

Kainate Receptor Gene Expression in the Developing Rat Brain

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Kainate-preferring receptors are a subclass of ionotropic glutamate receptors that might play a role in brain development. The expression of the five known genes encoding kainate receptor subunits (GluR-5, -6, -7, KA-1, and KA-2) was studied by *in situ* hybridization during pre- and postnatal development of the rat brain. We compared the combined expression patterns of these genes with autoradiography using ³H-kainate in the developing brain from embryonic day 12 (E12) through to adult. Although mRNAs for the receptor subunits (except KA-1) can be detected at stage E12, ³H-kainic acid binding (as an index of receptor protein) is not found at this stage. However, by E14 high-affinity kainate sites are found throughout the gray matter, but particularly in spinal cord, primordial cerebellum, and ventral forebrain structures. All genes undergo a peak in their expression in the late embryonic/early postnatal period. GluR-5 expression during development shows the most interesting features because the changes are qualitative. The GluR-5 gene shows peaks of expression around the period of birth in the sensory cortex (layers II, III, and IV), in CA1 hippocampal interneurons in the stratum oriens, in the septum, and in the thalamus. GluR-6 shows a prenatal expression peak in the cingulate gyrus of the neocortex. KA-1 transcripts appear with the development of the hippocampus and remain largely confined to discrete areas such as the CA3 region, the dentate gyrus, and subiculum. KA-2 transcripts are found throughout the CNS from as early as E12 and remain constant until adulthood. The GluR-5 and GluR-6 genes are coexpressed in multiple peripheral ganglia (e.g., cranial nerve ganglia, dorsal root ganglia, and mural ganglia) at E14.

[Key words: glutamate receptor, kainate, development, *in situ* hybridization, sensory cortex, thalamus]

Excitatory amino acid (EAA) receptor activation is critical for fine-scale sculpting of neuronal circuitry during brain development (Mattson et al., 1988a,b; McDonald and Johnston, 1990). For example, ocular dominance plasticity (Kleinschmidt et al., 1987; Tsumoto et al., 1987; Carmignoto and Vicini, 1992),

development of barrel fields in the sensory cortex (Schlaggar et al., 1993), stratification of dendrites in the developing retina (Bodnarenko and Chalupa, 1993), and the induction of some forms of neuronal differentiation (Kalb and Hockfield, 1990) all require EAA receptor activity. EAAs have trophic effects on neurons (Pearce et al., 1987; Balázs et al., 1988; Hack et al., 1993; Pizzi et al., 1994; reviewed by Gould and McEwen, 1993), are required for neuronal migration (Komuro and Rakic, 1993), and may regulate neuronal numbers during development by contributing to cell death via excitotoxicity (Cox et al., 1990; Mentis et al., 1993; Mount et al., 1993; O'Donoghue et al., 1993).

These developmental effects are mediated directly and indirectly by the same classes of receptors that are used for excitatory communication and the generation of plasticity in the adult brain, namely, AMPA/low-affinity kainate (AMPA-preferring), high-affinity kainate (kainate-preferring), NMDA-gated channels and metabotropic receptors (reviewed by Young and Fagg, 1990; Monaghan and Anderson, 1991; Gasic and Hollmann, 1992; Nakanishi, 1992; Monaghan, 1993; Wisden and Seeburg 1993a,b). Kainate-preferring receptors can be constructed from subunits GluR-5, GluR-6, GluR-7, KA-1, and KA-2/γ2 (Bettler et al., 1990, 1992; Egebjerg et al., 1991; Werner et al., 1991; Herb et al., 1992; Lomeli et al., 1992; Sakimura et al., 1992; Sommer et al., 1992; Partin et al., 1993; Wenthold et al., 1993; reviewed Wisden and Seeburg, 1993a,b). They can be generated *in vitro* from recombinant homomeric (GluR-5 and GluR-6) or heteromeric configurations (e.g., KA-2/GluR-6), and form channels with rapidly desensitizing responses to kainate (Herb et al., 1992; Sakimura et al., 1992; Sommer et al., 1992), and can further be distinguished from AMPA receptors because their desensitization rate can be modulated by ConA but not cyclothiazide (Partin et al., 1993). These kainate-gated channels are probably physiologically modulatable at the synapse by cAMP dependent phosphorylation (Raymond et al., 1993; Wang et al., 1993). The native agonist is presumed to be glutamate.

Since the expression of high-affinity kainate binding sites is markedly transient in some brain regions (Miller et al., 1990; Garcia-Ladona and Gombos, 1993), it is possible that kainate receptors are involved in some aspects of EAA dependent neuronal development outlined above. A marked peak in expression of high-affinity ³H-kainate binding sites occurs in the hippocampus and neocortex in the first 2 weeks after birth coinciding with periods of synaptogenesis (Miller et al., 1990; Garcia-Ladona and Gombos, 1993), and the deep cerebellar nuclei, inferior olive nucleus, pontine nuclei, inferior colliculi, and stratum oriens of CA1 have high numbers of sites around birth, with very little binding detectable in the adult (Garcia-Ladona and Gombos, 1993). A peak of gene expression of a kainate-

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selective ionotropic subunit similarly takes place in *Drosophila* during a major period of CNS neurogenesis (Ultsch et al., 1992). The occurrence of such phenomena across large distances of evolution might suggest a fundamental importance of kainate receptors in development.

Here, we examine the distribution of high-affinity kainate receptor subunit transcripts in the developing rat brain. In certain areas of the brain the composition of the five receptor subunits during embryonic and early postnatal development differ substantially from those in the adult. Although Bettler et al. (1990) first drew attention to the regulated expression of GluR-5 in the developing mouse brain, there are no systematic comparisons available with other related subunits. Further, we observed several striking features of GluR-5 expression in the developing rat brain not previously reported for the mouse.

Materials and Methods

Animals. Brains were taken from Sprague–Dawley rats using animals from embryonic ages E12, E14, E17, E19; postnatal ages P0, P5, P12; and adult males (150–180 d). Sex determination was only performed on adult rats. The occurrence of a vaginal plug was defined as E0, and the day of birth as P0. Uteri of pregnant females were rinsed and the embryos dissected out in ice-cold HEPES buffer (Laurie and Schrotz, 1994). The embryos/brains were frozen on dry ice and 14 μ m sections were cut on a cryostat and thaw mounted onto poly-L-lysine-coated slides. After briefly fixing in 4% (w/v) paraformaldehyde, sections were stored in 95% ethanol at 4°C (Wisden and Morris, 1994).

In situ hybridization. For each high-affinity kainate receptor subunit gene (GluR-5, GluR-6, GluR-7, KA-1, KA-2), unique 45-base antisense oligonucleotides were used. The oligonucleotide sequences and experimental procedures (labeling, hybridization, posthybridization washing) were exactly as described previously (Wisden and Seeburg, 1993b; Wisden and Morris, 1994). Probes were 3' end-labeled using terminal deoxynucleotidyl transferase (Boehringer-Mannheim) and a 30:1 molar ratio of α^{35} S-dATP (1200 Ci/mmol; New England Nuclear). *In situ* hybridization was performed on sagittal sections of whole embryos of E12 and E14 and on horizontal sections of brains taken of embryonic stages E17 and E19, as well as postnatal ages P0, P7, and P14 and from adults.

In order to confirm developmental changes, two sections from each of three animals at each age were hybridized and examined for each probe. After hybridization and washing, sections were exposed to Kodak XAR-5 film and dipped in photographic emulsion (Ilford K5). Anatomy of autoradiographs and thionin-stained sections was determined using the atlases of Paxinos and Watson (1986), Paxinos et al. (1991), and Kaufman (1992). Microscopic examination of emulsion-coated sections was performed for every described structure in order to confirm the cellular locations reported.

Signal specificity was assessed by competition experiments in which radiolabeled probes were hybridized to sections in the presence of excess (100-fold) unlabeled probe. This resulted virtually in blank autoradiographs, except for some nonspecific labeling of peripheral tissues. The assessment of the specificity of the probes has been described previously (Wisden and Seeburg, 1993b). Table 1 is designed to be a summary of the text and figures and does not reflect an accurate quantitative determination of mRNA copies/cell. The assignments (+) to +++ were made by two of the authors individually rating autoradiographic signal intensity using the original x-ray films and emulsion data for each of the areas mentioned in the table. These results were then pooled to produce a consensus. "Weakly detectable (+)" is distinguished from "undetectable" on the basis of comparing the autoradiographic signal with labeled probe alone to that of the same probe incubated with an excess of cold oligonucleotide for a given exposure time.

Kainate receptor autoradiography. Autoradiography with 3 H-kainic acid was performed as described previously (Monaghan and Cotman, 1982; Monaghan, 1993). Tissue slices were thaw mounted onto gelatin-coated slides and dried at room temperature. They were then desiccated at -20°C until use. After warming to room temperature, the slides were preincubated for 1 hr in freshly made 50 mM Tris-citrate buffer (pH 7.1) at 0°C and then incubated with 15 nM 3 H-kainic acid (50 Ci/mmol; New England Nuclear–Du Pont) in the same buffer at 0°C for 30 min. All slides were subsequently rinsed in ice-cold buffer and finally in ice-

cold deionized water. Sections were rapidly dried under a stream of cold air and exposed to tritium-sensitive film (Hyperfilm, Amersham) for 3 weeks. In order to define nonspecific binding, adjacent sections were incubated with 50 μM unlabeled kainic acid (Tocris Neuramin), in addition to the labeled ligand.

Results

During the development of the rat brain, a specific expression pattern was found for each of the five high-affinity kainate receptor subunit genes. These patterns changed during development. The total results are summarized in Table 1.

Kainic acid binding in development

To enable a useful side-by-side comparison and orientation with our *in situ* hybridization studies, we replicated the 3 H-kainate binding studies of Miller et al. (1990) on parallel sections, except that these authors did not report embryonic binding sites. Although the mRNA for most of the receptors (except KA-1) can be detected at stage E12 in for example dorsal root ganglia (GluR-6), spinal cord (GluR-5, -6, KA-2) (Fig. 3), 3 H-kainic acid binding (as an index of receptor protein) is not detectable until E14 (Figs. 1, 2). However, by E14 high-affinity kainate sites are found throughout the gray matter, but particularly in the spinal cord, primordial cerebellum, and in forebrain structures such as the striatum (Fig. 2). No specific binding is detectable in non-neuronal tissue of whole embryo sections. At E17, kainate sites are prevalent throughout the cortex, septum, hippocampus, and thalamus (Fig. 2).

The thalamus showed a very strong transient expression of kainic acid binding sites. At 2 weeks after birth and in the adult (Monaghan and Cotman, 1982; Unnerstall and Wamsley, 1983), only the reticular thalamic nucleus markedly expresses kainate sites (Fig. 1), yet at E19, P0, and P5, most thalamic nuclei are strongly labeled (Fig. 1). For example, at P5 both the medial geniculate and lateral geniculate nuclei are rich sources of sites compared to the same structures in the adult (Fig. 1). Miller et al. (1990) report that the laterodorsal thalamic nuclei also exhibit exuberant binding sites a day after birth with a strong decline in the number of sites found in the adult.

A clear developmental change is also seen in the hippocampus (Figs. 1, 2). At E17 diffuse kainate binding is present over the developing hippocampus, with the whole structure strongly labeled at E19 (Fig. 2). The characteristic pattern of kainate binding in the adult hippocampus is strong binding in the CA3 sector with relatively few sites in CA1 (Foster et al., 1981; Monaghan and Cotman, 1982; Monaghan et al., 1986; Unnerstall and Wamsley, 1983; Represa et al., 1987). However, in the late embryonic and early postnatal period, there is marked binding over the CA1 (stratum oriens) region (Fig. 1) that diminishes by P14. A similar observation was noted for the mouse (Garcia-Ladona and Gombos, 1993).

Kainate receptor gene expression in developing cortex

At E14, the cortex consists of a primordial plexiform layer that is split before E12 by the cortical plate of postmitotic neuroblasts (reviewed by Jacobson, 1991). The E14 telencephalon already has moderate amounts of KA-2 mRNA, but no expression for the other subunit mRNAs is seen (Fig. 4). By E17 and E19, GluR-5, GluR-6, and GluR-7 are the most abundantly expressed genes in the outer cortical layer (Figs. 5, 6). The KA-2 transcripts are found in both outer and inner (subplate) layers. Only KA-2 is expressed in the cortical plate at E12 and E14 (Fig. 4).

