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# Appearance of parvalbumin-specific immunoreactivity in the cerebral cortex and hippocampus of the developing rat and gerbil brain

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**Summary.** Developmental changes in the distribution of parvalbumin-specific immunoreactivity in the brain, in particular in the cerebral cortex and hippocampus, were followed immunohistochemically in two different species, the rat and the Mongolian gerbil (*Meriones unguiculatus*) using an antibody raised against for rat parvalbumin. The gerbil is known to develop its auditory and visual capacity later than rat. In both the rat and gerbil, parvalbumin-specific immunoreactivity appeared after birth in both the cerebral cortex and hippocampus. The timing of the development of expression of parvalbumin varied among different parts of the cerebral cortex. The parietal cortex showed evidence of the earliest expression of parvalbumin whilst the occipital and temporal cortices expressed parvalbumin at a later stage of a development. This feature was common to both the rat and gerbil but occurred at a relatively later stage in the gerbil. The profile of the distribution of parvalbumin in the brain of the developing and adult gerbil was similar to that of the rat, but there were some differences. The frequency of bead-like structures on the dendrites of the parvalbumin-positive cells in the CA1 region of the hippocampus was markedly lower in the gerbil; instead, straight non-beaded fibers which ran vertically into the pyramidal layer were stained. Parvalbumin-positive fibers were also found in the cerebral cortex of the gerbil.

## Introduction

Parvalbumin is a small, acidic protein which has a high affinity for calcium (Heizmann 1984; Heizmann 1988). Immunohistochemical studies have shown that this protein is present in distinct subpopulations of neurons in the brains of a wide range of animals, including birds and man (for reviews, see Heizmann and Braun 1989; Heizmann and Hunziker 1990). Parvalbumin is believed to participate in some of the calcium-dependent processes in neurons, and several physiological functions have been suggested for it (Kamphuis et al. 1989), however, its precise physiological roles are still unknown. There have been several studies of the expression of parvalbumin in the developing brain of various species: in the cerebrum, cerebellum and retina of the rat (Endo et al. 1985); in the reticular thalamic nucleus of the rat (Seto-Ohshima et al. 1989); in the vestibular fibers of the rat (Morris et al. 1988); in the cerebellum and visual nuclei of the zebra finch (Braun et al. 1986, 1988; Heizmann and Celio 1987); and in the visual cortex of the cat (Stichel et al. 1986). Detailed analysis of the temporal and spatial changes in the expression of parvalbumin may also provide hints as to the possible functions of parvalbumin in the brain. In order to obtain more information on the timing of the expression of parvalbumin, the appearance of parvalbumin, as well as that of GABA-like immunoreactivity in the cerebral cortex and hippocampus, was followed immunohistochemically during the development of the brain in two different species, the rat and the gerbil, which have distinctly different schedules of functional development.

Several processes during the developmental differentiation of the central nervous system are dependent on $Ca^{2+}$. These processes include elongation of neurites, formation of synapse and the development of neurotransmission. The gerbil and the rat each have their own individual time schedules for functional development. The gerbil is known to develop its auditory and visual capacity later than rat. Using these two species, we looked for a possible correlation between the appearance of the expression of parvalbumin and the functional maturation of the central nervous system.

## Materials and methods

### *Antibodies*

Specific antibodies raised in rabbit against parvalbumin from rat muscle (Kägi et al. 1987) and against GABA conjugated to rabbit serum albumin (Aoki et al. 1985) were used in this study.

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### *Animals*

Mongolian gerbils and Sprague-Dawley rats were bred in our animal facility and given free pelleted food and tap water ad libitum.

### *Muscle extracts*

Hind-leg skeletal muscles from gerbils or rats were homogenized in a Polytron (Kinematica, Luzern, Switzerland) in 2 volumes of 4 m*M* EDTA that contained 0.4 m*M* phenylmethylsulfonylfluoride. The homogenate was immediately heated to 80° C for 10 min, chilled on ice and then centrifuged at 20,000 × *g* for 30 min. The supernatant was dialyzed against distilled $H_2O$, centrifuged and then lyophilized.

### *Purification of muscle parvalbumin*

Parvalbumin from gerbil muscle was purified by a slightly modified version of the method of Endo et al. (1986). The hind-leg muscle was homogenized in a Polytron in 2.5 volumes of 0.05 *M* phosphate buffer (pH 7.1) that contained 0.1 m*M* EGTA, 0.4 m*M* phenylmethylsulfonylfluoride and 1 μ*M* pepstatin. The homogenate was immediately heated to 80° C for 10 min, chilled on ice and then centrifuged at 20,000 × *g* for 30 min; $(NH_4)_2SO_4$ was added to the supernatant to 55% saturation. After centrifugation of this mixture, one-tenth volume of 50% trichloroacetic acid was added to the supernatant and the precipitate obtained by centrifugation was dissolved in 0.05 *M* phosphate buffer (pH 7.1) that contained 1 m*M* EGTA and dialyzed against the same solution. The dialyzate was loaded onto a column of DEAE-Sephadex A-50 which had been equilibrated with the dialysis buffer. After washing of the column with the same solution, gerbil parvalbumin was eluted with 0.05 *M* phosphate buffer (pH 7.1) that contained 1 m*M* EGTA and 0.05 *M* NaCl, in the same way as parvalbumin from rat muscle. The fraction that contained the most parvalbumin was passed through a second column of DEAE-Sephadex under the same conditions. Gerbil parvalbumin was homogeneous when examined by SDS-polyacrylamide (15%) gel electrophoresis (Laemmli 1970).

### *Gel electrophoresis and immunoblotting*

Extracts of gerbil and rat muscle were subjected to electrophoresis on SDS-polyacrylamide (15%) gels and transferred onto nitrocellulose sheets (Towbin et al. 1979). Various amounts of authentic purified parvalbumins were also blotted on the nitrocellulose sheets. Protein concentrations were measured by Bradford's method (1976) using the dye reagent from Bio-Rad (Richmond, Calif., USA) with bovine serum albumin as a standard. Immunostaining was carried out essentially as described by Berchtold et al. (1984) except for use of 10% normal goat serum (NGS) instead of fetal calf serum. Briefly, each nitrocellulose sheet was washed with 0.05 *M* *Tris*-HCl buffer that contained 0.15 *M* NaCl (pH 7.0), blocked with 3% bovine serum albumin in the same buffer supple-

