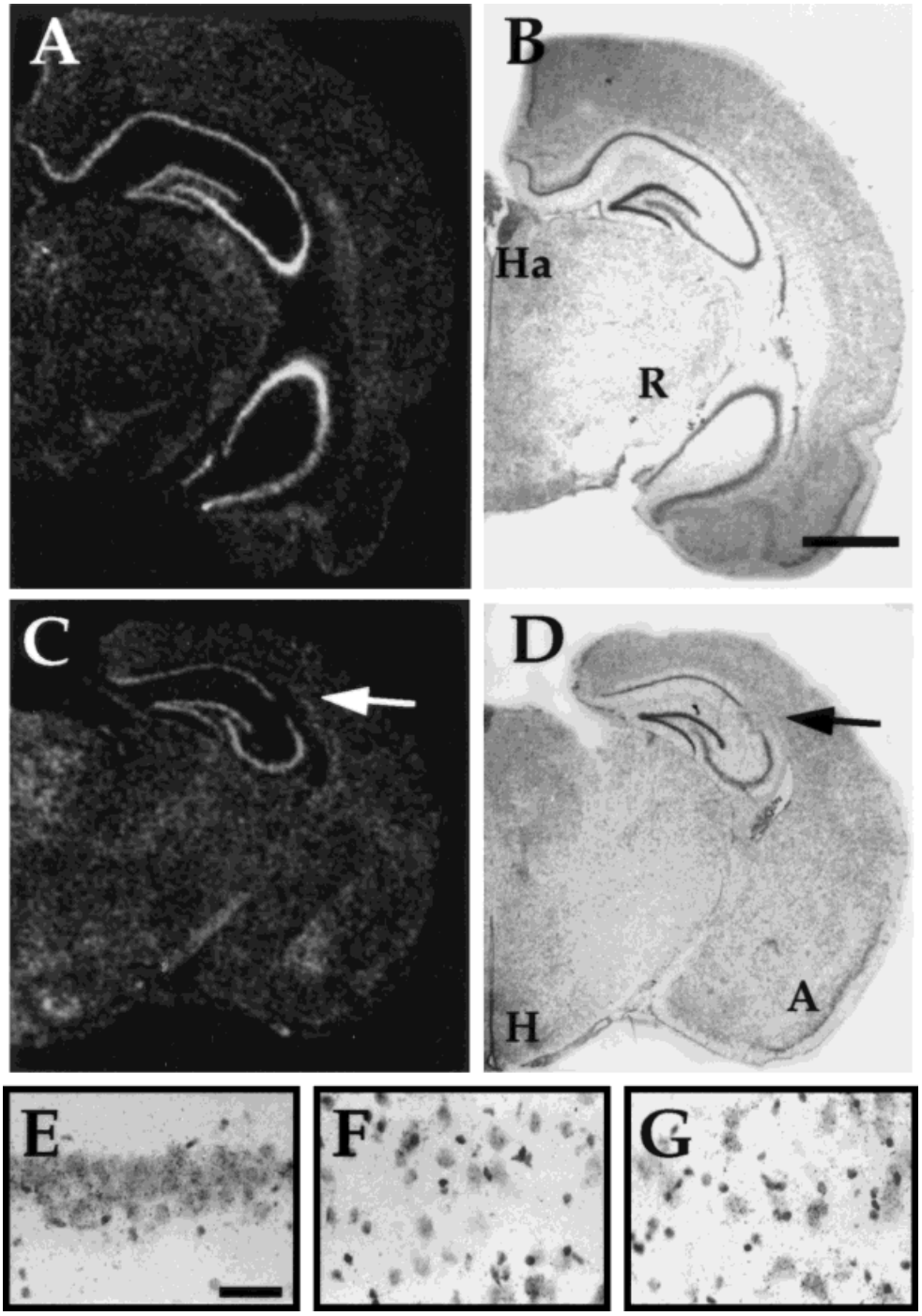

Fig. 6. **A–G:** Limbic-associated membrane protein (LAMP) mRNA expression in the brain of control and methylazoxymethanol (MAM) rats. Autoradiogram in a control (A) and a MAM (C) adult rat brain with cresyl violet of the same sections (B,D). Expression of the LAMP transcript is very prominent in limbic structures such as the hippocampus, the thalamic reticular nucleus (R), the amygdala (A), the habenula (Ha), the hypothalamus (H), and the entorhinal cortex. Note the absence of LAMP transcripts in the CA1 heterotopia (arrows in C and D). Emulsion analysis counterstained with cresyl violet shows the high expression of the LAMP transcript in CA1 (E), in the amygdala (G), but not in the CA1 heterotopia (F) of a MAM animal. Scale bar = 150 μm in A–D, 30 μm in E–G.

those in adjacent CA1 regions (Singh, 1980). Collier and Ashwell (1993) demonstrated that the topographical position of CA1 heterotopias was independent of the embryonic stage at which MAM injections were administered. Unlike PVH, CA1 heterotopias did not involve prenatal damage of the radial glia (Zhang et al., 1995). These investigators have suggested that CA1 heterotopias result from a second wave of migration of CA1 pyramidal cells.