Table 1. Summary of developmental changes of kainate receptor gene expression during rat brain development (see Materials and Methods): Expression of subunit mRNAs for high-affinity kainate receptors during rat ontogeny

| Brain region | E14 | | | | | | E17 | | | | | | E19 | | | | | |
|-------------------|--------|--------|--------|------|------|-----|--------|--------|--------|------|------|-----|--------|--------|--------|------|------|-----|
| | GluR-5 | GluR-6 | GluR-7 | KA-1 | KA-2 | B | GluR-5 | GluR-6 | GluR-7 | KA-1 | KA-2 | B | GluR-5 | GluR-6 | GluR-7 | KA-1 | KA-2 | B |
| Cortex | 0 | 0 | 0 | 0 | ++ | (+) | ++ | ++ | +++ | (+) | +++ | ++ | +++ | ++ | +++ | 0 | +++ | +++ |
| Layer II | | | | | | | | | | | | | | | | | | |
| Layer VI | | | | | | | | | | | | | | | | | | |
| Cingulate cortex | | | | | | | | | | | | | | | | | | |
| Entorhinal cortex | | | | | | | | | | | | | (+) | ++ | +++ | (+) | ++ | +++ |
| Hippocampus | + | + | 0 | + | ++ | (+) | (+) | ++ | 0 | + | +++ | ++ | | | | | | |
| Dentate gyrus | | | | | | | | | | | | | (+) | ++ | (+) | (+) | ++ | ++ |
| CA1 pyramidal | | | | | | | | | | | | | (+) | ++ | (+) | ++ | ++ | ++ |
| CA3 pyramidal | | | | | | | | | | | | | | | | | | |
| Caudate-putamen | 0 | + | ++ | (+) | + | ++ | (+) | +++ | ++ | 0 | +++ | ++ | (+) | +++ | ++ | 0 | ++ | +++ |
| Septum | ++ | 0 | 0 | ND | ++ | 0 | +++ | (+) | ++ | 0 | ++ | ++ | +++ | + | ++ | 0 | ++ | +++ |
| Thalamus | 0 | 0 | 0 | 0 | + | + | ++ | +++ | +++ | 0 | ++ | +++ | +++ | + | +++ | 0 | ++ | +++ |
| Cerebellum | 0 | 0 | (+) | 0 | ++ | ++ | + | ++ | (+) | 0 | ++ | ++ | ++ | + | (+) | 0 | ++ | ++ |
| Stellate/basket | | | | | | | | | | | | | | | | | | |
| Purkinje cells | | | | | | | | | | | | | | | | | | |
| Granule cells | | | | | | | | | | | | | | | | | | |
| Spinal cord | +++ | + | + | (+) | ++ | +++ | | | | | | | | | | | | |

| Brain region | P0 | | | | | | P5 | | | | | | P12 | | | | | | Adult | | | | | |
|-------------------|--------|--------|--------|------|------|-----|--------|--------|--------|------|------|-----|--------|--------|--------|------|------|-----|--------|--------|--------|------|------|-----|
| | GluR-5 | GluR-6 | GluR-7 | KA-1 | KA-2 | B | GluR-5 | GluR-6 | GluR-7 | KA-1 | KA-2 | B | GluR-5 | GluR-6 | GluR-7 | KA-1 | KA-2 | B | GluR-5 | GluR-6 | GluR-7 | KA-1 | KA-2 | B |
| Cortex | | | | | | | | | | | | | | | | | | | | | | | | |
| Layer II | +++ | + | (+) | (+) | +++ | +++ | +++ | (+) | + | + | +++ | ++ | ++ | (+) | + | (+) | +++ | ++ | + | (+) | + | (+) | +++ | ++ |
| Layer VI | + | (+) | +++ | (+) | ++ | +++ | + | (+) | +++ | (+) | +++ | ++ | + | (+) | +++ | (+) | +++ | +++ | (+) | (+) | ++ | (+) | +++ | ++ |
| Cingulate cortex | ++ | +++ | ++ | (+) | ++ | +++ | ++ | ++ | +++ | + | +++ | + | ++ | ++ | +++ | 0 | +++ | +++ | + | ++ | ++ | 0 | +++ | ++ |
| Entorhinal cortex | ++ | ++ | +++ | (+) | +++ | +++ | + | + | +++ | (+) | +++ | ++ | + | +++ | 0 | +++ | ++ | + | + | ++ | 0 | +++ | ++ | |
| Hippocampus | | | | | | | | | | | | | | | | | | | | | | | | |
| Dentate gyrus | (+) | ++ | + | + | +++ | +++ | + | + | +++ | + | +++ | +++ | + | ++ | ++ | + | +++ | + | + | +++ | + | + | +++ | ++ |
| CA1 pyramidal | ++ | ++ | + | (+) | +++ | +++ | ++ | + | + | (+) | +++ | (+) | ++ | + | + | (+) | +++ | (+) | + | + | (+) | (+) | +++ | (+) |
| CA3 pyramidal | + | ++ | (+) | +++ | +++ | +++ | + | + | 0 | +++ | +++ | +++ | + | ++ | 0 | +++ | +++ | +++ | (+) | +++ | 0 | +++ | +++ | |
| Caudate-putamen | (+) | +++ | + | 0 | +++ | +++ | 0 | + | ++ | 0 | +++ | +++ | 0 | + | + | 0 | +++ | +++ | 0 | ++ | + | 0 | +++ | +++ |
| Septum | +++ | 0 | + | 0 | ++ | ++ | ++ | 0 | + | 0 | +++ | +++ | ++ | 0 | + | 0 | ++ | ++ | + | 0 | + | 0 | ++ | + |
| Thalamus | +++ | + | ++ | 0 | ++ | +++ | +++ | 0 | + | 0 | ++ | +++ | +++ | (+) | + | 0 | ++ | + | + | 0 | + | 0 | ++ | (+) |
| Cerebellum | | | | | | +++ | | | | | | | | | | | | | | | | | | 0 |
| Stellate/basket | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | ++ | 0 | 0 | + | 0 | 0 | ++ | 0 | 0 | + | 0 | 0 | ++ | 0 | ++ | |
| Purkinje cells | ++ | 0 | 0 | 0 | 0 | 0 | ++ | 0 | 0 | (+) | 0 | 0 | +++ | 0 | 0 | (+) | 0 | +++ | 0 | 0 | + | 0 | 0 | |
| Granule cells | (+) | +++ | 0 | 0 | ++ | (+) | +++ | 0 | 0 | +++ | +++ | (+) | +++ | 0 | 0 | +++ | +++ | 0 | +++ | 0 | +++ | 0 | +++ | |

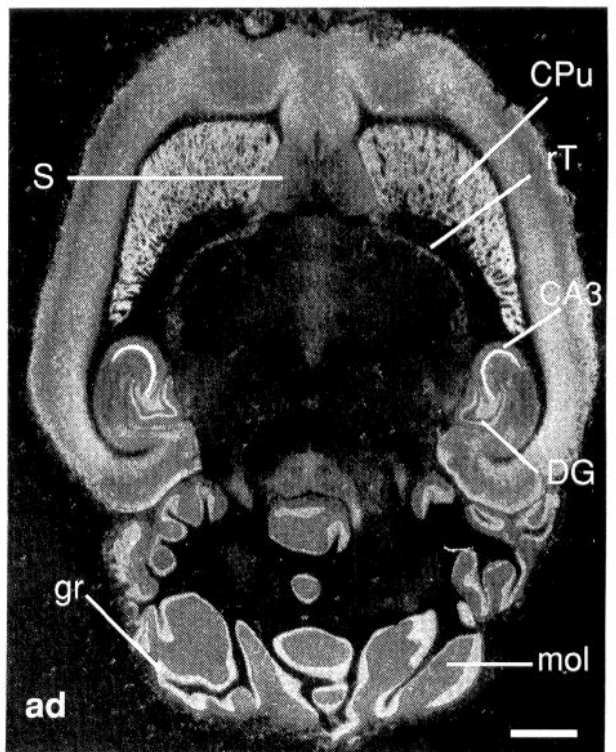
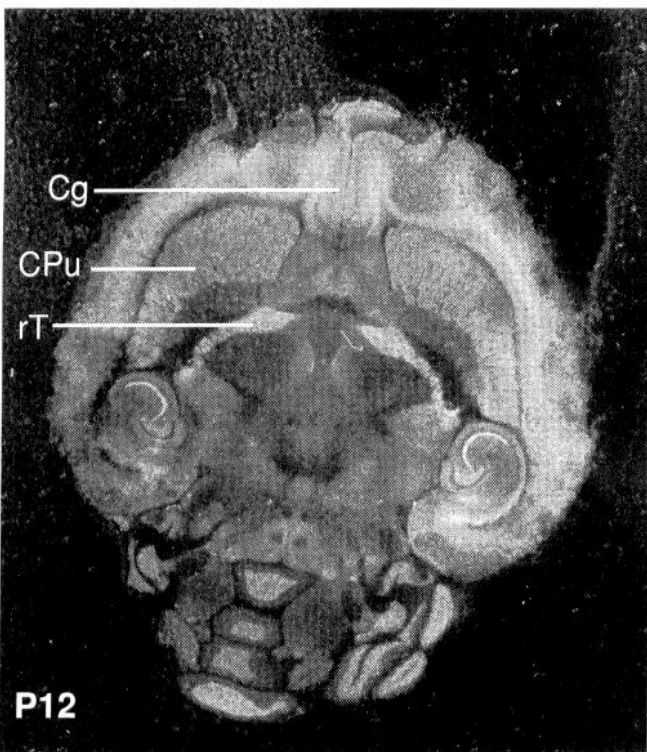
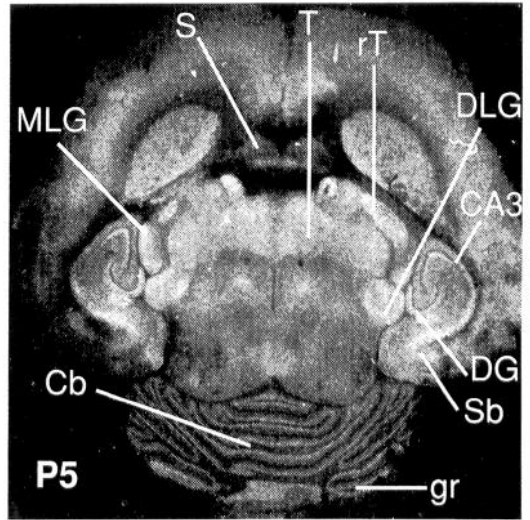
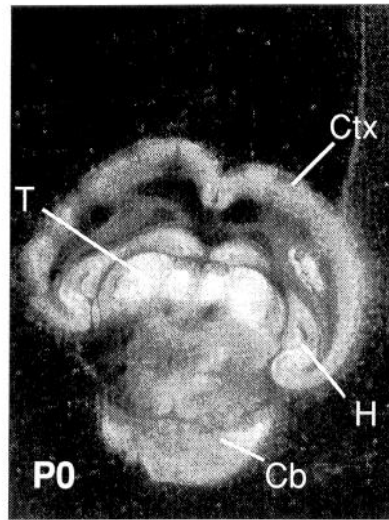
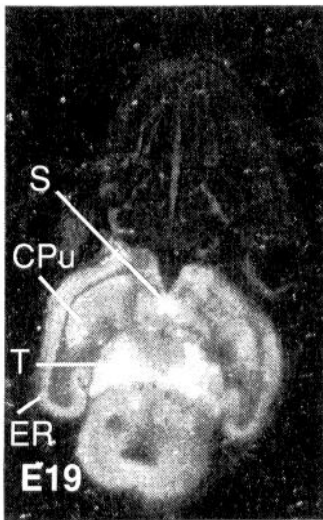
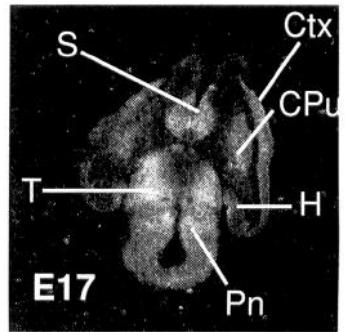
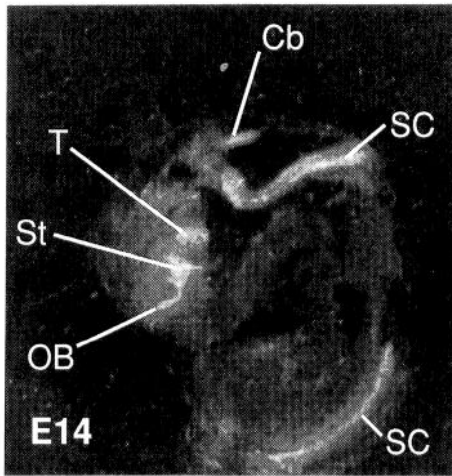
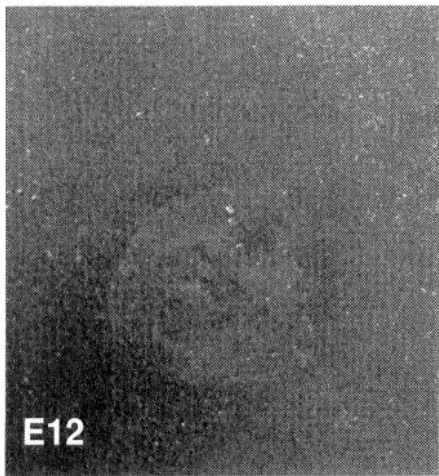
0, not detectable; (+) weakly detectable; +, detectable; ++, moderate; +++, abundant. B, ³H-kainic acid autoradiography.