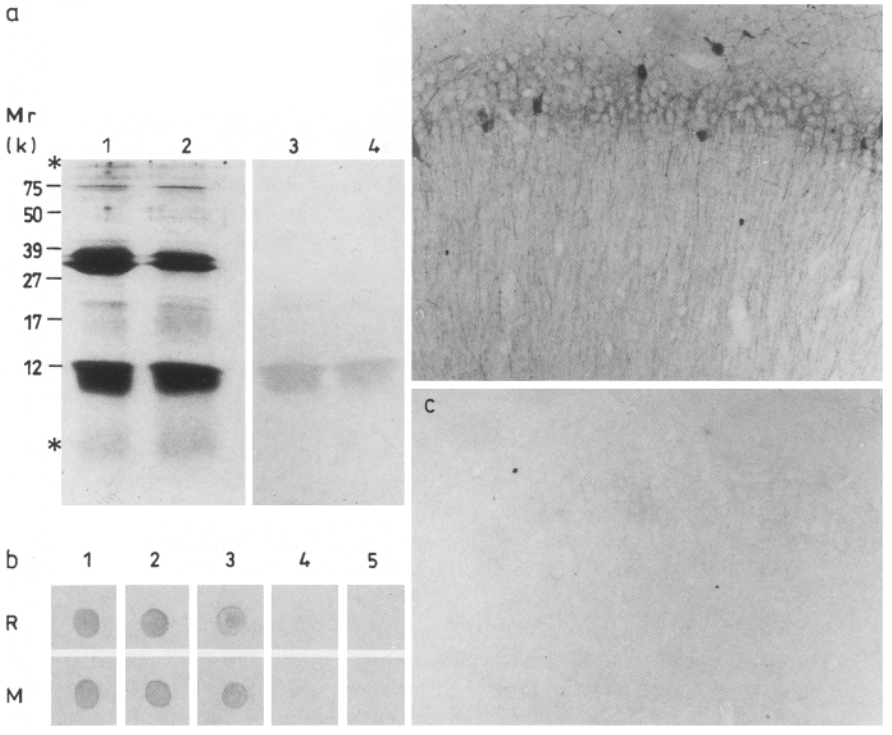


**Fig. 1a–c.** Cross-reactivity of an antiserum raised in rabbit against parvalbumin from rat muscle with parvalbumin from gerbil. (**a**) Extracts of gerbil muscle (*lane 1*, 1.4 μg protein) and rat muscle (*lane 2*, 1.3 μg protein) were separated on a one-dimensional polyacrylamide (15%) gel in the presence of SDS. On a parallel gel, the corresponding proteins (*lane 3*, extract of rat muscle; *lane 4*, extract of gerbil muscle) were transferred onto a nitrocellulose membrane and incubated with antibody specific for rat parvalbumin. Under these conditions, the three major proteins in each lane were transferred completely. *Asterisks* denote the top of the gel and the front. (**b**) Decreasing concentrations of purified parvalbumins (1, 200 ng; 2, 67 ng; 3, 22 ng; 4, 7.4 ng; 5, 2.5 ng) from rat (*R*) and gerbil (*M*) muscle were spotted on a nitrocellulose sheet. Immunostaining was carried out as described in Materials and methods. (**c**) Consecutive sections of gerbil hippocampus were incubated with antiserum against parvalbumin from rat muscle (1:1000; *upper*) or with the antiserum that had been preincubated with parvalbumin ($4 \times 10^{-5}$ M) from gerbil muscle at 4° C for 20 h, centrifuged and then diluted to the same concentration (lower). Then they were processed for immunohistochemistry in the same way

mented with 10% NGS and then treated with antiserum against rat parvalbumin (1:1000) in the same solution. Immunoreactive bands or spots were detected by incubation with peroxidase-conjugated antiserum raised in goat against rabbit IgG (1:1000, Miles-Yeda Ltd., Rehovot, Israel), followed by the peroxidase reaction with 4-chloro-1-naphthol as substrate.

As shown in Fig. 1a, the extract of gerbil muscle contained a protein that comigrated with the rat parvalbumin with Mr of 12,000, found in extract of rat muscle, on SDS-polyacrylamide gel and reacted with the antiserum against parvalbumin from rat muscle. The immunoreaction of this protein band and of the fraction of the extract of gerbil muscle which was passed through a column of Sephadex G-100 to concentrate the parvalbumin, with the antiserum specific for rat parvalbumin showed a similar dose-response relationship to that of the fractions prepared in the same way from rat muscle (data not shown). Figure 1b shows that there are no apparent differences in the capacity to react with the antiserum against rat parvalbumin, between the purified parvalbumins from the gerbil and the rat.

### *Immunohistochemistry*

Pregnant rats were anaesthetized with chloral hydrate. The embryos were quickly removed and their brains were fixed in fixative P (4% paraformaldehyde in 0.1 *M* phosphate buffer, pH 7.4) for more than 24 h at 4° C or in fixative G (1% sucrose, 10% formalin, 1% glutaraldehyde, 0.2% picric acid in 0.1 *M* sodium acetate buffer, pH 6.0; Schmechel et al. 1980) for 3 h at room temperature, after being cut into several blocks of appropriate size in each fixative. Both fixatives preserved parvalbumin-specific immunoreactivity well, but the use of the glutaraldehyde-containing fixative was required for detection of the presence of GABA-like immunoreactivity. The day on which a vaginal plug was detected was designated as being gestation day 0 (E0). Tissues were then washed with cold PBS (0.1 *M* phosphate-buffered saline) and finally transferred into 10–20% sucrose in PBS for cryoprotection. To examine postnatal development, gerbils and rats of different ages (gerbils on P0 (the day of birth), P1, P3, P7, P11, P15, P19, P38 and P90 and rats on P0, P1, P3, P4, P7, P9, P11, P16, P33 and P90) were anesthetized with chloral hydrate and perfused with cold saline and then with cold fixative P or cold fixative G. The amount of fixative used ranged from 20 to 250 ml according to the body weight. Brains were quickly removed and further fixed again in fixative P for more than 24 h at 4° C, or in fixative G for 3 h at room temperature, after being cut into several blocks. These brains were treated in the same way as brains of embryo.

Serial sections (35–45 μm thick) of the tissue preparations were cut on a freezing microtome. The sections were washed in cold PBS, blocked with 8% NGS in PBS for 1 h at room temperature and then incubated with primary antibody (parvalbumin-specific antiserum at a dilution of 1:1,000–1:8,000 in 1% NGS, 0.001% Triton X-100 and 0.02% $NaN_3$ or GABA-specific antibody at a dilution of 1:400–1:1000 in the same solution but without Triton X-100) for 2 days at 4° C. The antigen-antibody complex was visualized by either the ABC method (Vectastain kit, Vector Labs., Burlingame, Calif., USA) or by using a peroxidase-labelled second antibody, antibody raised in goat against rabbit IgG (dilution, 1:100–1:150, Cappel Laboratories, West Chester, Pa, USA) and subsequent peroxidase reaction with 0.1% 3,3′-diaminobenzidine (DAB) and 0.045% hydrogen peroxide. Some sections were treated with 4-chloro-1-naphthol instead of DAB. In some cases, sections were incubated with 0.05% 3,3′-diaminobenzidine, 0.033% hydrogen peroxide and 0.01 *M* imidazole in 0.05 *M Tris* buffer (pH 7.6) to enhance the peroxidase reaction (Straus 1982).