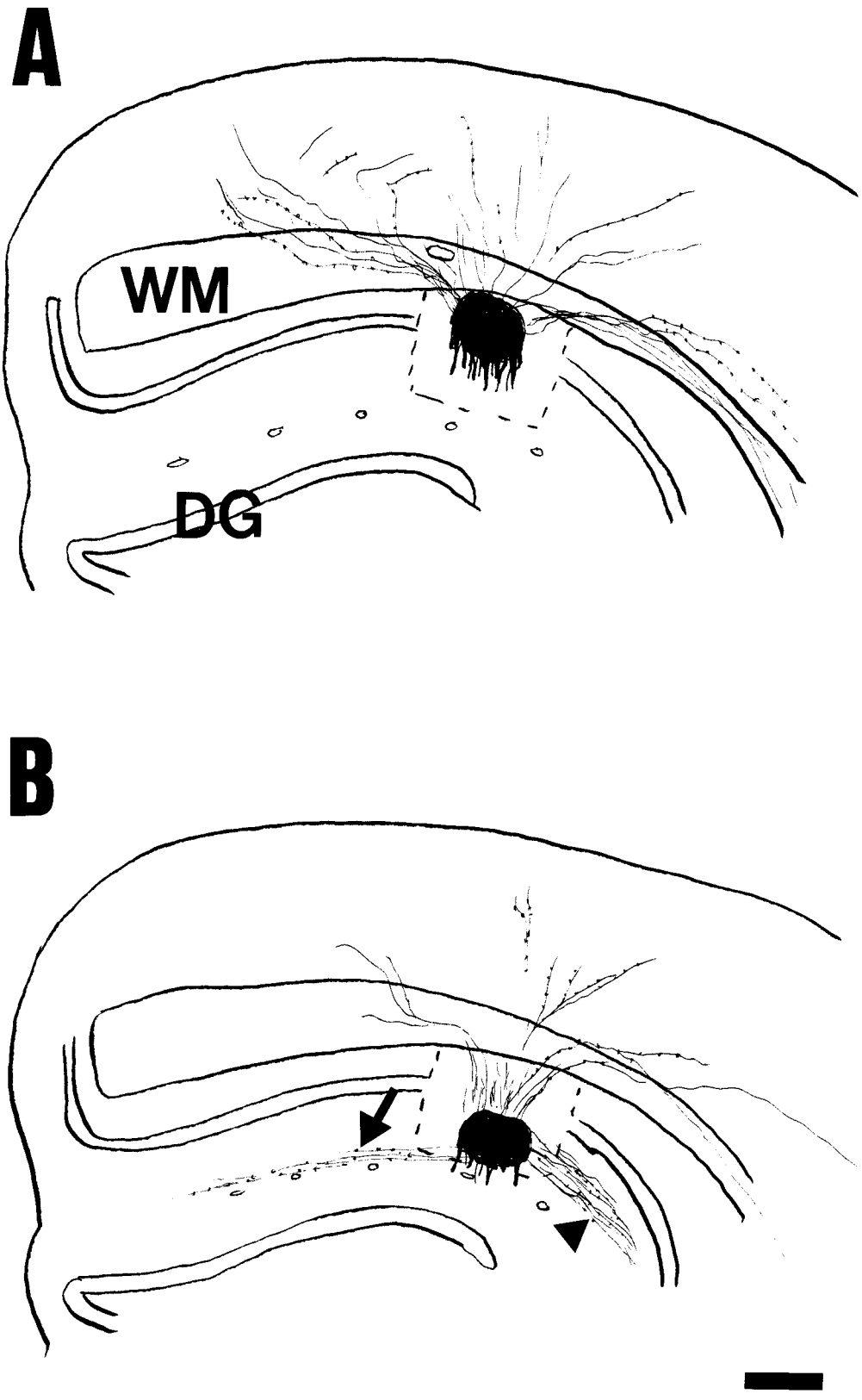

Fig. 7. Labeling of heterotopia projections in a slice preparation. Low power magnification drawings of horseradish peroxidase (HRP)-labeled fibers in a 60-μm-thick section of a 400-μm slice injected with HRP in the dorsal (**A**) or ventral (**B**) part of the heterotopia. In B, arrow points to the afferent fibers of the temporoammonic pathway, and the arrowhead points to Schaffer collaterals. WM, white matter; DG, dentate gyrus. Scale bar = 1 mm.

TABLE 1. Behavioral Effects of Convulsants in Adult MAM Animals[1]

Convulsant	Behavioral manifestations	Control	MAM
PTZ (60 mg/kg)	No change	16*	3*
	Mild clonic seizure	15	23
	Violent tonicoclonic seizure	10	15
KA (8 mg/kg)	No change	4	2
	Minor change	3	2
	Limbic seizure	14	17

[1]Adult rats treated with methylazoxymethanol (MAM) have an increased sensitivity to pentylenetetrazole (PTZ) but an unaltered sensitivity to kainic acid (KA).
*$P < 0.01$, chi-square test.

In the present study, we show that CA1 heterotopias share several developmental features with the supragranular neocortex, namely (1) similar period of neurogenesis from E16 to E20 with a rudimentary gradient; (2) postnatal migration, as opposed to prenatal migration, in CA1; (3) similar cell types, including previously undescribed in CA1 spinous stellate neurons; (4) a similar pattern and ontogenesis of calbindin and parvalbumin expression; and (5) a similar projection in the subcortical white matter and adjacent neocortex.

Therefore, we propose that CA1 heterotopias contain neocortical committed cells, similar to those of PVH, that settle secondarily in the hippocampus. We interpret the absence of CA1 heterotopias at birth to be a consequence of the incomplete resorption of the ventricle at this age, which would serve to separate physically the neocortex and the hippocampus, making CA1 invasion impossible. Between P0 and P5, the progressive junction of the hippocampus with the neocortex allows the most medial and caudal PVH to invade the CA1 region, whereas PVH, situated more laterally and rostrally, would remain physically separated from the hippocampus by the ventricle. These heterotopias thus would remain PVH. This location specificity explains the conserved position of CA1 heterotopias when the topography of the initial lesion to the germinative ventricular zone is shifted rostrally by delaying the MAM injection by 3 days from E13 to E16 (Collier and Ashwell, 1993).

At P5, we observed an abundance of pyknotic profiles in CA1 heterotopias. These dying cells may represent either heterotopic neocortical cells that failed to make synaptic connections after completion of their abnormal migration