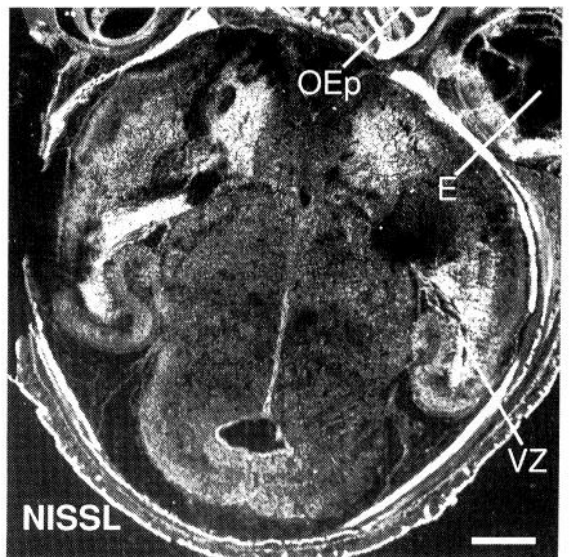
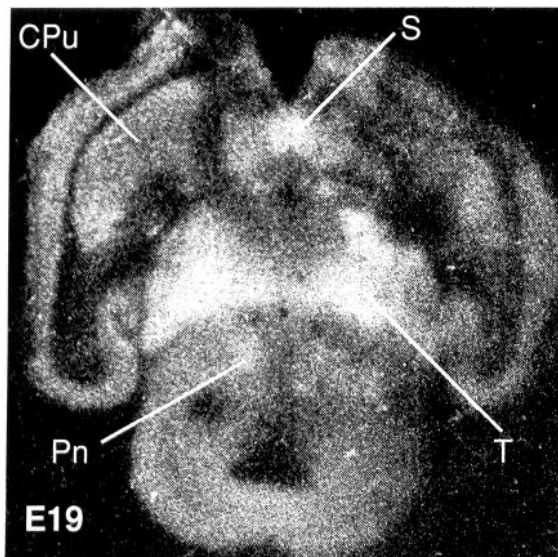
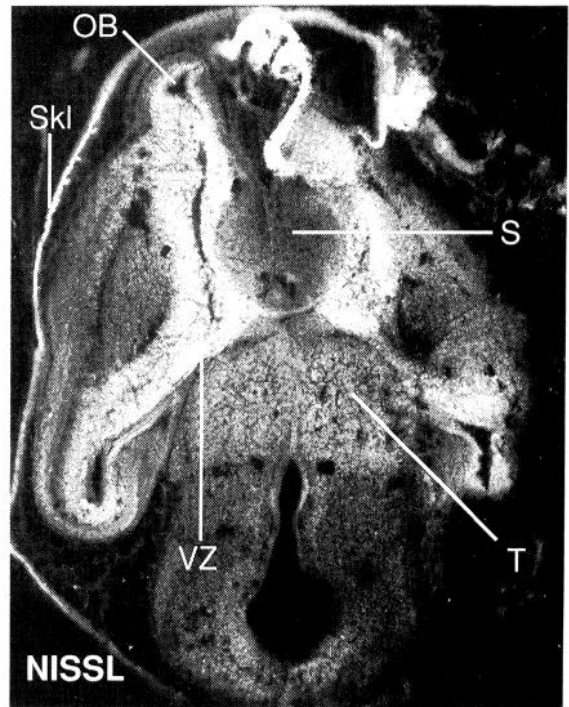
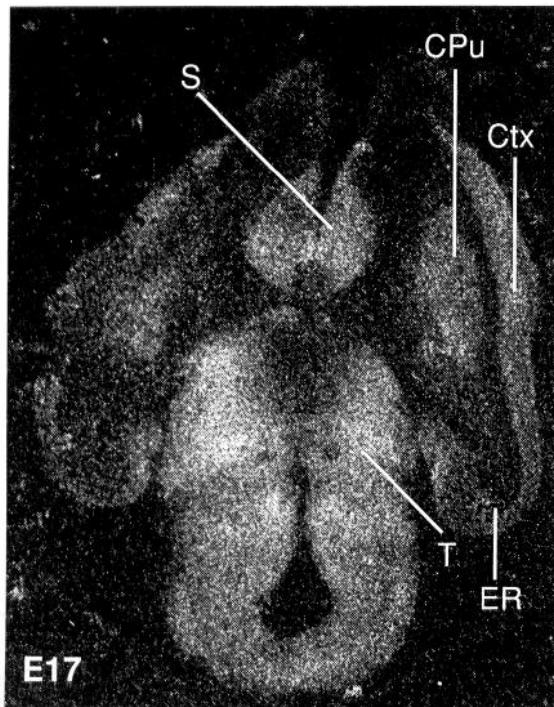
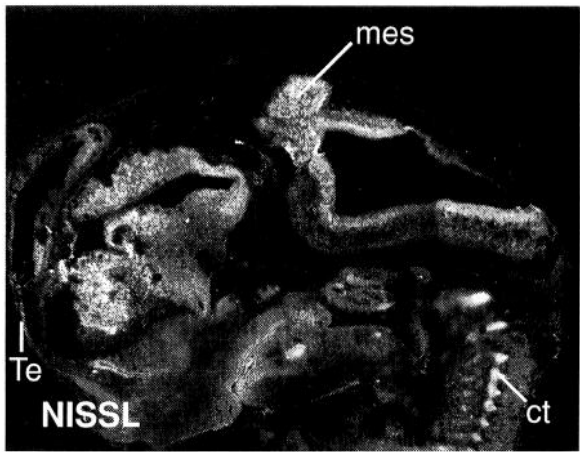
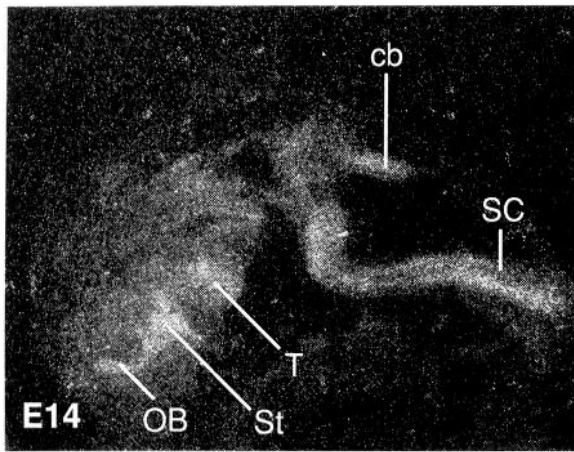
Cortical GluR-5 expression during development shows the most interesting features (Fig. 11). Unlike the other subunit genes, for any given age GluR-5 subunit gene expression varies depending on the region of the neocortex examined. In particular, the somatosensory cortex expresses more GluR-5 transcript than other cortical areas in the same horizontal section.

Transcripts are predominantly observed in the outer layers of the sensory cortex. A very obvious peak occurs for GluR-5 gene expression around birth in this region. The expression is especially high in layer II/III of the cortex (lamina granularis externa), where on the x-ray film the positive signal appears as a very dense, bright band (Fig. 11). Emulsion autoradiography

→
Figure 1. Complete developmental ³H-kainic acid binding profile to sagittal (E12, E14) and horizontal (all others) sections from rats. The E17 and E19 sections are horizontal sections through the whole head including the skull and snout. Representative sections for total binding and nonspecific binding (i.e., binding in the presence of an excess of unlabeled kainic acid) were not above film background. Note the increase in binding in E19 and P0 sections. No binding was detectable at E12. All images are printed to the same scale, reflecting the increase in brain size with development. See Appendix for abbreviations. Scale bar, 2 mm.

Figure 2. Enlargement of ³H-kainic acid binding autoradiographs at E14 (sagittal section), E17, and E19 (horizontal sections through the whole head). The corresponding Nissl stains for each of the autoradiographs are illustrated to the right. The ventricular zone (VZ) is unlabeled. See Appendix for abbreviations. Scale bar, 0.8 mm.





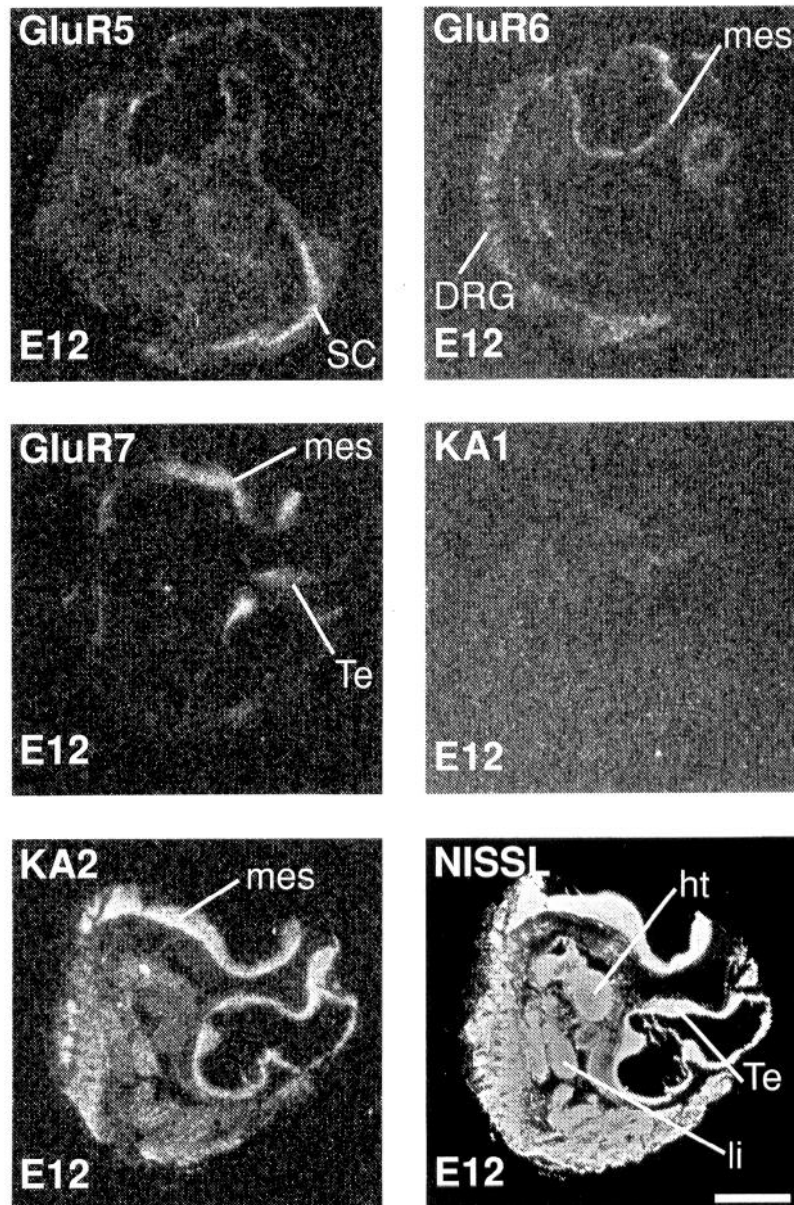


Figure 3. X-ray film autoradiographs illustrating distribution of kainate-preferring receptor subunit mRNAs encoding GluR-5, -6, -7, KA-1, and KA-2 subunits in sagittal sections of E12 rat embryos. GluR-6 mRNA is present in dorsal root ganglia. No KA-1 signal is detectable at E12. The *bottom right panel* shows a Nissl (thionin) stain of the whole embryo section. See Appendix for abbreviations. Scale bar, 1.75 mm.

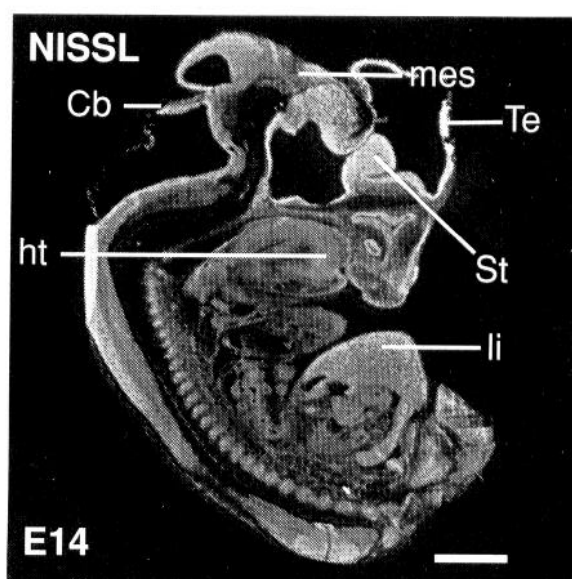
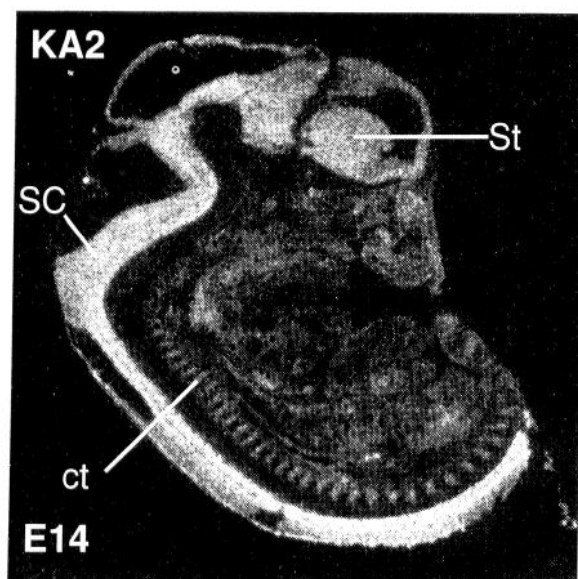
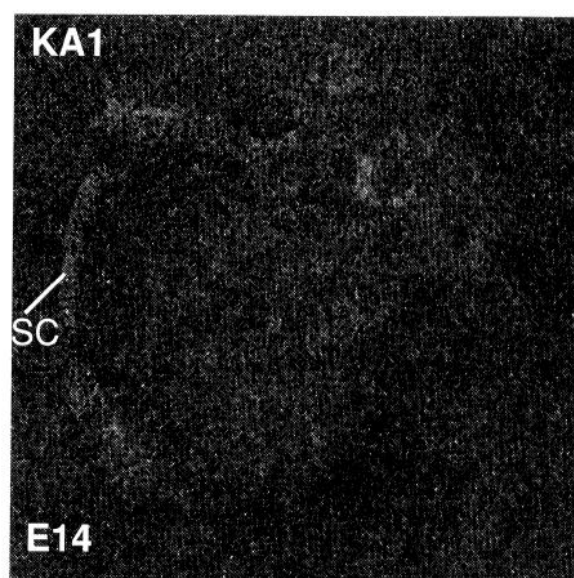
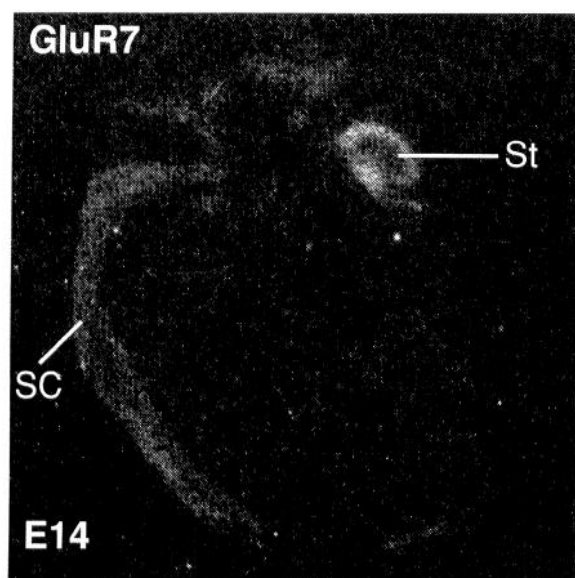
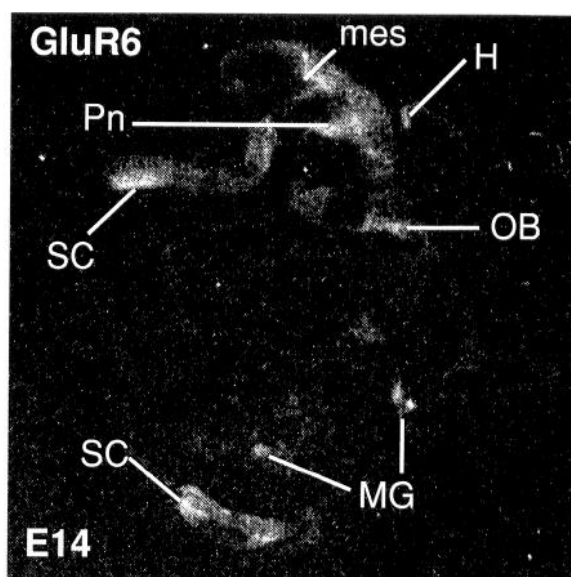
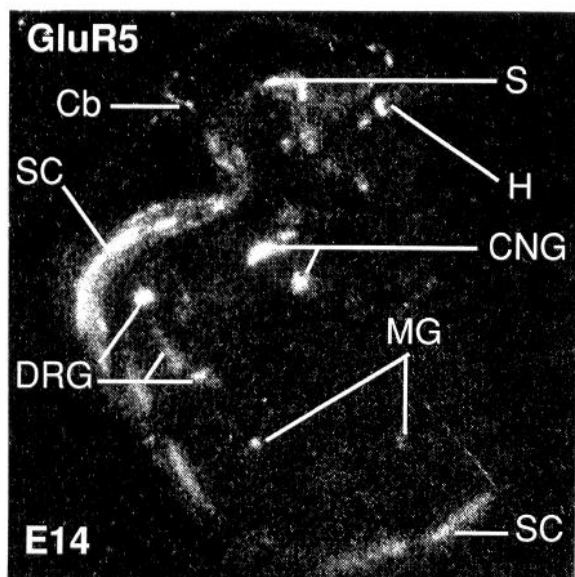
confirms that this cell dense layer of the E19 cortex (layer II/III) has a high accumulation of silver grains after being hybridized with the GluR-5 probe (Fig. 12). Cells more interior or exterior to this layer are unlabelled (Fig. 12). By P12, this layer II/III band is diminished in intensity for the GluR-5 signal and has vanished in the adult (Fig. 12). In contrast, the KA-2 gene is expressed in very many cells of the E19 cortex, regardless of their laminar position (Fig. 13) and continues to be expressed in many cortical neurons of the adult.