Specificity of the immunoreaction was tested by treatment of the antiserum against rat parvalbumin with parvalbumin from rat or gerbil ($4 \times 10^{-5}$ *M*) or antibody for GABA with rabbit serum albumin-conjugated GABA ($1 \times 10^{-5}$ *M*). After such treatments, the specific immunostaining vanished completely, demonstrating the specificity of the staining procedures. Figure 1c shows the specificity of the staining of gerbil brain treated with antiserum against rat parvalbumin.

## Results

At early stages of development, parvalbumin-specific immunoreactivity was localized in specific areas of the rat brain. At E18, several fiber systems in the hindbrain, including the oculomotor fibers and neurons of the nucleus mesencephalic trigemini, showed strong immunoreactivity (Fig. 2a). At this time, neurons of the nucleus reticular thalami showed only faint staining for parvalbumin and then a gradual increase in immunostaining became apparent (Fig. 2b).

At E20, some scattered cells in the midbrain and in the medulla oblongata showed light to moderate staining. After birth, parvalbumin-positive cells increased rapidly in number and in intensity of staining, but the timing of this increase in staining varied between areas. In the substantia nigra, some cells showed moderate staining at P0 and the number of stained cells increased, in particular after P7 (Fig. 2c). At P3, some cells of the ventral nucleus of the lateral lemniscus showed moderate immunoreactivity and some in the colliculus inferior showed faint staining. By P7, the numbers of stained cells in these areas had increased. Staining in cells of the dorsal nucleus of the lateral lemniscus and in fibers of the lateral lemniscus became detectable. Then the intensity of their staining increased (Fig. 2d). A group of neurons in the zona incerta also showed faint staining at P3 and moderate staining by P11.

The relatively late appearance of parvalbumin-specific immunostaining was observed in the following areas. The nucleus anterior pretectalis showed weak staining at P7 which became moderate in intensity by P11. In the corpus striatum, very faint staining of a few cells was detected at the most lateral region at P7. The number of these cells and the intensity of staining increased thereafter but, even in adults, there was a clear gradient in the density of stained cells with a higher density being apparent in the more lateral region of the corpus striatum (Fig. 2e). The Purkinje cells in the cerebellum showed weak staining at P7 and a strong staining by P11 (Fig. 2f).

In the cerebral cortex and hippocampus, parvalbumin-specific immunoreactivity in neuronal cell somata also developed relatively late. The details of the timing of the appearance of parvalbumin-specific immunoreactivity in these areas in the rat were compared with those for the corresponding areas in the gerbil.

### *Cerebral cortex*

In the cerebral cortex of the rat, faint immunostaining was found in the straight fibers that ran vertically to the presumptive cortical layers in the cortical plate at P3. Stained fibers could be traced from the area that corresponded to layer II down to layer V. However, this