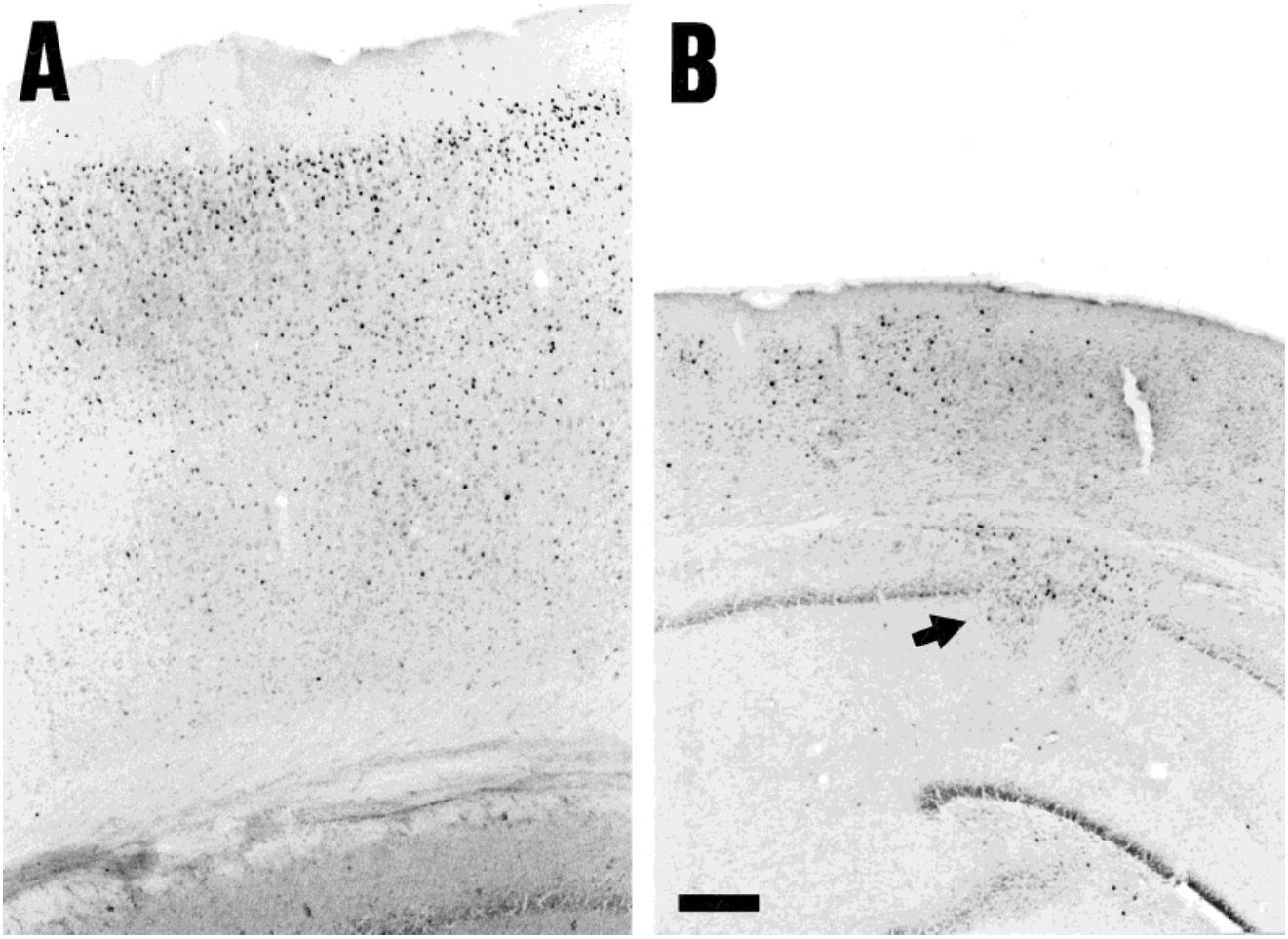

Fig. 8. Fos expression 60 minutes after a pentylenetetrazole (PTZ)-induced clonic seizure. Fos immunostaining with neutral red counterstaining in adult control (**A**) and methylazoxymethanol (MAM) rat (**B**) after PTZ-induced clonic seizures. CA1 heterotopias (arrow) are the only hippocampal region to contain Fos-like immunoreactive (FLIR) cells. Note the marked microcephaly of the MAM rat. Scale bar = 250 μm.