As the width of the neocortex increases between E19 and P5 a further population of GluR-5-expressing cells becomes apparent. The area around layer IV is heavily labeled in a more punctate fashion at P0, P5, and P12 (Fig. 11). Although the

majority of cells are unlabelled, a small number of cells express the GluR-5 gene at high levels (Fig. 12*ef*, arrowheads). This population of GluR-5-expressing cells is transient or expresses the gene transiently, since examination of the corresponding region in the adult reveals little or no expression (Fig. 12*g,h*). Again in contrast to the regulated expression of GluR-5, the KA-2 transcripts are found in these cell layers in an invariant manner throughout development (Fig. 13).

The hybridization signal for GluR-6 in the cortex is generally low; but in the cingulate cortex this subunit is very prominent, especially at age P0 (Fig. 7), where it undergoes a clear peak in expression. In contrast to GluR-6, the GluR-7 gene is rather abundant in the inner cortical layers. However, the qualitative

Figure 4. X-ray film autoradiographs illustrating distribution of kainate-preferring receptor subunit mRNAs encoding GluR-5, -6, -7, KA-1, and KA-2 subunits in sagittal sections of E14 rat embryos. GluR-5 and -6 are colocalized in some peripheral ganglia (e.g., the MG ganglion). GluR-7 and KA-2 are the only genes expressed in the striatum at this age. Nissl (thionin) stain of whole embryo section is shown in the *bottom right panel*. See Appendix for abbreviations. Scale bar, 1.6 mm.



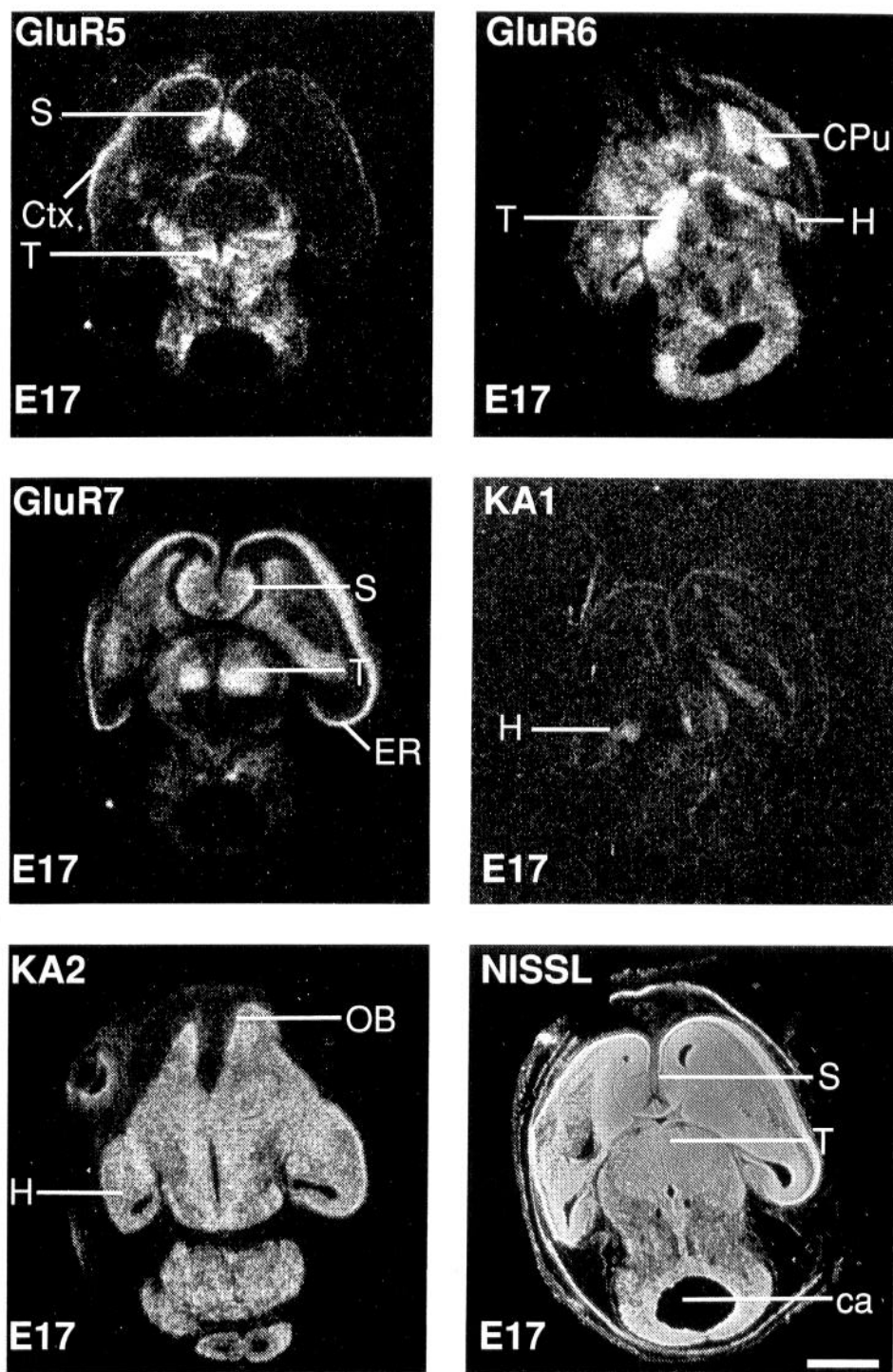


Figure 5. X-ray film autoradiographs illustrating distribution of kainate-prefering receptor subunit mRNAs encoding GluR-5, -6, -7, KA-1, and KA-2 subunits in horizontal sections of E17 rat embryo heads. Nissl (thionin) stain of the whole embryo head is shown in the *bottom right panel*. See Appendix for abbreviations. Scale bar, 1.4 mm.

expression of GluR-7 during ontogeny does not seem to change remarkably, although it also shows a perinatal expression peak (E19, P0, and P5; see Table 1).

The ^3H -kainic acid binding in the developing cortex might correlate especially with the expression of GluR-6 (gyrus cingli), GluR-7 (inner cortical layers, entorhinal cortex) and KA-2. Perinatally the binding is diffuse without restriction to any layer. There is no obvious accumulation of ^3H -kainate binding sites corresponding to GluR-5 in the somatosensory cortex.

Kainate receptors in the developing hippocampus

The formation of the hippocampal structures begins around E14 (Stanfield and Cowan, 1988), and kainate receptor subunit gene expression also begins around this age. However, the signals are very faint (Fig. 4). The dentate granule cells are largely born postnatally (reviewed Gould and McEwen, 1993). The complement of kainate receptor subunits transcripts found in these dentate granule cells in the early postnatal period is essentially

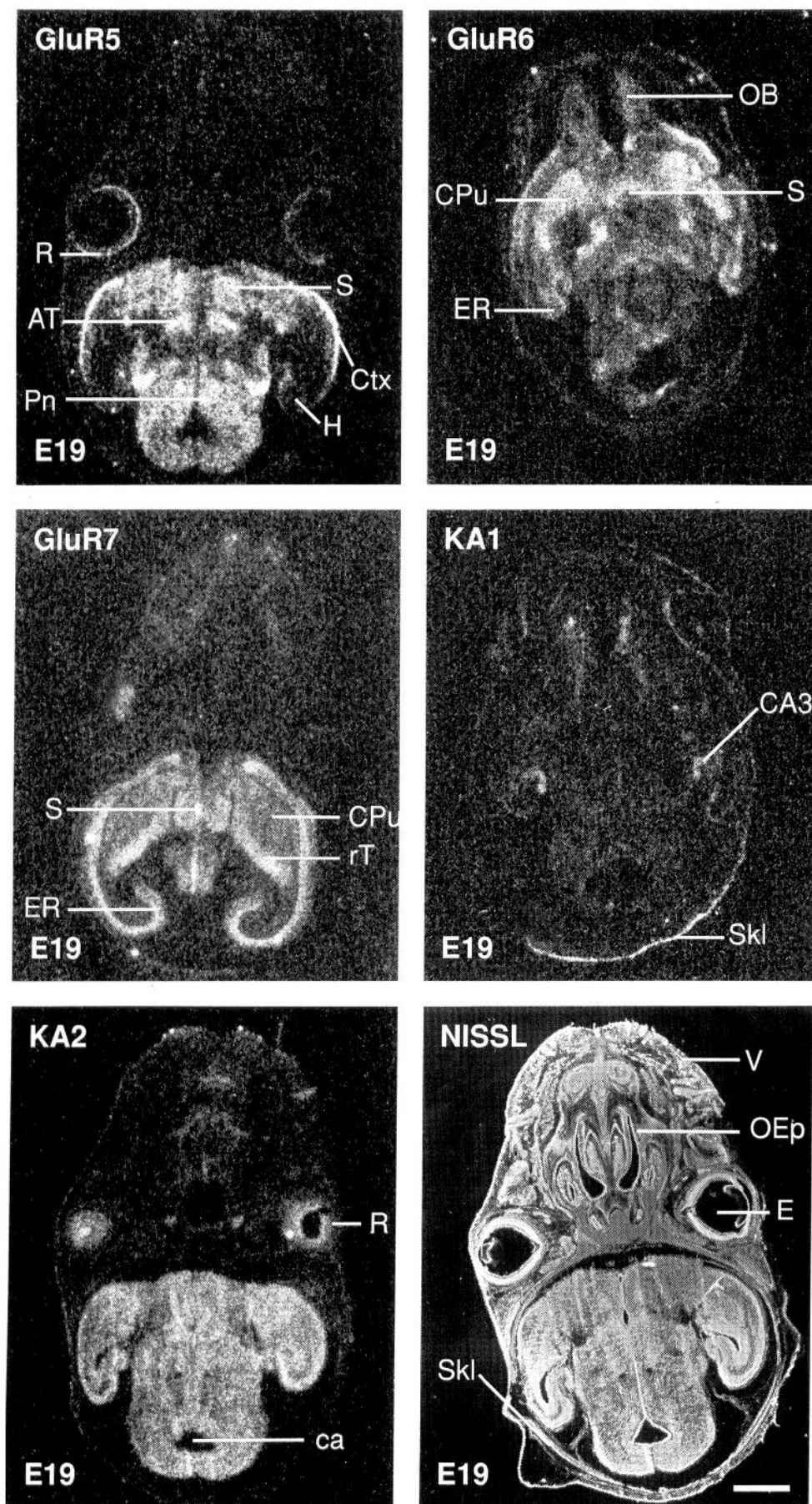


Figure 6. X-ray film autoradiographs illustrating distribution of kainate-prefering receptor subunit mRNAs encoding GluR-5, -6, -7, KA-1, and KA-2 subunits in horizontal sections through the skull of E19 rat embryos. Note that specific GluR-5, -7, and KA-2 expression can be seen in the retina. Nissl (thionin) stain of the whole embryo head is shown in the *bottom right panel*. See Appendix for abbreviations. Scale bar, 1.5 mm.