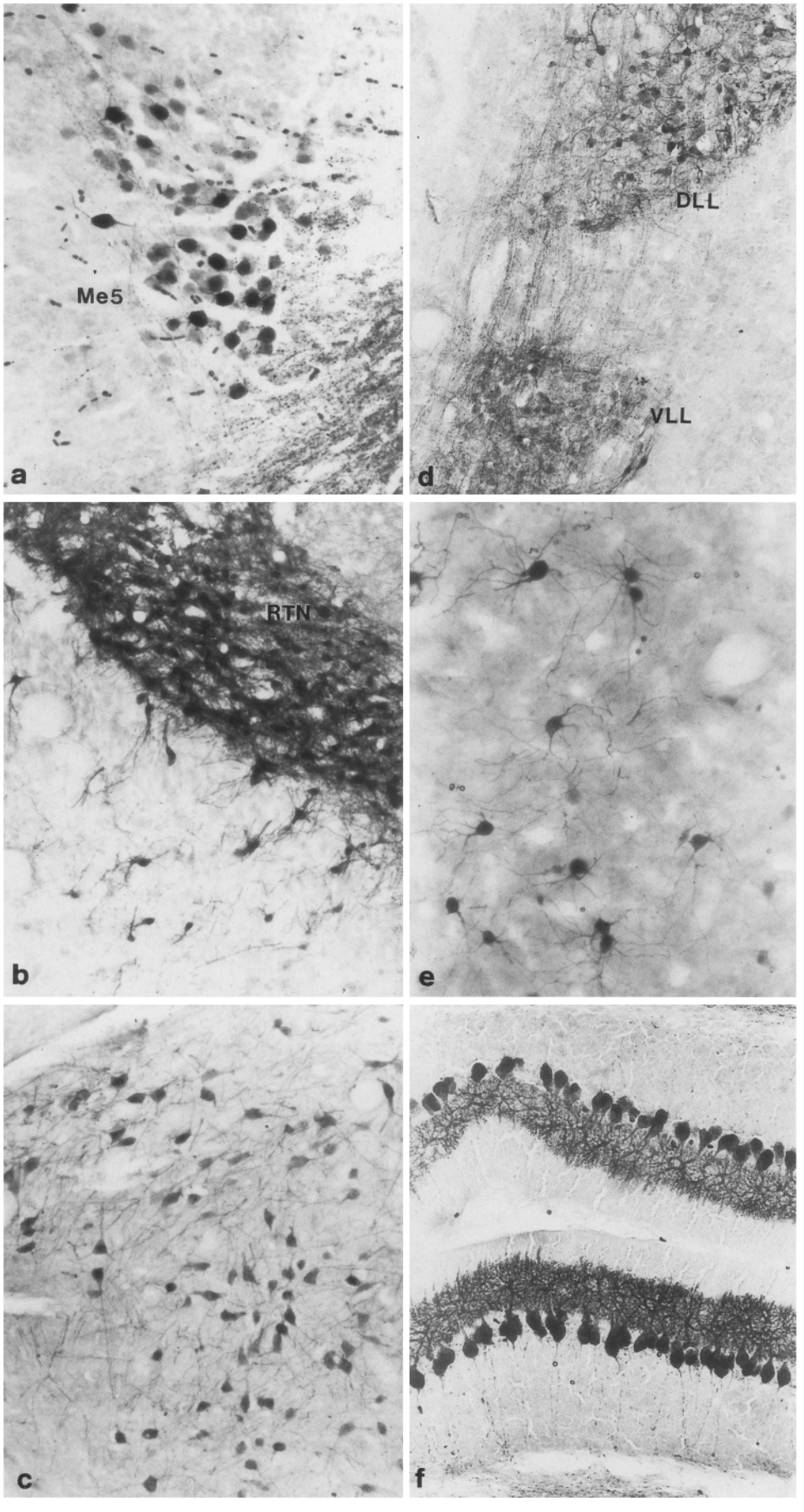


**Fig. 2a–f.** Parvalbumin-specific immunoreactivity in several areas of the developing rat brain. (**a**) The neurons of the mesencephalic nucleus of the trigeminus (*Me5*) showed intense parvalbumin-specific immunoreactivity (E20). (**b**) In the nucleus reticularis thalami (*RTN*) and its adjacent areas, many cells were stained heavily (P9). (**c**) In the substantia nigra, the neurons with processes had parvalbumin-specific immunoreactivity (P16). (**d**) Many cells in the dorsal nucleus (*DLL*) and ventral nucleus (*VLL*) of the lateral lemniscus and fibers connecting these nuclei were stained (P11). (**e**) In the corpus striatum, several neurons were parvalbumin-positive (P16). (**f**) The Purkinje cells were immunostained in their cell somata, dendrites and axons (P11). (**a**) ×222; (**b**) and (**c**) ×111; (**d**) ×183; (**e**) ×222; (**f**) ×130

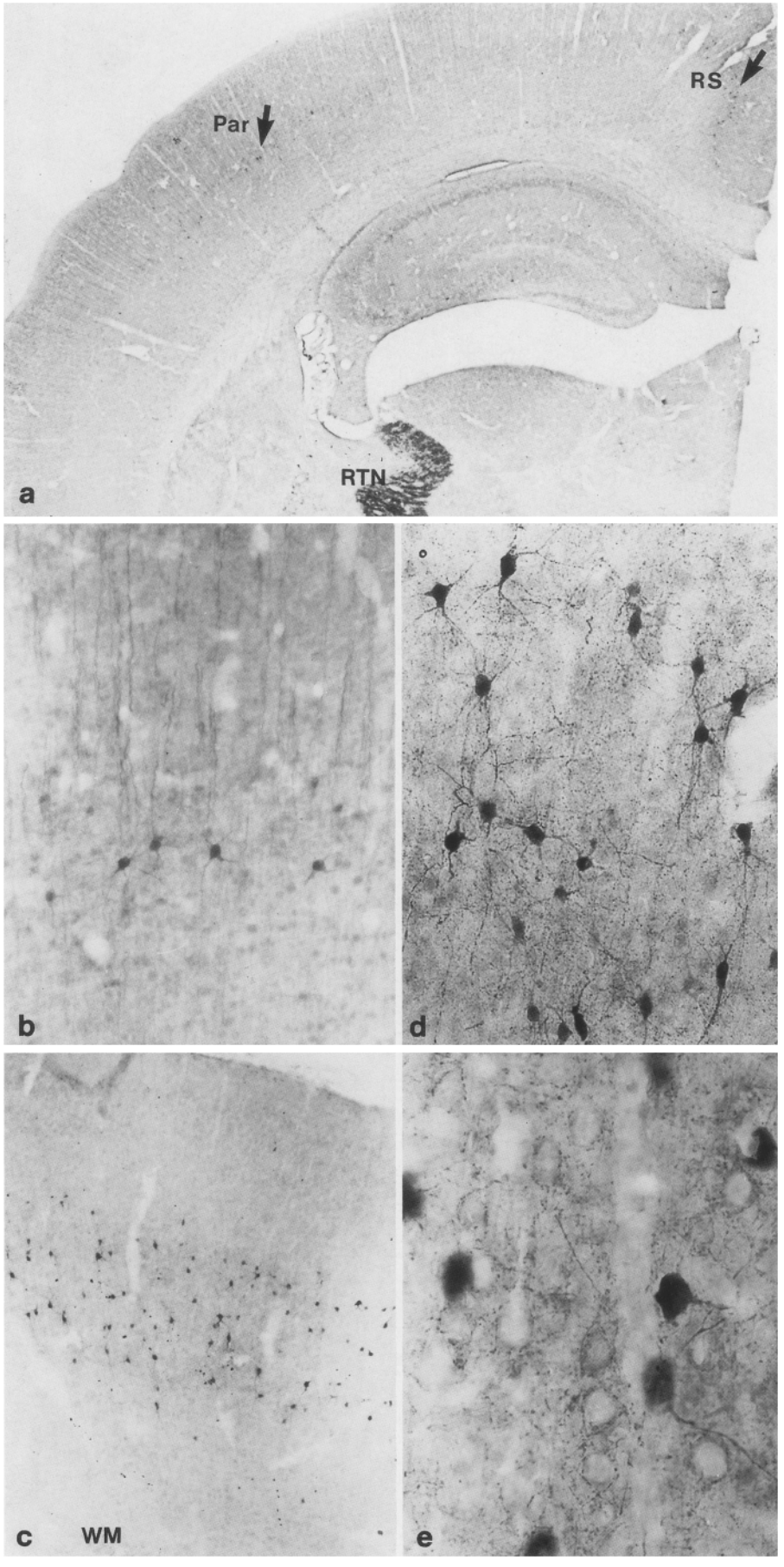


**Fig. 3a–e.** Immunohistochemical localization of parvalbumin in the cerebral cortex of the developing rat. (**a**) Frontal section of the cerebral cortex of a rat at P9 showing parvalbumin-stained cells in restricted areas, the parietal cortex (*Par*) and retrosplenial cortex (*RS*). Notice that the stained cells were located at the midcortical region (*arrows*). Intense immunostaining was found in the nucleus reticularis thalami (*RTN*). The section was counterstained with methyl green. (**b**) High-magnification view of the parietal cortex at P9. At this age, stained long fibers which ran vertically to the cortical layers were found in addition to the stained cell somata. (**c**) At P11, the number of stained cells increased but they were still distributed mostly at the midcortical region. *WM*, white matter. (**d**) At P11, many stained dots were found around stained cell somata, while the stained fibers which were observed at P9 were hardly seen. (**e**) At P33, numerous immunopositive dots, some of which surrounded immunonegative cells, were found (*arrows*). (**a**) ×22.5; (**b**) ×160; (**c**) ×41; (**d**) ×180; (**e**) ×360