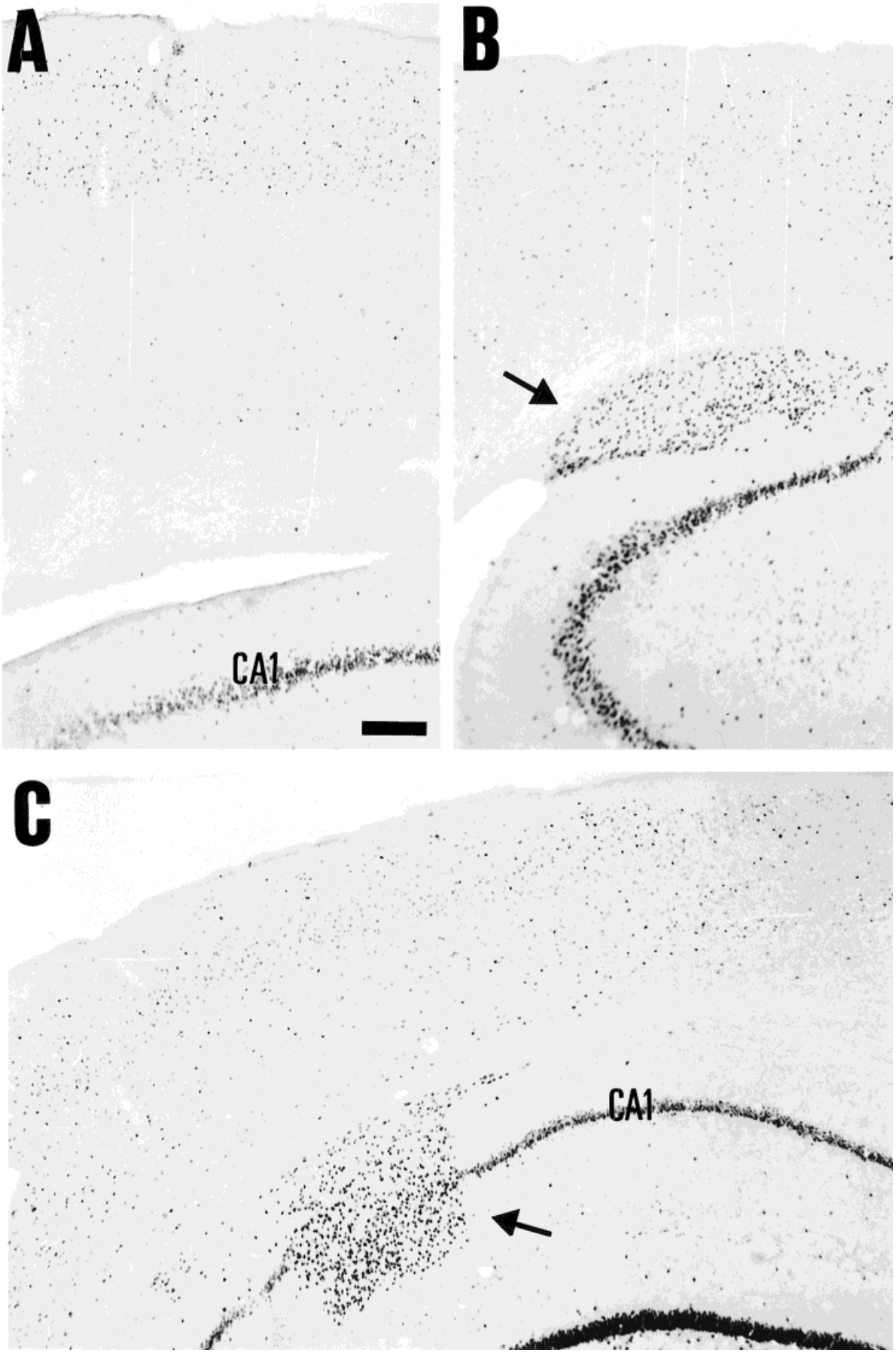

Figure 9

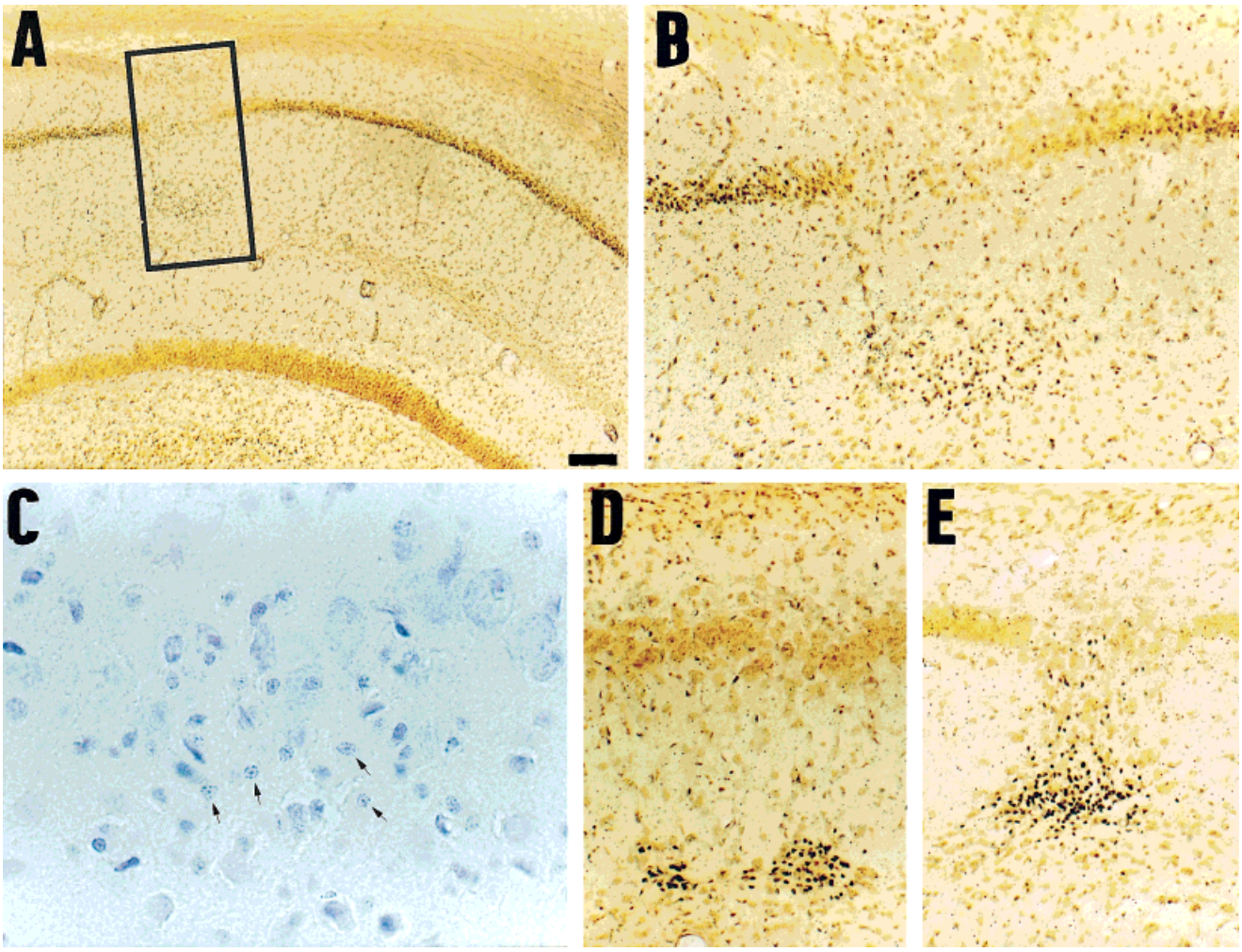

Fig. 10. Gallyas staining for degenerating cells three days after kainic acid (KA)-induced status epilepticus. Note the presence of degenerating cells in the CA1 field (**A**) and mostly in the ventral part of the heterotopic mass (**B;** higher magnification of the boxed area in **A**). In cresyl violet, these cells (arrows) have a small round nucleus with chromatin condensation (**C**). Cytoplasm, when distinguished, appeared normal. In two cases, no cell death was found in CA1 except in the ventral part of the heterotopic areas (**E**), even when the continuity of the CA1 layer was conserved (**D**). Scale bar = 250 μm in A, 100 μm in B, 25 μm in C, 50 μm in D,E.

or endogenous CA1 pyramidal cells that were displaced by the cortical invasion and lost their synaptic contacts. Double labeling with a hippocampal marker such as LAMP and a cell death marker is required to clarify this point. Alternatively, CA1 pyramidal cells may have been pushed by the cortical invasion and displaced to the border of the heterotopias. Further developmental studies are clearly required to determine the mechanisms of the supposed migration of PVH cells to the hippocampus and the fate of CA1 pyramidal neurons.

Fig. 9. Fos expression 180 minutes after a kainic acid (KA)-induced limbic seizure. Cortical Fos-like immunoreactive (FLIR) 180 minutes after KA injection in control (**A**) and methylazoxymethanol (MAM) (**B,C**) rats. FLIR cells are present in the supragranular layers of the neocortex of control rats and in all layers of the neocortex in MAM rats. Note the very high density of FLIR cells (arrow) in the PVH and CA1 heterotopias of MAM rats. Scale bar = 250 μm.

Early commitment of heterotopic neurons to a neocortical phenotype

The mechanisms underlying the phenotype specification of neocortical neurons have been widely discussed (Kennedy and Dehay, 1993; Götz and Price, 1994; Levitt et al., 1997). Several lines of evidence, including the study of the projection pattern in the *reeler* mutant (Caviness, 1976; Terashima et al., 1987) and in genetic (Lee et al., 1997) or experimentally induced heterotopias (Jones et al., 1982; Jensen and Killackey, 1984; Yurkewicz et al., 1984; Miller, 1987) support the idea of an early "genetic" specification of several aspects of the neuronal phenotype.