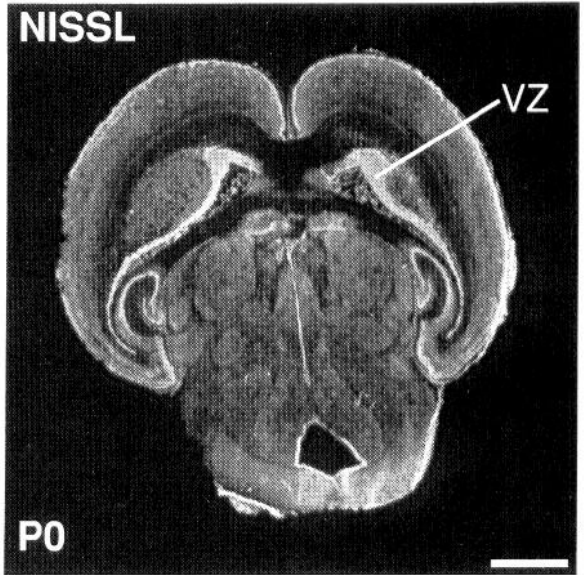
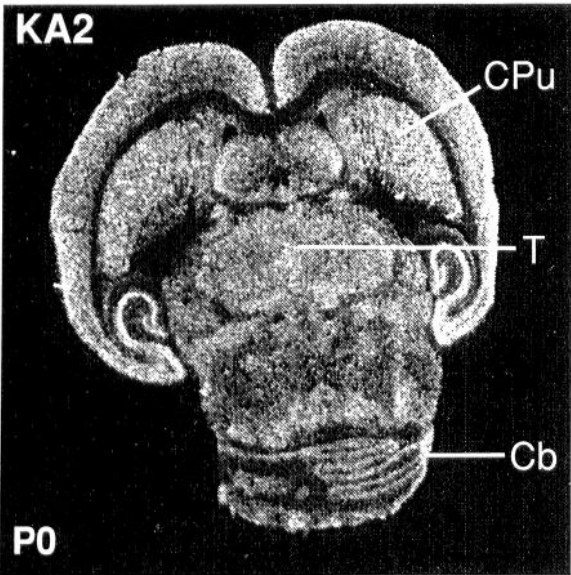
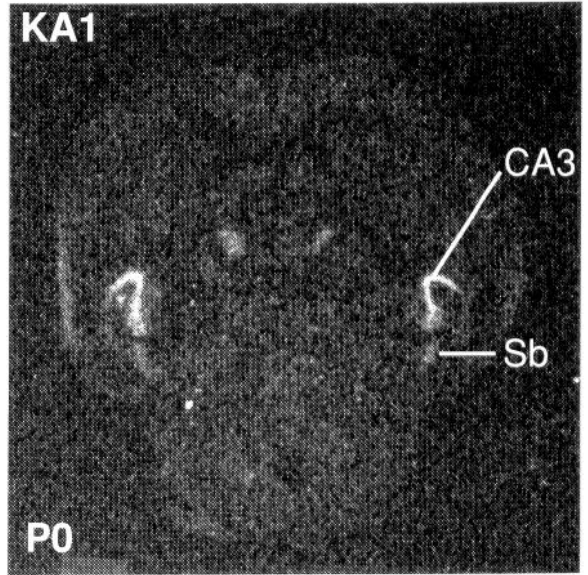
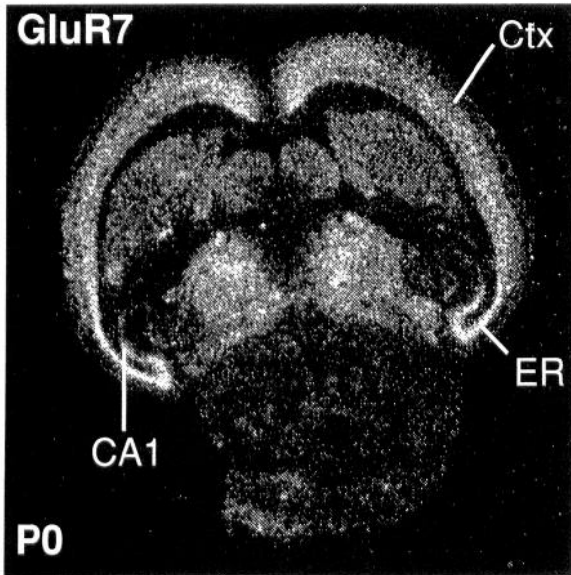
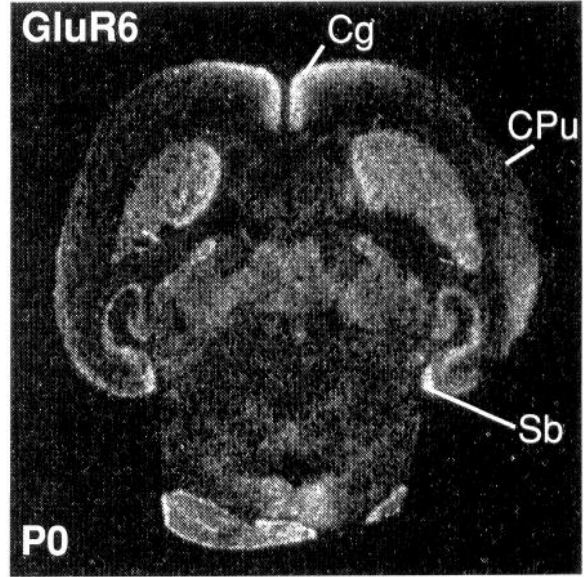
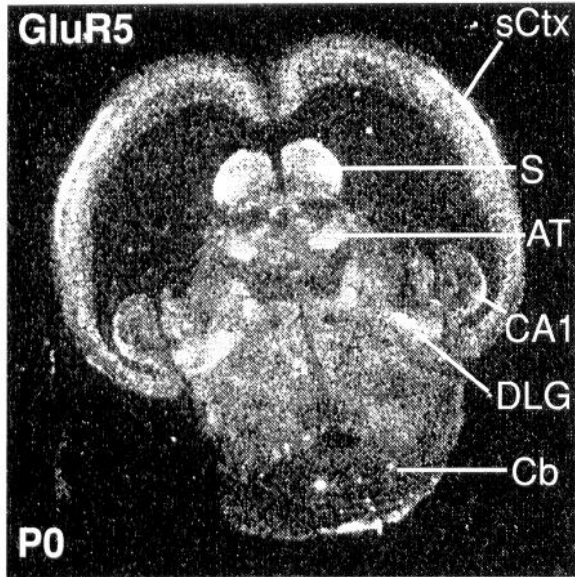


Figure 7. X-ray film autoradiographs illustrating distribution of kainate-preferring receptor subunit mRNAs encoding GluR-5, -6, -7, KA-1, and KA-2 subunits in horizontal sections of P0 rat brains. Nissl (thionin) stain of a corresponding section is shown in the *bottom right panel*. See Appendix for abbreviations. Scale bar, 1.7 mm.

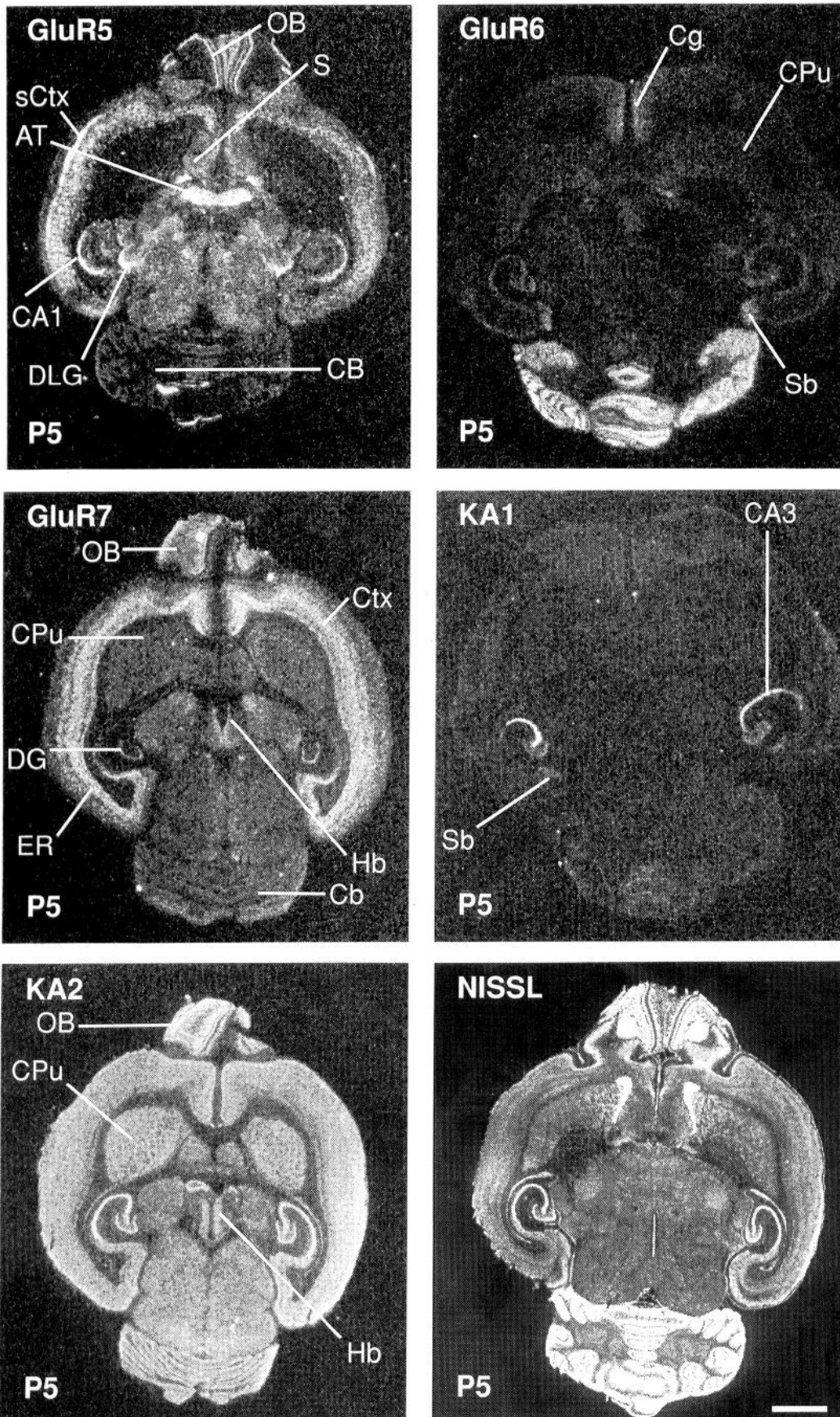


Figure 8. X-ray film autoradiographs illustrating distribution of kainate-preferring receptor subunit mRNAs encoding GluR-5, -6, -7, KA-1, and KA-2 subunits in horizontal sections of P5 rat brains. Nissl (thionin) stain of a corresponding section is shown in the *bottom right panel*. See Appendix for abbreviations. Scale bar, 2.1 mm.

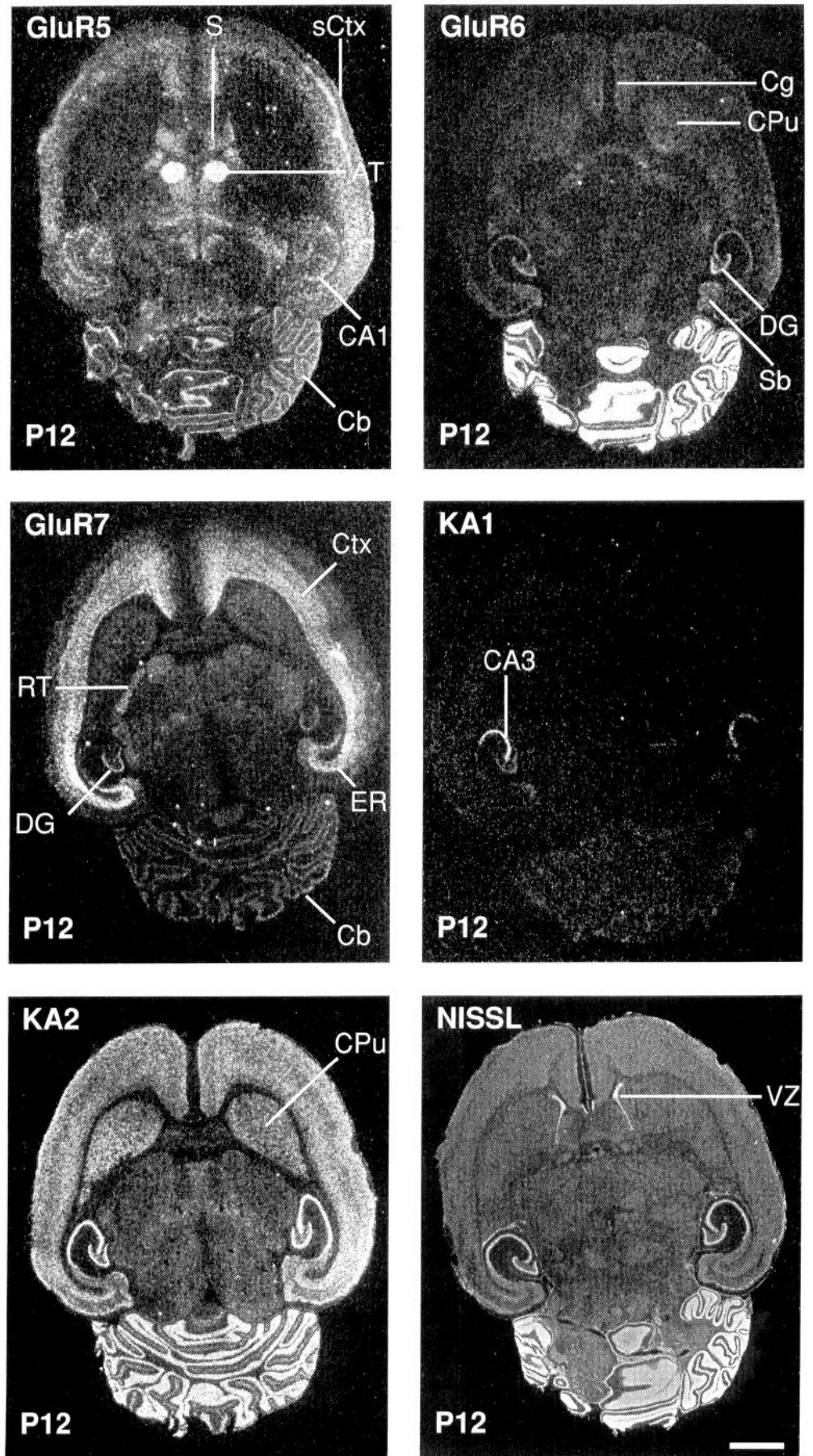


Figure 9. X-ray film autoradiographs illustrating distribution of kainate-prefering receptor subunit mRNAs encoding GluR-5, -6, -7, KA-1, and KA-2 subunits in horizontal sections of P12 rat brains. Nissl (thionin) stain of a corresponding section is shown in the *bottom right panel*. See Appendix for abbreviations. Scale bar, 2.1 mm.