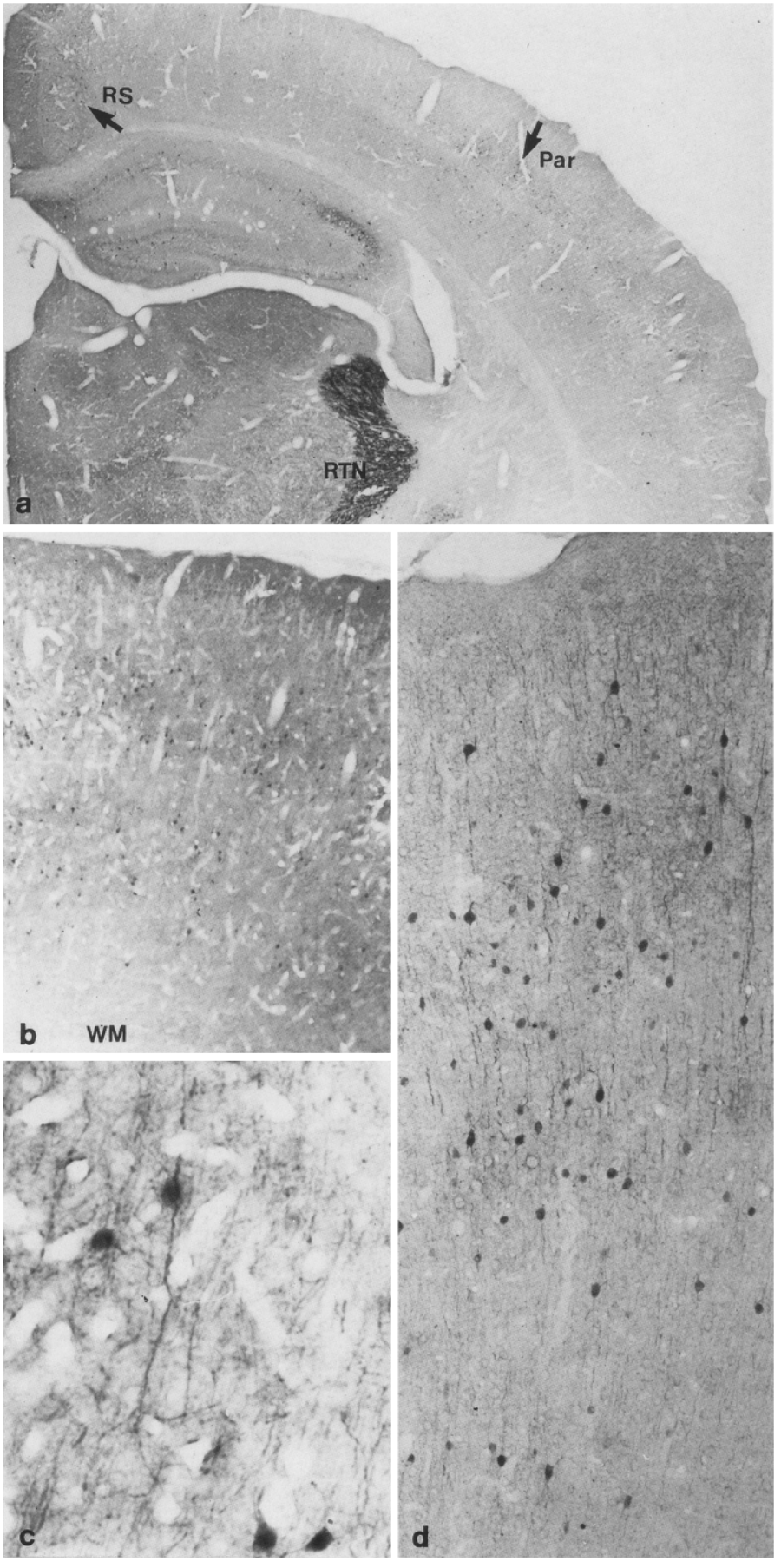


**Fig. 4a–d.** Immunohistochemical localization of parvalbumin in the cerebral cortex of the developing gerbil. (**a**) Frontal section of the cerebral cortex of a gerbil at P19 showing parvalbumin-stained cells in restricted areas, the parietal cortex (*Par*) and retrosplenial cortex (*RS*). The nucleus reticularis thalami (*RTN*) showed intense staining. The cells in the hippocampus and the neuropile in the CA3 region were also stained. (**b**) High-magnification view of the parietal cortex at P19. Notice that there is no tendency for the accumulation of stained cells in the midcortical area. *WM*, white matter. (**c**) At P38, stained dots were hardly seen. (**d**) There were many segments of stained fibers that ran vertically to the cortical layers at P38. (**a**) ×21; (**b**) ×49; (**c**) ×200; (**d**) ×128

staining diminished gradually after P7. At P11, it persisted only in areas where parvalbumin-specific immunoreactivity in cell somata had not developed. At P16, such fibers were barely detectable in the cerebral cortex. In addition to these fibers, several lightly stained cell somata were first detected at P7 in distinct areas of the parietal cortex. At P9, some stained cells were found in the retrosplenial cortex as well as in the parietal cortex (Fig. 3a, b). They were located mostly in midcortical area. At P11, the number of stained cells increased further but there were still striking differences in numbers of stained cells among the various cortical area. In sagittal sections, there seemed to be a gradient in the density of stained cells, with the largest number of stained cells being in the frontal cortex and a decrease in numbers of stained cells being apparent both posterior to and anterior to the frontal cortex. In area 1 of the occipital cortex, there were a few stained cells in the region near area 2 of the occipital cortex but, in the cingulate cortex, stained cells were not detectable at P11. An exception to this observation was provided by part of the retrosplenial cortex in which the very early appearance of parvalbumin-specific immunoreactivity was detected, as described above. The neurons which showed well-developed morphology with high immunoreactivity at P11 were still located mostly in the midcortical region (Fig. 3c) and they were surrounded by numerous "dots" (Fig. 3d). At P16, the number of parvalbumin-positive dots and stained cells with clearly stained processes increased further and they became to distribute widely throughout the cerebral cortex. Stained cells were distributed widely from layer II to near the white matter but the density of cells with well-developed morphology and stained dots was still high in the midcortical region. At P33, the adult pattern of staining was fully developed, with stained cells being found throughout all the cortical layers with the exception of layer I. Parvalbumin-positive dots were scattered throughout the cortical layers and some immunonegative cells were regularly surrounded by these parvalbumin-immunoreactive dots (Fig. 3e).