Another popular approach to investigate the mechanisms underlying phenotype specification consists of heterochronic and/or heterotopic transplantation of progenitor cells to a new environment (O'Leary, 1989; Ebrahimi-Gaillard and Roger, 1996; Jankovski et al., 1996). As such, CA1 heterotopias may be considered as naturally occur-

ring transplants of neocortical undifferentiated neurons into a limbic structure. This offers an opportunity to compare the biochemical characteristics of neurons in the heterotopias and in the adjacent CA1 pyramidal layer (new environment) or supragranular neocortex (to which they were committed). We have observed an absence of LAMP transcripts in CA1 heterotopias, and this could result from failure of heterotopic CA1 pyramidal neurons to express this marker. However, studies by Levitt and collaborators (Barbe and Levitt, 1991; Ferri and Levitt, 1993; Barbe and Levitt, 1995) have shown that presumptive limbic cortex transplanted to the neocortex after E13 conserves LAMP expression and its typical pattern of projection. Therefore, we assume that the absence of LAMP expression in heterotopic neurons is an other argument in favor of their neocortical origin.

Using Golgi analysis of CA1 heterotopias, we found small pyramidal neurons very similar to the small pyramidal cells typical of the rodent supragranular neocortex (Ramon y Cajal, 1911; Larkman and Mason, 1990; Schröder and Luhmann, 1997). The various types of nonpyramidal neurons we have observed have also been described in the rat neocortex (Feldman and Peters, 1978). These results support the idea of an early "genetic" control of neuronal morphology that is independent of the direction of migration.

Similar conclusions have been drawn from the phenotype of ectopic granule neurons in the molecular layer of the cerebellum in normal rats (Stoughton et al., 1978; Griffin et al., 1980; Lafarga and Berciano, 1985) and, to a much larger extent, following postnatal irradiation (Altman, 1973) or injection of various toxins (Sotelo and Rio, 1980; Robain et al., 1985) including MAM (Garcia-Ladona et al., 1991). These cells were found to conserve their characteristic shape and dendritic arborization and to express several molecules typical of normally positioned granule cells (Lafarga and Berciano, 1985; Sievers et al., 1985; Garcia-Ladona et al., 1991). These data fit very well with our demonstration that neocortical neurons conserve their morphological properties when heterotopically situated in the hippocampus. In both these situations, the neuronal phenotype is acquired very early in development and is independent of the final localization of the cell.

Neocortical heterotopias in CA1 may be integrated into both neocortical and hippocampal circuitry

As observed with extracellular HRP injections, heterotopia projections appeared in the neocortex and subcortical white matter, independent of the injection, and in the main afferent pathways to CA1, when the injection site was in the ventral portion of the heterotopia.

As previously observed (Burkhalter, 1989), HRP tracing in slices does not allow one to distinguish afferent from efferent projections because of the absence of cell somata retrograde labeling. Despite this technical limitation, our data indicate that CA1 heterotopias are integrated in both the neocortical (afferent and/or efferent projections to the neocortex and white matter) and hippocampal (likely to be afferent connections) networks. We tested this hypothesis further in vivo with a functional approach based on the effects of seizures on Fos expression (Dragunow and Faull, 1989; White and Price, 1993). This technique has been used to study the functional connectivity of intrahippocampal grafts (Vicario-Abejon et al., 1995).

After PTZ-induced seizures, we observed FLIR cells in the neocortex and in the thalamus. This observation is in agreement with previous studies with mild clonic seizures (Smeyne et al., 1992; Jensen et al., 1993). A striking feature in MAM animals was that CA1 heterotopias were the only hippocampal region to contain FLIR cells after PTZ-induced seizures. This result shows that CA1 heterotopias can be activated together with the neocortex and confirms our hypothesis that CA1 heterotopias conserve neocortical connections. Similar conclusions have been drawn from ectopic cerebellar granule cells that were shown to receive normal afferent mossy fibers connections and to send efferent connections to the Purkinje cells (Berciano and Lafarga, 1988).

However, KA-induced limbic seizures cause intense Fos expression during the seizure and prominent cell death after 3 days. Both of these features have been observed in the CA1 layer (for Fos expression, see LeGal La Salle, 1988; Popovici, et al., 1990; White and Price, 1993; Kasof et al., 1995; for cell death, see Ben-Ari et al., 1980; Weiss et al., 1996; Kotti et al., 1997) and are direct and indirect consequences of the KA-induced paroxysmal activity i nthe limbic circuitry (reviewed in Khrestchatisky et al., 1995; Represa et al., 1995). Together with our tracing experiments and previous anatomical data (Singh, 1978), these data suggest that CA1 neocortical heterotopias are also integrated into the hippocampal network. Electrophysiological experiments are in progress to provide direct evidence for this hypothesis.

ACKNOWLEDGMENTS

We are grateful to Drs. P. Gaspar, J.-L. Gaïarsa, O. Robain, and C. Sotelo for helpful discussions and to Drs. G.L. Holmes and H. McLean for linguistic assistance. N.C.-A.-L. received a fellowship from Ecole Normale Supérieure de Lyon and A.R. received a fellowship from Association Française contre les Myopathies.

LITERATURE CITED

Alcantara, S., I. Ferrer, and E. Soriano (1993) Postnatal development of parvalbumin and calbindin D28K immunoreactivities in the cerebral cortex of the rat. Anat. Embryol. *188:*63–73.

Altman, J. (1973) Experimental reorganization of the cerebellar cortex: III Regeneration of the external germinal layer and granule cells ectopia. J. Comp. Neurol. *149:*123–152.

Angevine, J.B. and R.L. Sidman (1961) Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. Nature *192:*766–768.

Anton, E.S., R.S. Cameron, and P. Rakic (1996) Role of neuron-glial junctional domain proteins in the maintenance and termination of neuronal migration across the embryonic cerebral wall. J. Neurosci. *16:*2283–2293.

Baraban, S.C. and P.A. Schwartzkroin (1996) Flurothyl seizure susceptibility in rats following prenatal methylazoxymethanol treatment. Epilepsy Res. *23:*189–194.

Barbe, M.F. and P. Levitt (1991) The early commitment of fetal neurons to the limbic cortex. J. Neurosci. *11:*519–533.

Barbe, M.F. and P. Levitt (1995) Age-dependent specification of the corticocortical connections of cerebral grafts. J. Neurosci. *15:*1819–1834.

Barkovitch, A.J. and B.O. Kjos (1992) Gray matter heterotopias: MR characteristics and correlation with developmental and neurological manifestations. Radiology *182:*493–499.

Ben-Ari, Y. (1985) Limbic seizure and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy. Neuroscience *14:*375–403.