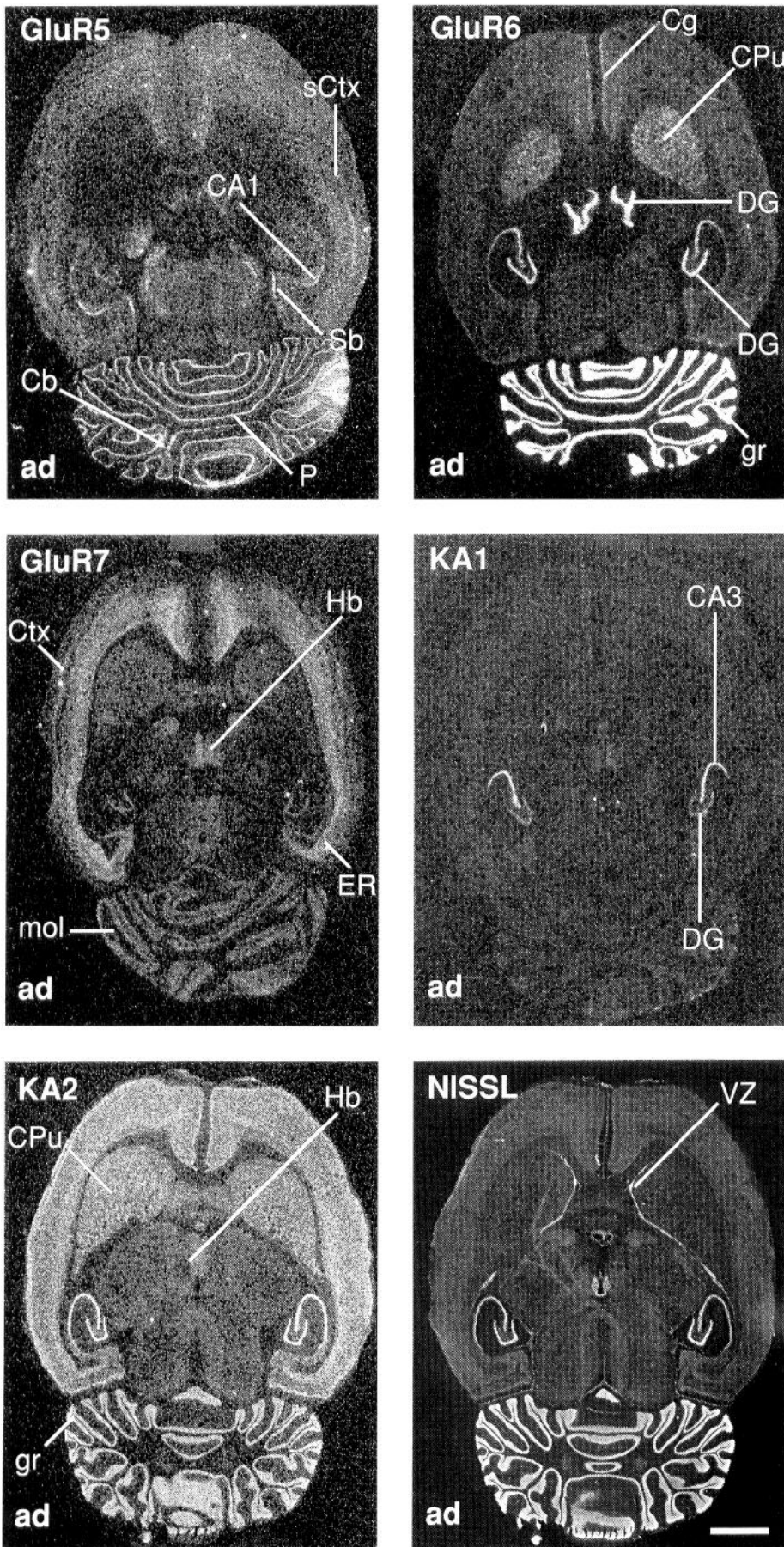


Figure 10. X-ray film autoradiographs illustrating distribution of kainate-preferring receptor subunit mRNAs encoding GluR-5, -6, -7, KA-1, and KA-2 subunits in horizontal sections of adult rat brains. Nissl (thionin) stain of a corresponding section is shown in the bottom right panel. See Appendix for abbreviations. Scale bar, 2.6 mm.

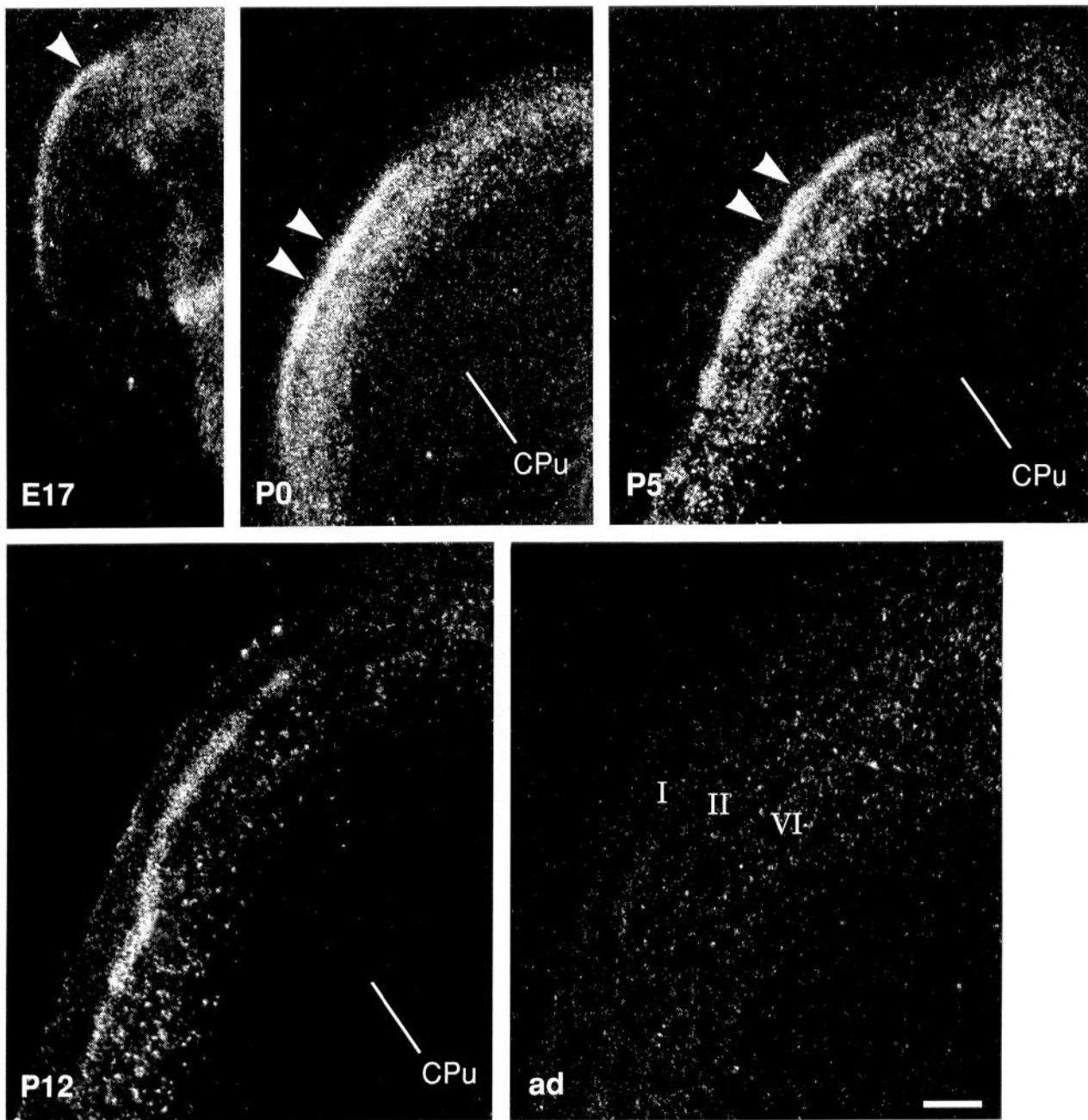


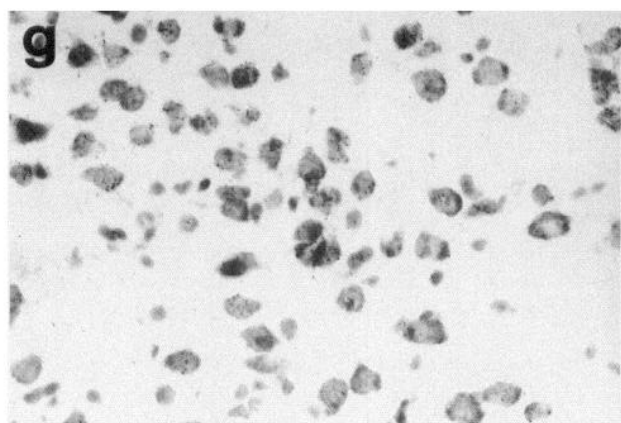
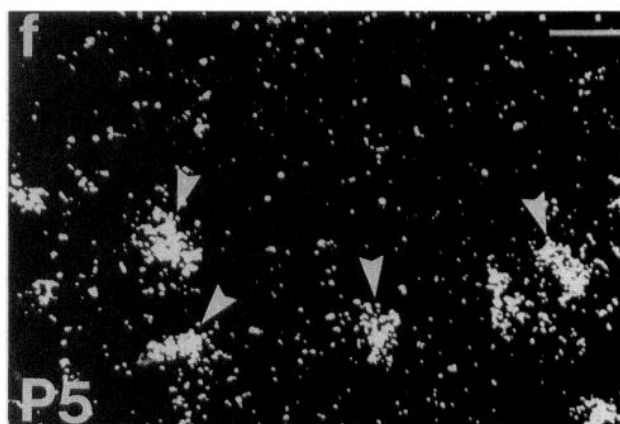
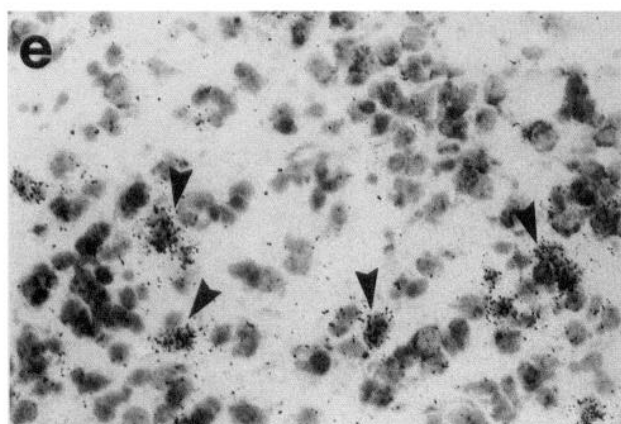
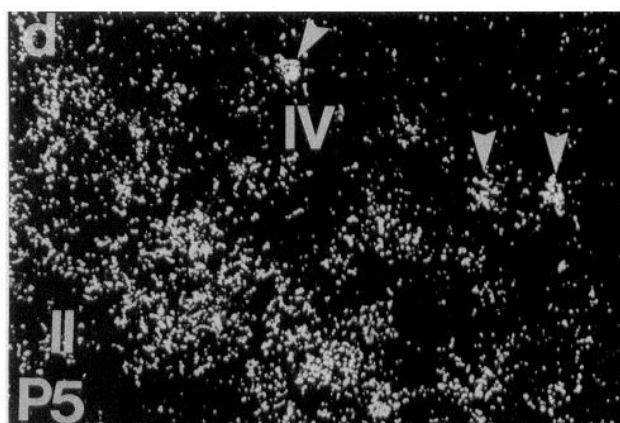
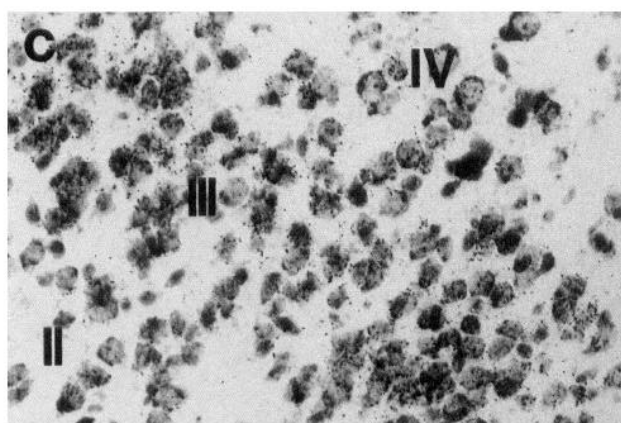
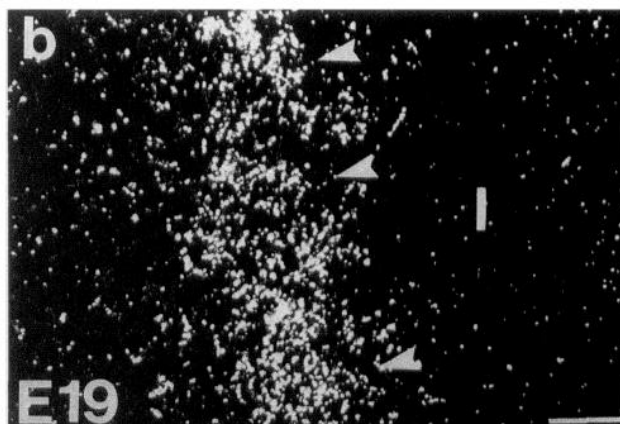
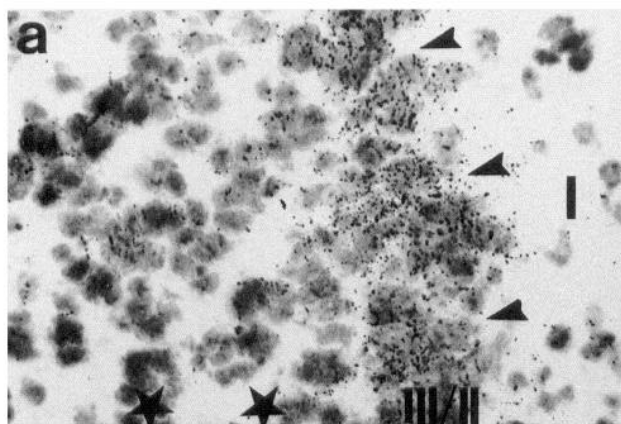
Figure 11. Enlargement of x-ray autoradiographs illustrating the expression of GluR-5 mRNA in the sensory cortex at different stages of brain development (*E17*, *P0*, *P5*, *P12*, adult). White arrowheads indicate the particularly intense line of expression in layer II/III cells in postnatal development. This line is absent in the adult. See Appendix for abbreviations. Scale bar, 0.7 mm.

the same as that of the adult (Table 1), that is, KA-1, KA-2, GluR-6, GluR-7, and some GluR-5.