In gerbils, the timing of the appearance of stained cell somata was uniformly later than that in the rat. Thus, at P15, there were only a few stained cell somata in the parietal cortex. At P19, more cells displayed immunoreactivity but their numbers varied between cortical areas, as noted in the cerebral cortex of the developing rat. Figure 4a shows that, in the parietal cortex and retrosplenial cortex, there were stained cells at P19, but at this time no stained cells were found in the temporal cortex. In this respect, the cerebral cortex of the gerbil at P19 seems to correspond developmentally to the stage reached by the rat at P11. However, in contrast to our observations in the rat, these cells were not restricted to the midcortical region but were much more widely scattered (Fig. 4b). Clearly stained dots could hardly be detected at this age and even later in development, a clear difference from our observations in the rat. Instead, immunostained fibers were noted that ran vertically to the cortical layers and these fibers tended to remain detectable even in the adult although, in many areas, it

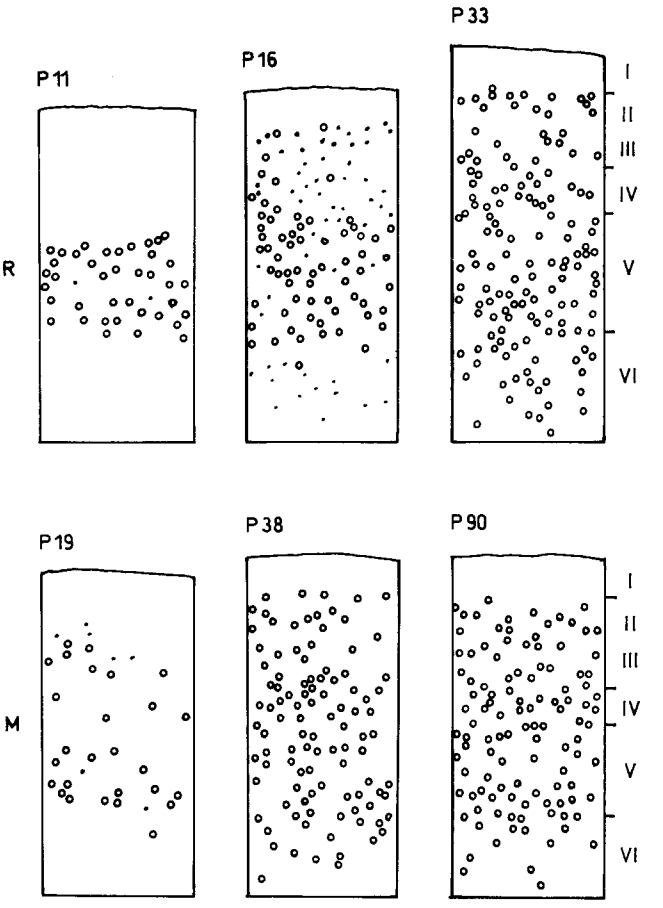


**Fig. 5.** Illustration of the developmental changes in the distribution of parvalbumin-positive cells in the developing cerebral cortex of the rat and the gerbil. The distribution of stained cells in the parietal cortex of the rat (*R*) and the gerbil (*M*) are illustrated. Weakly stained cells are indicated by dots

became progressively more difficult to follow all of their processes. At P38, stained cells were found to be distributed throughout the different cortical areas and cortical layers, with the exception of layer I. Between stained cells, stained fibers were still found (Fig. 4c, d). Developmental changes in the distribution of parvalbumin-positive cells in the frontal cortex of rat and gerbil are illustrated in Figure 5.

*Hippocampal formation*

In the hippocampus of rats, there were lightly stained fibers that crossed vertically to the layer of stratum pyramidale and lightly stained dots were seen in zones immediately adjoining the stratum radiatum in CA1 at P1. At P7, staining of such fibers became faint (Fig. 6a) and became difficult to detect subsequently.

At P7, a few cell somata were immunoreactive (Fig. 6a). They were mostly in the stratum pyramidale and in the adjoining layer in CA3, but some were also present in CA1 or CA2. At P11, the stained cells increased in number and they had clearly stained processes. Most parvalbumin-positive cells in CA1 had