Ben-Ari, Y., E. Tremblay, O.P. Ottersen, and B.S. Meldrum (1980) The role of epileptic activity in hippocampal and "remote" cerebral lesions induced by kainic acid. Brain Res. *191:*79–97.

Ben-Ari, Y., E. Tremblay, D. Riche, G. Ghilini, and R. Naquet (1981) Electrographic, clinical and pathological alterations following systemic administration of kainic acid, bicuculline or pentetrazole: Metabolic mapping using the deoxyglucose method with special reference to the pathology of epilepsy. Neuroscience *6:*1361–1391.

Berciano, M.T. and M. Lafarga (1988) Colony forming ectopic granule cells in the cerebellar primary fissure of normal adult rats: A morphologic and morphometric study. Brain Res. *439:*169–178.

Bernardo, K.L. and T.A. Woolsey (1987) Axonal trajectories between mouse somatosensory thalamus and cortex. J. Comp. Neurol. *258:*542–564.

Bernardo, K.L., P.M. Ma, and T.A. Woolsey (1986) In vitro labeling of axonal projections in the mammalian nervous system. J. Neurosci. Methods *16:*89.

Berry, M., A.W. Rogers, and J.T. Eayrs (1964) Pattern of cell migration during cortical histogenesis. Nature *203:*591–593.

Burkhalter, A. (1989) Intrinsic connections of rat primary visual cortex: Laminar organization of axonal projections. J. Comp. Neurol. *279:*171–186.

Cattabeni, F. and M. Di Luca (1997) Developmental models of brain dysfunctions induced by targeted cellular ablations with methylazoxymethanol. Physiol. Rev. *77:*199–215.

Cattaneo, E., B. Reinach, A. Caputi, F. Cattabeni, and M. Di Luca (1995) Selective in vitro blockade of neuroepithelial cells proliferation by methylazoxymethanol acetate, a molecule capable of inducing long lasting functional impairments. J. Neurosci. Res. *41:*640–647.

Caviness, V.S., Jr. (1976) Patterns of cell and fiber distribution in the neocortex of the reeler mutant mice. J. Comp. Neurol. *170:*435–448.

Chen, S. and D.E. Hillman (1986) Selective ablation of neurons by methylazoxymethanol during pre- and postnatal brain development. Exp. Neurol. *94:*103–119.

Collier, P.A. and K.W.S. Ashwell (1993) Distribution of neuronal heterotopias following prenatal exposure to methylazoxymethanol. Neurotoxicol. Teratol. *15:*439–444.

Dambska, M., R. Haddad, P.B. Kozlowski, M.H. Lee, and J. Shek (1982) Telencephalic cytoarchitectonics in the brains of rats with graded degrees of micrencephaly. Acta Neuropathol. (Berl.) *58:*203–209.

De Feo, M.R., O. Mecarelli, and G.F. Ricci (1995) Seizure susceptibility in immature rats with micrencephaly induced by prenatal exposure to methylazoxymethanol acetate. Pharmacol. Res. *31:*109–114.

Des Portes, V., J.-M. Pinard, P. Billuart, M.-C. Vinet, A. Koulakoff, A. Carrié, A. Gélot, A. Dupuis, J. Motte, Y. Berwald-Netter, M. Catala, A. Kahn, C. Beldjord, and J. Chelly (1998) Identification of a novel CNS gene required for neuronal migration and involved in X-linked subcortical heterotopias and lissencephaly syndrome. Cell *92:*51–61.

Dragunow, M. and R. Faull (1989) The use of c-Fos as a metabolic marker in neuronal pathway tracing. J. Neurosci. Methods *29:*261–265.

Dubeau, F., D. Tampieri, N. Lee, E. Andermann, S. Carpenter, R. Leblanc, A. Olivier, R. Radtke, J.-G. Villemure, and F. Andermann (1995) Periventricular and subcortical nodular heterotopia. A study of 33 patients. Brain *118:*1273–1287.

Ebrahimi-Gaillard, A. and M. Roger (1996) Development of spinal cord projections from neocortical transplants heterotopically placed in the neocortex of newborn hosts is highly dependant on the embryonic locus of origin of the graft. J. Comp. Neurol. *365:*129–140.

Eksioglu, Y.Z., I.E. Scheffer, P. Cardenas, J. Knol, F. DiMario, G. Ramsby, M. Berg, K. Kamuro, S.F. Berkovic, G.M. Duyk, J. Parisi, P.R. Huttenlocher, and C.A. Walsh (1996) Periventricular heterotopia: An X-linked dominant epilepsy locus causing aberrant cerebral cortical development. Neuron *16:*77–87.

Feldman, M.L. and A. Peters (1978) The forms of non-pyramidal neurons in the visual cortex of the rat. J. Comp. Neurol. *179:*761–794.

Ferrer, I., I. Fabregues, and G. Palacios (1982) An autoradiographic study of methylazoxymethanol acetate-induced cortical malformation. Acta Neuropathol. (Berl.) *57:*313–315.

Ferrer, I., A. Xumetra, and J. Santamaria (1984) Cerebral malformation induced by prenatal X-irradiation: An autoradiographic and Golgi study. J. Anat. *138:*81–93.

Ferrer, I., S. Alcantara, M.J. Zujer, and C. Cinos (1993) Structure and pathogenesis of cortical nodules induced by prenatal X-irradiation in the rat. Acta Neuropathol. (Berl.) *85:*205–212.

Ferri, R. and P. Levitt (1993) Cerebral cortical progenitors are fated to produce region-specific neuronal populations. Cereb. Cortex *3:*187–198.

Freund, T.F. and G. Buzsaki (1996) Interneurons of the hippocampus. Hippocampus *6:*347–370.

Gallyas, F., J.R. Wolff, H. Böttcher, and L. Zaborsky (1980) A reliable and sensitive method to localize terminal degeneration and lysosomes in the central nervous system. Stain. Technol. *55:*299–306.

Garcia-Ladona, F.J., J. de Barry, C. Girard, and G. Gombos (1991) Ectopic granule cell layer in mouse cerebellum after methylazoxymethanol (MAM) treatment. Exp. Brain Res. *86:*90–96.

Götz, M. and J. Price (1994) Cell fate and axonal projection from the cerebral cortex. Sem. Dev. Biol. *5:*359–369.

Gressens, P., B.E. Kosofsky, and P. Evrard (1992) Cocaine-induced disturbances of corticogenesis in the developing murine brain. Neurosci. Lett. *140:*113–116.