Of all the kainate receptor subunit genes, that of KA-1 has the most restricted expression in the CNS. In the adult, KA-1

subunit gene expression is a marker for CA3 pyramidal cells and dentate granule cells (Werner et al., 1991). KA-1 gene expression in the hippocampus first faintly appears at *E17* (Fig. 5), and becomes better resolved at *E19* (Fig. 6), where a tiny

Figure 12. Emulsion autoradiographs illustrating the cellular resolution of GluR-5 expression in the developing somatosensory cortex. Left column shows bright-field images, and right column shows the corresponding dark-field images. In *a* and *b*, the arrowheads indicate the layer of positively hybridizing cells in the *E19* sensory cortex. Neither the deeper layers of *E19* cortex (marked by the stars), or cells in layer I express the GluR-5 gene. *c-f* illustrate positive cells (arrowheads) that have appeared by *P5* in the deeper layers of sensory cortex as well as the continued labeling of cells in layer II/III. Most of the cells in the deeper layer are unlabeled. *e* and *f* are an enlargement of *c* and *d*. The same area of adult cortex (*g, h*) has far fewer labeled cells altogether, and none in the frame illustrated. Roman numerals indicate the cortical layers. Scale bars: *a* and *b*, 30 μ m; *c-h*, 50 μ m.



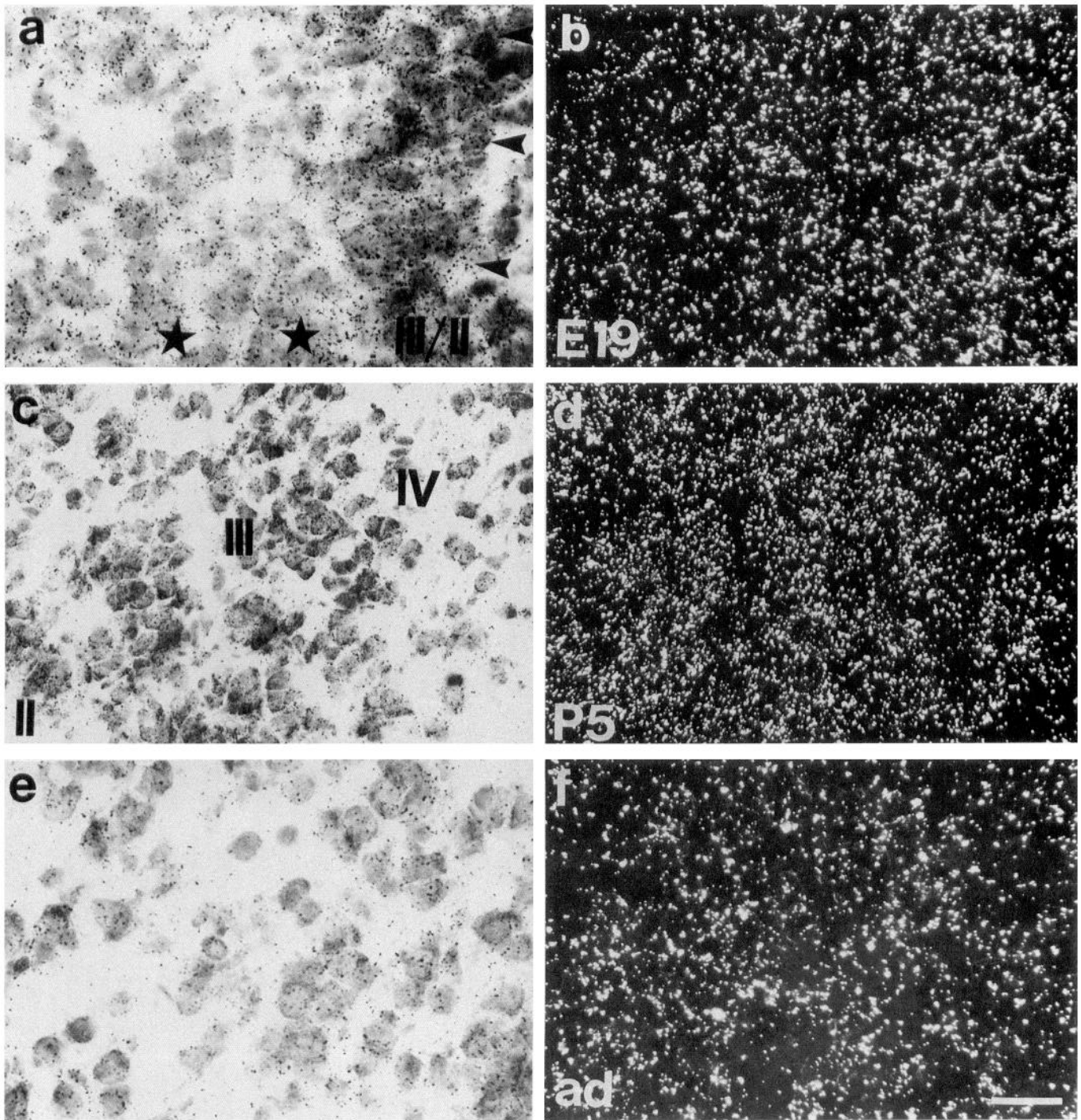


Figure 13. Emulsion autoradiographs illustrating the cellular resolution of KA-2 expression in the developing somatosensory cortex. *Left column* shows bright-field images, and *right column* shows the corresponding dark-field images. This plate is for comparison with Figure 12, and shows that in contrast to GluR-5, KA-2 is heavily and ubiquitously expressed in all cortical layers (except I) at all ages (*E19*, *P5*, adult) as seen by the profusion of silver grains. *Roman numerals* indicate cortical layers. Scale bar, 50 μ m.

arch of expression marks out the CA3 region and a dot of signal marks the presumptive dentate (Fig. 6). The characteristic KA-1 pattern is already clearly defined at P0 (Fig. 7) and remains constant throughout development (Figs. 8–10). GluR-6 is also present in a CA3 to CA1 gradient in the adult (Egebjerg et al., 1991; Wisden and Seeburg, 1993b), but the development of this gradient is retarded compared with that for KA-1. For example, at P0 and P5 when KA-1 expression is already clearly parti-

tioned between CA1 and CA3, GluR-6 is equiabundant in both sectors (Figs. 7, 8). GluR-6 mRNA increases in CA3 pyramidal cells during the late postnatal period (Figs. 9, 10).

Expression peak of GluR-5 in the hippocampus

In the CA1 region and the adjacent subiculum, there is a clear expression peak of GluR-5 at stages P0 and P5 as assessed from x-ray film (Fig. 14). This signal declines markedly by P12 and

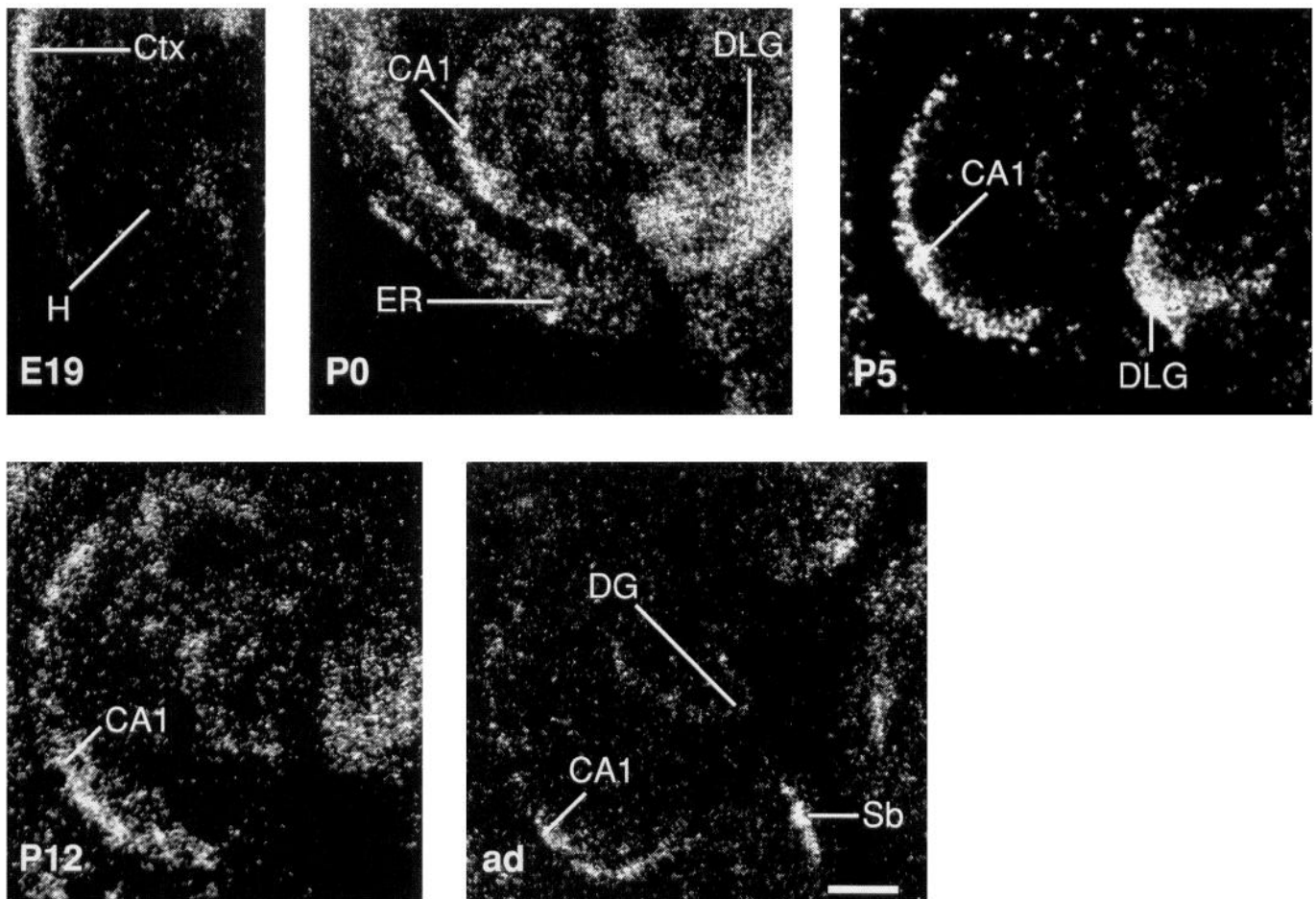


Figure 14. Enlargement of x-ray autoradiographs illustrating the expression of GluR-5 mRNA in the hippocampal region at different stages of brain development (E19, P0, P5, P12, adult). At E19 there is very little expression in the hippocampus as a whole. All images are printed to the same scale. See Appendix for abbreviations. Scale bar, 0.6 mm.

is very diminished in the adult. Relatively little GluR-5 signal is present in CA3 or the dentate gyrus at any age (Fig. 14). At E17 there is very little GluR-5 expression in the hippocampus as a whole, whereas GluR-6 and KA-2 transcripts are already abundant at this stage (Fig. 5). This emphasizes that the GluR-5 signal in the developing hippocampus has a peak, rather than a decline in expression from some higher point.