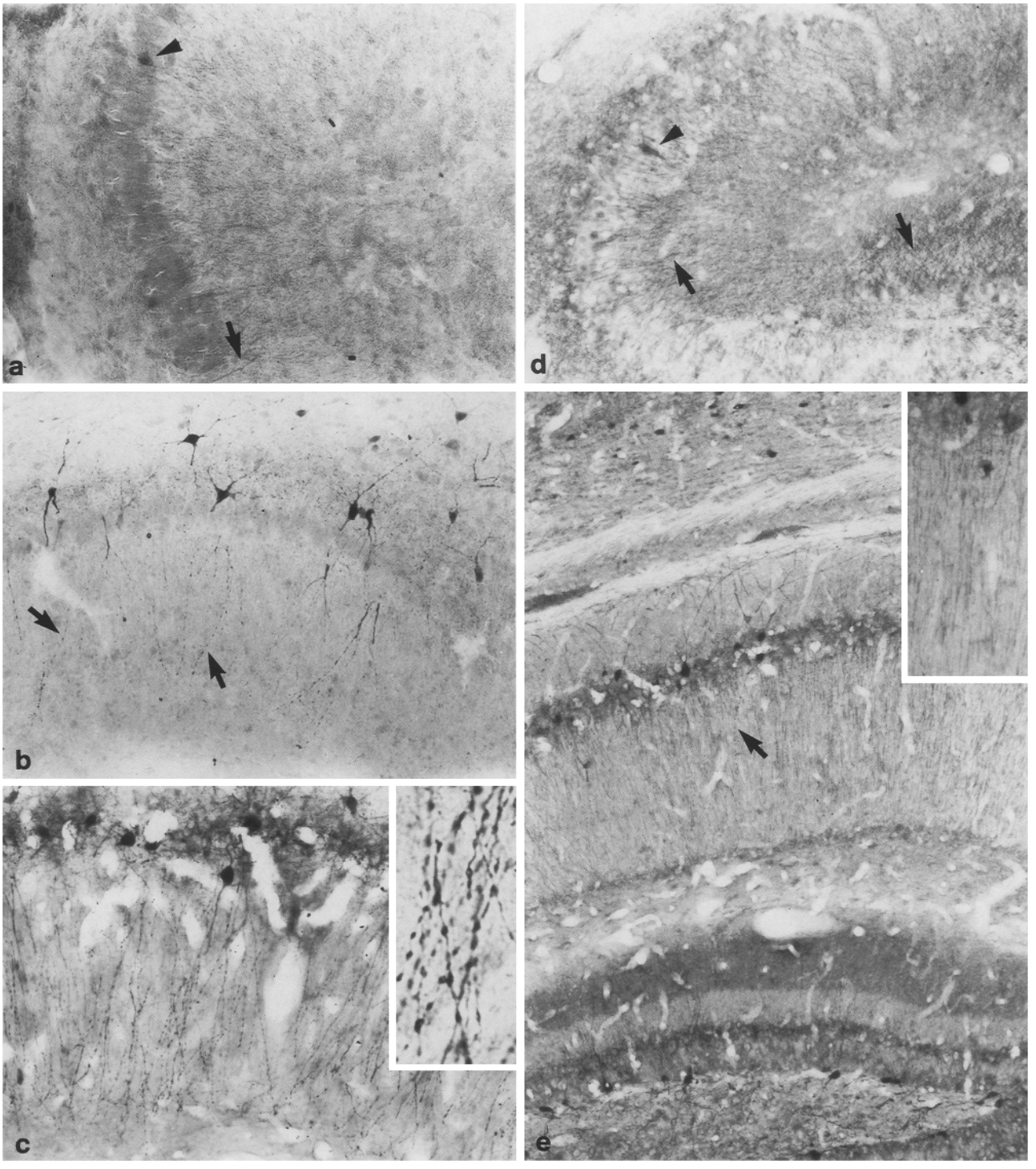


**Fig. 6a–e.** Immunohistochemical localization of parvalbumin in the hippocampus of the developing rat and gerbil. **(a)** In the rat at P7, a faintly stained cell was found in CA3 (*arrowhead*). At this age, weak staining was found in fibers (*arrow*) which later diminished. **(b)** At P11, the stained cells in CA1 already had a number of bead-like structures on their dendrites (*arrows*). **(c)** Dendrites with bead-like structures increased as the number of stained cells in CA1 increased (P90). The *inset* shows a high-magnification view of bead-like structure. **(d)** In the gerbil hippocampus, parvalbumin-stained cells were detectable at P11 (*arrowhead*). Stained fibers were found in many areas including CA3 (*arrows*). **(e)** The number of dendrites with bead-like structures in CA1 (*arrow*) was small even at P90. Instead, numerous straight fibers with immunostaining were found to run vertically to the layer of pyramidal cells as shown in the *inset*. **(a)** ×140; **(b)** ×110; **(c)** ×130; **(d)** ×140; **(e)** ×93

bead-like structures on their dendrites, which were characteristic structures observed throughout development and were also found in the adult (Fig. 6b). In addition, stained dots were found in the stratum pyramidale, in particular around well-developed, highly immunoreactive cells in CA3. At this age, several cells with staining of various intensities were found in the granular cell layer of the dentate gyrus. At P16, the profile of parvalbumin-specific immunoreactivity was almost the same as that seen in the adult. Parvalbumin-positive cells with long processes were found in the hilus. In the granular cell layer of the dentate gyrus, immunostained cells increased in number and many immunostained dots were found. A large fraction of the dendrites of parvalbumin-positive cells in CA1 had many immunoreactive bead-like structures, as can be found in this area in the adult (Fig. 6c).

In gerbils, faintly stained fibers were found in the stratum radiatum at P7. Lightly stained fibers were also found in the hilus and in the molecular layer near the granular cell layer of the dentate gyrus. At P11, a few faintly stained cells were detected (Fig. 6d). Their location and morphology were similar to those in the rat at P7. The number of stained cells and their intensity of staining increased somewhat at P15 but was highest at P19. At P15, parvalbumin-specific staining was found between unstained pyramidal cells in CA3. At P19, such staining was recognized as parvalbumin-positive fibers that surrounded immunonegative cells. At P38, this type of staining was found in the pyramidal layer of all the areas and in the granular layer of the dentate gyrus.

The distribution of stained cell somata in the hippocampus of the gerbil were similar to that seen in the rat but there were some differences in the profiles of parvalbumin-specific staining. In gerbils, bead-like structures on the dendrites of the parvalbumin-positive cells in the CA1 region were only occasionally found; instead, numerous straight parvalbumin-positive fibers were observed (Fig. 6e). The staining of these fibers was abolished by absorption of the rat parvalbumin-specific antiserum with parvalbumin from the gerbil (Fig. 1c) or the rat. In CA3, numerous parvalbumin-positive fibers were found running along the plane of the sagittal sections, another difference from the rat.

In the brain of both the rat and the gerbil, parvalbumin was colocalized with GABA in many areas, such as the cerebral cortex, hippocampus and nucleus reticularis thalami (data not shown). As development proceeded, in the cerebral cortex and hippocampus, many neurons showed evidence of strong or moderate GABA-like immunoreactivity, which was detectable by E20 in the rat and by P0 in the gerbil, the earliest stages examined. To investigate a possible relationship between parvalbumin and GABA-like immunoreactivity, sections of rat brain at P11, in which the cerebral cortex had a heterogeneous distribution of parvalbumin-positive cells, were treated first with GABA-specific antibody and then processed for immunohistochemistry with DAB as chromogen, which gave a brown color. After washing, these sections were treated with parvalbumin-specific antiserum and the location of the antigen-antibody complex was visualized using 4-chloro-1-naphthol, which gave a purple color. By this procedure, cells that contained parvalbumin but little or no GABA-like immunoreactivity were stained purple. The large round cells in the mesencephalic nucleus of the trigeminus, for example, which were typically parvalbumin-positive but were not GABAergic cells, were clearly purple but such purple cells were hardly found at all in the cerebral cortex at P11, when a high concentration of GABA-specific antibody was used (dilution of 1:300) (Fig. 7); there were a few cells that gave ambiguous results when the GABA-specific antibody was used at a dilution of 1:900.