Griffin, W.S.T., M.A.E. Eriksson, M. del Cerro, D.J. Woodward, and N. Stampfer (1980) Naturally occurring alterations of cortical layers surrounding the fissura prima of cerebellum. J. Comp. Neurol. *192:*109–118.

Harding, B. (1996) Gray matter heterotopia. In R. Guerrini, E. Andermann, R. Canapicchi, J. Roger, B. Zilfkin, and P. Pfanner (eds): Dysplasias of the Cerebral Cortex and Epilepsy. Philadelphia: Lippincott-Raven, pp. 81–88.

Hicks, S.P., C.J. D'Amato, and M.J. Lowe (1959) The development of the mammalian nervous system: I. Malformation of the brain, especially the cerebral cortex, induced in rats by radiation. II. Some mechanisms of the malformations of the cortex. J. Comp. Neurol. *113:*435–469.

Jankovski, A., F. Rossi, and C. Sotelo (1996) Neuronal precursors in the postnatal mouse cerebellum are fully committed cells: Evidence from heterochronic transplantations. Eur. J. Neurosci. *8:*2308–2319.

Jensen, F.E., I.R. Firkusny, and G.D. Mower (1993) Differences in c-Fos immunoreactivity due to age and mode of seizure induction. Mol. Brain Res. *17:*185–193.

Jensen, K.F. and H.P. Killackey (1984) Subcortical projections from ectopic neocortical neurons. Proc. Natl. Acad. Sci. USA *81:*964–968.

Johnston, M.V. and J.T. Coyle (1979) Histological and neurochemical effects of fetal treatment with metylazoxymethanol on rat neocortex in adulthood. Brain Res. *170:*135–155.

Jones, E.G., K.L. Valentino, and J.W. Fleshman (1982) Adjustment of connectivity in rat neocortex after destruction of precursor cells of layers II–IV. Dev. Brain Res. *2:*425–431.

Kasof, G.M., A. Mandelzys, S.D. Maika, R.E. Hammer, T. Currran, and J.I. Morgan (1995) Kainic acid-induced neuronal death is associated with DNA damage and a unique immediate-early gene response in *c-Fos-lacZ* transgenic rats. J. Neurosci. *15:*4238–4249.

Kennedy, H. and C. Dehay (1993) Cortical specification of mice and men. Cereb. Cortex *3:*171–186.

Khrestchatisky, M., L. Ferhat, G. Charton, A. Bernard, H. Pollard, A. Represa, and Y. Ben-Ari (1995) Molecular correlates between reactive and developmental plasticity in the rat hippocampus. J. Neurobiol. *26:*426–436.

Kotti, T., T. Halonen, J. Sirviö, P. Riekkinen, and R. Miettinen (1997) Comparison of NADPH diaphorase histochemistry, somatostatin immunohistochemistry, and silver impregnation in detecting structural and functional impairment in experimental status epilepticus. Neuroscience *80:*105–117.

Lafarga, M. and M.T. Berciano (1985) A golgi and morphometric study of the ectopic granule cells of the molecular layer of the rat cerebellum. Brain Res. *345:*398–401.

Larkman, A.U. and A. Mason (1990) Correlations between morphology and electrophysiology of pyramidal neurones in slices of rat visual cortex. I. Establishment of cell classes. J. Neurosci. *10:*1407–1414.

Lee, K.S., F. Schottler, J.L. Collins, G. Lanzino, D. Couture, A. Rao, K.-I. Hiramatsu, Y. Goto, S.-C. Hong, H. Caner, H. Yamamoto, Z.-F. Chen, E. Bertram, S. Berr, R. Omary, H. Scrable, T. Jackson, J. Goble, and L. Eisenman (1997) A genetic model of human neocortical heterotopia associated with seizures. J. Neurosci. *17:*6236–6242.

LeGal LaSalle, G. (1988) Long-lasting and sequential increase of c-Fos oncoprotein expression in kainic acid-induced status epilepticus. Neurosci. Lett. *88:*127–130.

Levitt, P. (1984) A monoclonal antibody to limbic system neurons. Science *223:*299–301.

Levitt, P. (1985) Relating molecular specificity to normal and anormal brain development. Ann. N.Y. Acad. Sci. *450:*239–246.

Levitt, P., M.F. Barbe, and K.L. Eagleson (1997) Patterning and specification of the cerebral cortex. Annu. Rev. Neurosci. *20:*1–24.

Marret, S., P. Gressens, and P. Evrard (1996) Arrest of neuronal migration by excitatory amino acids in hamster developing brain. Proc. Natl. Acad. Sci. USA *93:*15463–15468.

Matsumoto, H., M. Spatz, and G.L. Laqueur (1972) Quantitative changes with age in the DNA content of methylazoxymethanol-induced microcephalic rat brain. J. Neurochem. *19:*297–306.

Miller, M.W. (1986) Effects of alcohol on generation and migration of cerebral cortex neurons. Science *233:*1308–1311.

Miller, M.W. (1987) Effect of prenatal exposure to alcohol on the distribution and time of origin of corticospinal neurons in the rat. J. Comp. Neurol. *257:*372–382.

Morgan, J.I., D.R. Cohen, J.L. Hempstead, and T. Curran (1987) Mapping patterns of *c-Fos* expression in the central nervous system after seizure. Science *237:*192–197.

Moshe, S.L., L. Velisek, and G.L. Holmes (1994) Developmental aspects of experimental generalized seizures induced by pentylenetetrazol, bicuculline and flurothyl. In A. Malafosse, P. Genton, E. Hirsch, C. Marescaux, D. Broglin, and R. Bernasconi (eds): Idiopathic Generalized Epilepsies: Clinical, Experimental and Genetic Aspects. London: John Libbey & Co Ltd., pp. 201–213.

Nadler, J.V. and D.A. Evenson (1983) Use of excitatory amino acids to make axon-sparing lesions of hypothalamus. Methods Enzymol. *103:*393–400.

Nitsch, R., I. Bergmann, K. Kuppers, M. Mueller, and M. Frotscher (1990) Late appearance of parvalbumin-immunoreactivity in the development of GABAergic neurons in the rat hippocampus. Neurosci. Lett. *118:*147–150.

O'Leary, D. (1989) Do cortical areas emerge from a protocortex? Trends Neurosci. *12:*400–406.

Popovici, T., A. Represa, V. Crépel, G. Barbin, M. Beaudoin, and Y. Ben-Ari (1990) Effects of kainic acid-induced seizures and ischemia on c-*Fos* like proteins in rat brain. Brain Res. *536:*183–194.

Rafiki, A., N. Chevassus-au-Louis, Y. Ben-Ari, M. Khrestchatisky, and A. Represa (1998) Glutamate receptors in rat with diffuse cortical dysplasia: An in situ hybridization and immunohistochemical study. Brain Res. *782:*141–154.

Rakic, P. (1988) Defects of neuronal migration and the pathogenesis of cortical malformations. Prog. Brain Res. *73:*15–37.

Ramon y Cajal, S. (1911) Histologie du système nerveux central de l'homme et des vertébrés. Paris: Maloine.

Reinoso, B.S., A.F. Pimenta, and P. Levitt (1996) Expression of the mRNAs encoding the limbic system-associated membrane protein (LAMP): I. Adult rat brain. J. Comp. Neurol. *375:*274–288.

Represa, A., J. Niquet, H. Pollard, and Y. Ben-Ari (1995) Cell death, gliosis and synaptic remodeling in the hippocampus of epileptic rats. J. Neurobiol. *26:*413–425.

Robain, O., L. Lanfumey, J. Adrien, and E. Farkas (1985) Developmental changes in the cerebellar cortex after locus coeruleus lesion with 6-hydroxydopamine in the rat. Exp. Neurol. *88:*150–164.

Rosen, G.D., G.F. Sherman, J.M. Richman, L.V. Stone, and A.M. Galaburda (1992) Induction of molecular layer ectopias by puncture wounds in newborn rats and mice. Dev. Brain Res. *67:*285–291.

Sanchez, M.P., C. Frassoni, G. Alvarez-Bolado, R. Spreafico, and A. Fairen (1992) Distribution of calbindin and parvalbumin in the developing somatosensory cortex and its primordium in the rat: An immunocytochemical study. J. Neurocytol. *21:*717–736.

Saper, C.B. (1996) Any way you count it: a new journal policy for the use of unbiased counting methods. J. Comp. Neurol. *364:*5.

Schröder, R. and H.J. Luhmann (1997) Morphology, electrophysiology and pathophysiology of supragranular neurons in rat primary somatosensory cortex. Eur. J. Neurosci. *9:*163–176.

Senba, E., K. Matsunaga, M. Tohyama, and K. Noguchi (1993) Stress-induced *c-Fos* expression in the rat brain: Activation mechanism of sympathetic pathway. Brain Res. Bull. *31:*329–344.

Sharp, F.R., S.M. Sagar, K. Hicks, D. Lowenstein, and K. Hisanaga (1991) c-Fos mRNA, FOS, and Fos-related antigen induction by hypertonic saline and stress. J. Neurosci. *11:*2321–2331.

Sherman, G.F., G.D. Rosen, L.V. Stone, D.M. Press, and A.M. Galaburda (1992) The organization of radial glial fibers in spontaneous ectopias of newborn New Zealand Black mice. Dev. Brain Res. *67:*279–283.

Sievers, J., U. Mangold, and M. Berry (1985) 6-OHDA-induced ectopia of external granule cells in the subarachnoid space covering the cerebellum. III. Morphology and synaptic organization of ectopic cerebellar neurons: A scanning and transmission electron microscopic study. J. Comp. Neurol. *232:*319–330.

Singh, S.C. (1977) Ectopic neurons in the hippocampus of the postnatal rat exposed to methylazoxymethanol during fetal development. Acta Neuropathol. (Berl.) *40:*111–116.

Singh, S.C. (1978) Redirected perforant and commissural connections of eutopic and ectopic neurons in the hippocampus of methylazoxymethanol-acetate treated rats. Acta Neuropathol. (Berl.) *44:*197–202.

Singh, S.C. (1980) Deformed dendrites and reduced spine numbers on ectopic neurones in the hippocampus of rats exposed to methylazoxymethanol-acetate. Acta Neuropathol. (Berl.) *49:*193–198.

Smeyne, R.J., K. Schilling, L. Robertson, D. Luk, J. Oberdick, T. Curran, and J. Morgan (1992) Fos-lacZ transgenic mice: Mapping sites of gene induction in the central nervous system. Neuron *8:*13–23.

Soriano, E., J.A. DelRio, I. Ferrer, C. Auladell, L. De Lecea, and S. Alcantara (1992) Late appearance of parvalbumin-immunoreactive neurons in the rodent cerebral cortex does not follow an inside-out sequence. Neurosci. Lett. *142:*147–150.

Sotelo, C. and J.P. Rio (1980) Cerebellar malformation obtained in rats by early postnatal treatment with 6-aminonicotinamide: Role of neuron glia interaction in cerebellum development. Neuroscience *5:*1737–1759.

Stoughton, R.L., M. DelCerro, J.R. Walker, and J.R. Swarz (1978) Presence of displaced neuronal elements within rat cerebellar fissures. Brain Res. *148:*15–29.

Sun, X.-Z., M. Inouye, Y. Takagishi, S. Hayasaka, and T. Yamamura (1995) Follow-up study on histogenesis of microcephaly associated with ectopic gray matter induced by prenatal γ-irradiation in the mouse. J. Neuropathol. Exp. Neurol. *55:*357–365.

Terashima, T., K. Inoue, Y. Inoue, and K. Mikoshiba (1987) Thalamic connectivity of the primary motor cortex of normal and reeler mutant mice. J. Comp. Neurol. *257:*405–421.

Vicario-Abejon, C., M.G. Cunningham, and R.D.G. McKay (1995) Cerebellar precursors transplanted to the neonatal dentate gyrus express features characteristic of hippocampal neurons. J. Neurosci. *15:*6351–6363.

Weiss, S., O. Cataltepe, and A.J. Cole (1996) Anatomical studies of DNA fragmentation in rat brain after systemic kainate administration. Neuroscience *74:*541–551.

White, L.E. and J.L. Price (1993) The functional anatomy of limbic status epilepticus in the rat. I. Patterns of ^{14}C-2-deoxyglucose uptake and Fos immunocytochemistry. J. Neurosci. *13:*4787–4809.

Yurkewicz, L., K.L. Valentino, M.K. Floeter, J.W. Fleshman, and E.G. Jones (1984) Effects of cytotoxic deletions of somatic sensory cortex in fetal rats. Somatosen. Res. *1:*303–327.

Zhang, L.L., P.A. Collier, and K.W.S. Ashwell (1995) Mechanisms in the induction of neuronal heterotopias following prenatal cytotoxic brain damage. Neurotoxicol. Teratol. *17:*297–311.