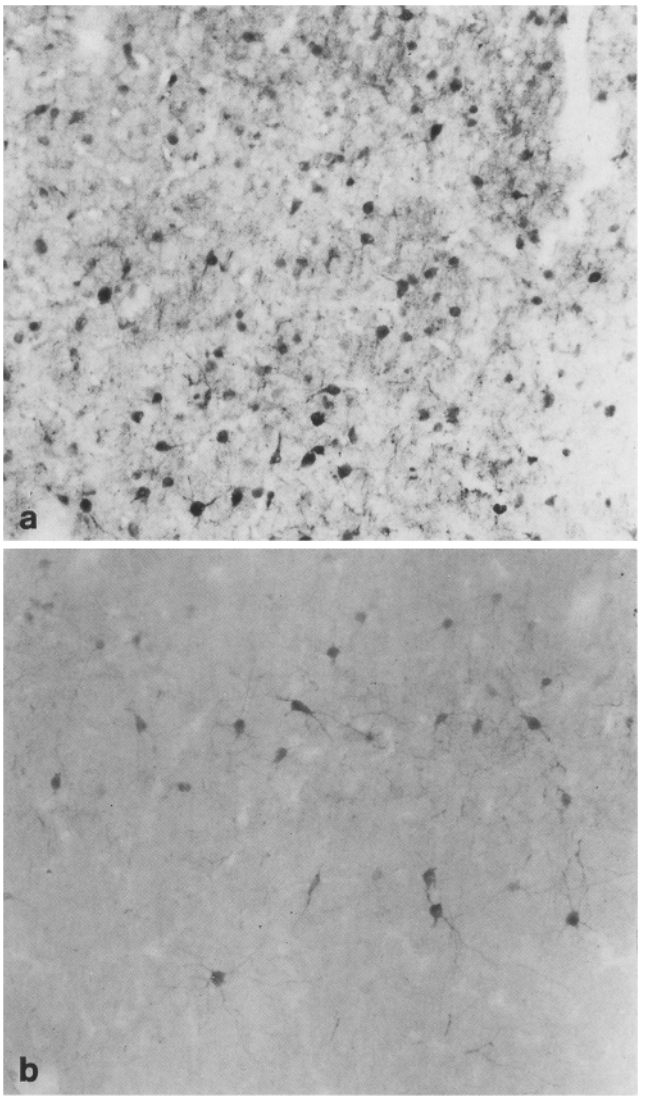


**Fig. 7a, b.** Distribution of cells with GABA-like and parvalbumin-specific immunoreactivity. (**a**) A section of cerebral cortex from a rat at P11 was first treated with GABA-specific antibody and stained with DAB. Then, parvalbumin-specific antiserum and 4-chloro-1-naphthol were used to localize parvalbumin-positive cells. There were many cells with GABA-like immunoreactivity but there was no cell with only purple staining, which would be evidence of parvalbumin but little or no GABA. (**b**) The distribution of parvalbumin-positive cells in the corresponding area is shown. (**a**), (**b**) ×111

## Discussion

In this study, developmental changes of parvalbumin in two rodents, the rat and gerbil, were followed immunohistochemically to see whether the appearance of parvalbumin in the brain is correlated with functional maturation. These two animal species have distinct patterns of neurological maturation.

In the adult rat brain, parvalbumin was localized in distinct sub-populations of neurons, as reviewed previously (Heizmann and Braun 1989). Details of its distribution were described recently (Celio 1990). Our present study revealed a similar distribution of parvalbumin in the gerbil brain. Each area of the brain of each of these two species has its own temporal profile of expression of parvalbumin during ontogeny. It is noteworthy that the different areas of the cerebral cortex have different but parallel patterns of appearance of parvalbumin, whether in the rat or in the gerbil. The order of appearance, namely, the appearance of parvalbumin first in the parietal and retrosplenial cortex, and only later in the occipital and temporal cortex, was common to both the gerbil and the rat. The factors that support such sequential expression of parvalbumin are unknown but should clearly also show regional differences during development.

Parvalbumin is known to be localized in not all but in a considerable fraction of the GABAergic interneurons in the cerebral cortex (Kosaka et al. 1987; Demeulemeester et al. 1989; Hendry et al. 1989). Our observation that the distribution of cells with GABA-like immunoreactivity shows no obvious regional differences in the cerebral cortex at P0, as well as later during development, seems to suggest that expression of parvalbumin in at least most GABAergic basket cells in the cerebral cortex is regulated independently of expression of immunohistochemically detectable GABA.

In comparing the gerbil with the rat, we found that parvalbumin-specific immunoreactivity in the various regions of the gerbil brain tended to appear later than in corresponding areas of the rat brain, especially in the cerebral cortex. Since the antibody used in this study was raised against parvalbumin from rat muscle (Kägi et al. 1987), it was possible that the late appearance of parvalbumin-specific immunoreactivity in the gerbil could have been due to a lower reactivity because of some differences in antigenicity. However, the results presented in Figure 1 suggest that this possibility is unlikely. Application of the more sensitive imidazole method or use of high concentrations of antibody for immunohistochemistry also gave similar results. Furthermore, parvalbumin-specific immunoreactivity in the Purkinje cells of the cerebellum of rat and gerbil appeared simultaneously at P7. Therefore, the observed differences in the timing of the appearance of parvalbumin-specific immunoreactivity in the rat and gerbil brain are not an artifact but seem to reflect real and intrinsic difference between the two species. Again, the factors that control such difference are unknown.

Braun et al. (1985a) noted that some visual nuclei in the zebra finch developed parvalbumin-specific immunoreactivity only with the opening of the eyes, suggesting that expression of parvalbumin is related to the onset of certain neuronal activity that requires parvalbumin. The function of parvalbumin in the brain is unclear as yet. However, since parvalbumin can bind calcium and magnesium ions with high affinity and is found in fast-firing neurons (Kawaguchi et al. 1987), it is thought to act as a calcium and/or magnesium mediator, for example as a genuine calcium buffer that acts to reduce temporarily the intracellular concentration of free calcium and/or as a supplier of magnesium ions which are important for the electronic and metabolic processes of the neurons. Thus, the suggestion of Braun et al. that parvalbumin is expressed when the neurons begin to fire actively seems quite plausible. In the rat, onset of hearing (for rat, P11; for gerbil, P14–15) and eye opening (for rat, about P14; for gerbil, P18–20) occur earlier than in the gerbil. The distinct differences in ontogenetic appearance of parvalbumin in the cerebral cortex between gerbils and rats may reflect alterations in the requirement for $Ca^{2+}$ or $Mg^{2+}$ of individual neurons, as a result of the development of such cortical functions and neurotransmission. More information, however, needs to be accumulated to clarify the possible relationship between neuronal development and appearance of parvalbumin.

We observed some differences between parvalbumin-positive structures in rat brain and those in gerbil brain. In the hippocampus parvalbumin-positive, bead-like structures on dendrites in the CA1 region appeared as early as P11 in the rat but they were far less abundant in the gerbil. Katsumaru et al. (1988) found that many boutons made synapses on these bead-like structures in the rat. Parvalbumin-positive cells in the hippocampus are almost always GABAergic (Kosaka et al. 1987) and the smaller number of bead-like structures in the gerbil might suggest less effective inhibitory functions in this animal, which is used as a model for epilepsy (Loskota and Lomax 1975). This point, as well as characterization of parvalbumin-positive straight fibers in the hippocampus and those in the cerebral cortex of the gerbil is the focus of further experiments in our laboratory.

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