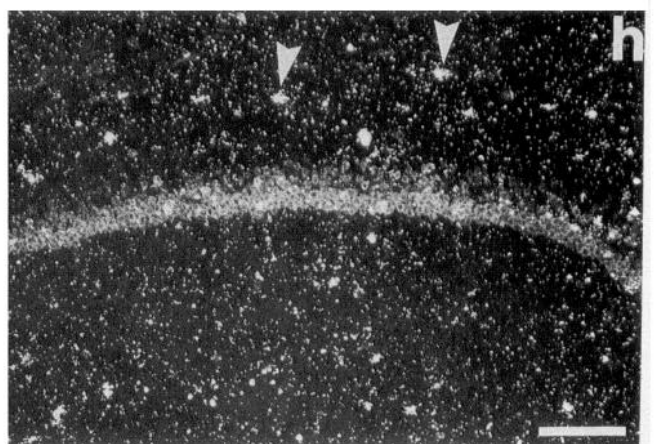
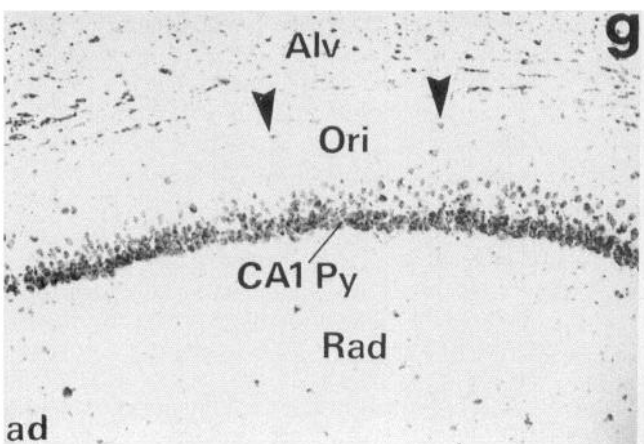
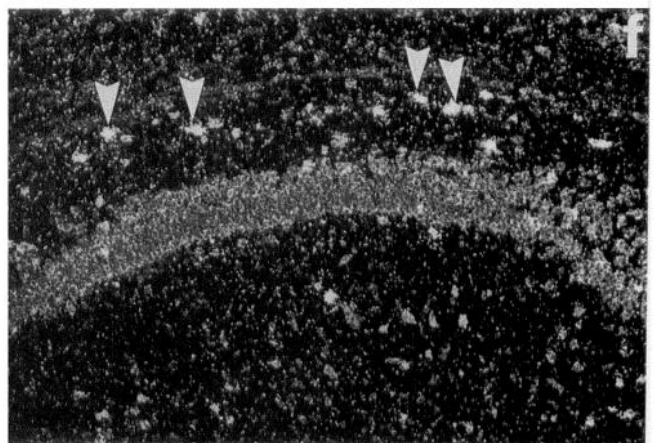
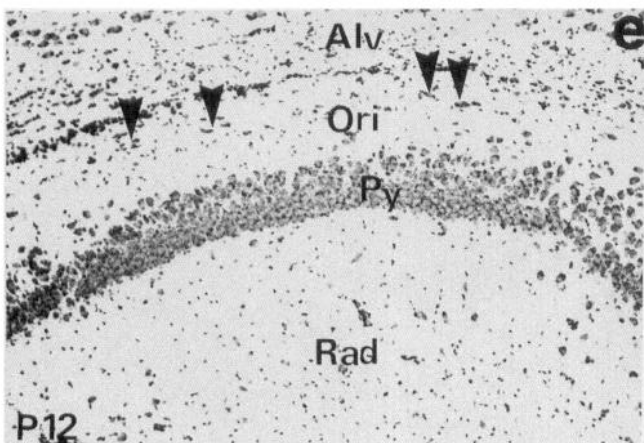
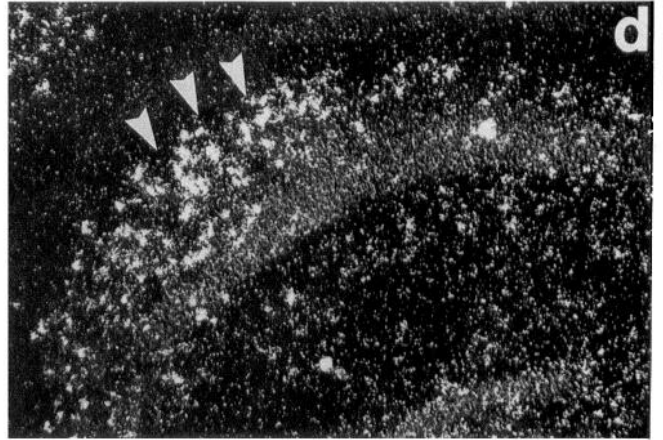
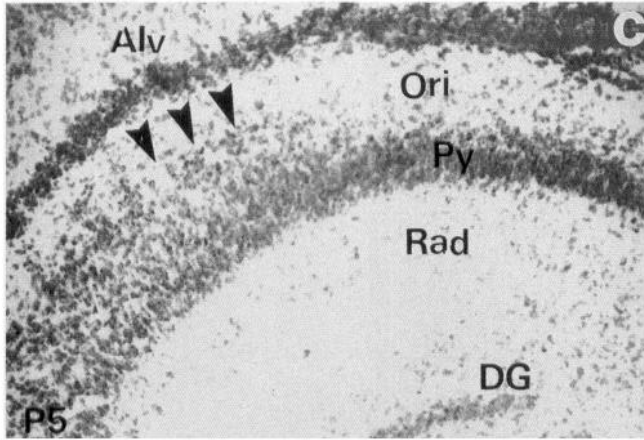
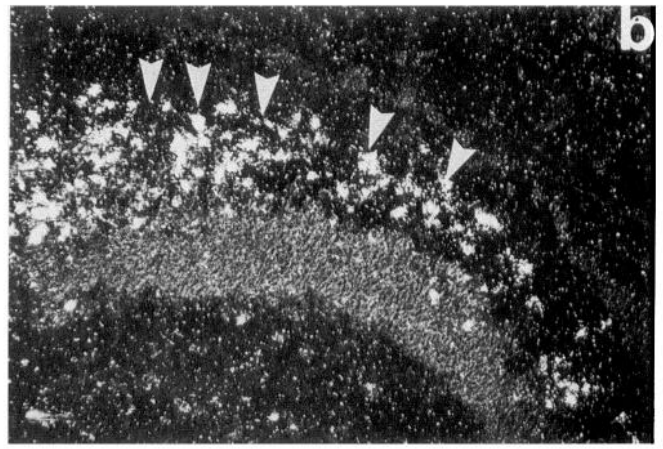
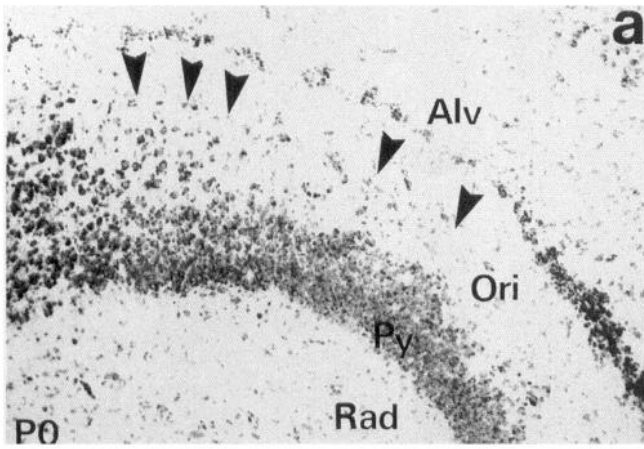
Emulsion autoradiography reveals that the cells responsible for the transient GluR-5 signal are located in the stratum oriens (Fig. 15), and are probably nonpyramidal interneurons. At P0, many cells are strongly positive in the oriens layer (Fig. 15*b*, arrowheads), with the pyramidal cell layer at best lightly labeled. Through consequent stages, the number of GluR-5-positive oriens cells greatly diminishes until only a few remain in the adult (Fig. 15*h*, arrowheads). Consistent with our interpretation of these cells as interneurons, Craig et al. (1993) observe that GluR-5-expressing cells from cultured embryonic rat hippocampus have the morphological characteristics of GABAergic interneurons. However, in these hippocampal primary cultures, no transient GluR-5 expression was seen, even though the cultures were maintained for 2 weeks from E19. Although, as is the case *in vivo*, the GluR-5-expressing cells in culture were a minority (approximately 20%), GluR-5 expression in individual cells increased with age (Craig et al., 1993).

Septum

Only three of the five high-affinity kainate receptors are expressed in septal areas. The hybridization signal for GluR-5 is especially high, and as early as E14 already prominent. From E17 through to P5, the septum is a bright beacon of GluR-5 signal (Figs. 5–8). After P5 the labeling with the GluR-5 probes decreases significantly in the septum. The GluR-7 transcripts in this region show a peak at E17 and gradually decline thereafter from moderate (E19, P0) to low levels. The expression of the KA-2 gene does not change during development of the septum. The ³H-kainic acid binding is detectable in the septum at E17 (Fig. 2), is very high at P0 and declines to moderate/low levels in the adult (Fig. 1). It might correlate with the GluR-5 gene expression pattern (i.e., a perinatal peak).

Kainate receptor expression in the developing thalamus

The developing diencephalon provides a further example of the heterogeneous expression of high-affinity kainate receptors. The KA-2 subunit transcripts are already ubiquitous in the mesencephalon at E12 and E14 and are expressed throughout this structure (Figs. 3, 4). In the following stages almost all subunits (except KA-1) are found in the thalamus. There is a very high expression of GluR-5 mRNA around birth (E19, P0) in certain



nuclei, for example, the dorsal lateral geniculate and the anterior thalamic nuclei (Fig. 7), with a strong decline after P12.

In the adult thalamus, only the reticular area has an appreciable number of binding sites and GluR-7 predominates in this nucleus (Monaghan and Cotman, 1982; Unnerstall and Wamsley, 1983; Miller et al., 1990; Bettler et al., 1992; Lomeli et al., 1992; Wisden and Seeburg, 1993; and Figs. 1, 10). However, in the early postnatal period, the reticular thalamus expresses GluR-6 and KA-2 at comparable levels to GluR-7 (Fig. 7).

Kainate receptor expression in the developing cerebellum

The adult cerebellum has three cortical layers: the molecular layer, the Purkinje cell layer, and the granular layer (named in order from the outer surface), with the final structures of the cerebellum being formed by neuronal migration largely during postnatal development (Jacobson, 1991). Therefore, the expression of most high-affinity kainate receptors is restricted to the period of postnatal development. However, ³H-kainate binding is found over the primordial cerebellum (Fig. 2).

The localization of GluR-5 mRNA in the P0 cerebellum is diffuse (Fig. 7). By P5 a rather faint band over the multilayered Purkinje cells has appeared (cellular emulsion not shown). By P12, the hybridization signal is clearly over the single layered Purkinje cells; this pattern is maintained during adulthood. Purkinje cells also show KA-1 gene expression. In the mouse, it has been observed that GluR-5 mRNA is abundant in immature granule cells, which then markedly downregulate the gene at a later period (Bettler et al., 1990). This does not seem to be the case in the rat, with no obviously strong expression of GluR-5 by granule cells at any age.

The GluR-6 gene has its highest level of expression in granule cells in both the adult (Egebjerg et al., 1991, Fig. 10) and throughout development (e.g., at P5, Fig. 10). Transcripts for GluR-6 and KA-2 are observed in granule cells, with no striking developmental change. The pattern of labeling corresponds with the migration of these cells. At P0 the external granular cell layer is heavily labeled by the GluR-6 and KA-2 probes as a thick band (Fig. 7); in the following stages P5 and P12, the signal increases in migrating cells and the internal granular cell layer with a slight expression peak of GluR-6 at P12 (Figs. 8, 9). In the cerebellum of the adult rat, GluR-6 and KA-2 signals are restricted to the internal layer. The GluR-7 gene is expressed in the basket/stellate cells of the molecular layer throughout the postnatal period.

Perinatally, the ³H-kainic acid binding in the cerebellum is diffuse (Fig. 1); at P5 the granule cell and molecular layer labeling becomes more distinct increases with age (see also Cambray-Deakin et al., 1990). Consistent with the expression of GluR-7 in the stellate/basket cells in the molecular layer, the possible expression of KA-1/GluR-5 on Purkinje cell dendrites, and GluR-6/KA-2 in the granule cell layer, both the granule and the molecular layers are labeled with ³H-kainate, with the granule cell layer always containing more sites at any age (see Monaghan and Cotman, 1982; Cambray-Deakin et al., 1990).

Discussion

We performed a systematic comparison of the distribution of the five high-affinity kainate receptor subunits (GluR-5, GluR-6, GluR-7, KA-1, and KA-2) during development of the rat brain. Each of these subunits has a characteristic ontogenetic expression profile that begins as early as E12 (summarized in Table 1). The fact that kainate binding (as an index of protein formation) is not detectable until E14 (Fig. 1), even though transcripts are present at E12, may reflect a lag between the appearance of mRNA and significant accumulation of protein. In the mouse, where embryonic stages are approximately 2 d ahead of the rat (reviewed Laurie and Schrotz, 1994), GluR-5 mRNA is weakly detectable at E10, which is compatible with our results (Bettler et al., 1990). At rat embryonic stages E12 and E14, the migration and differentiation of the majority of neurons in the telencephalon and mesencephalon has not yet occurred (Jacobson, 1991), so that kainate receptors are likely to be synthesized on immature postmitotic neurons. At this very early stage, many synapses have not formed, so receptors might be extrasynaptic or located on dendritic growth cones. Similarly, in the early postnatal cerebellum, GluR-6 and KA-2 transcripts are detected in the external granule cell layer prior to their inward migration.

The role of kainate-preferring receptors in the brain is opaque. Based on their recombinant properties, they are predicted to form glutamate-gated cation channels that constitute a parallel system to those of the AMPA-preferring variety, but which rapidly desensitize to kainate. Kainate-preferring receptors can be distinguished from AMPA receptors by differential sensitivities to ConA and cyclothiazide (Partin et al., 1993). However, with the exception of the dorsal root ganglia in the periphery (Huettner, 1990; Partin et al., 1993), responses of kainate-preferring channels have not been reported when patch clamping CNS cells in slices (Jonas and Sakmann, 1992; reviewed by Wisden and Seeburg, 1993a,b). One possible reason for this is that functional kainate receptors could be largely located on distal dendrites (Vickers et al., 1993), and so elude detection by standard patch-clamping techniques. However, kainate has been found to activate a fully desensitizing current in a subset of cultured hippocampal cells of a small appearance (22% of all cells studied) dissociated from E17 rat embryos when examined 4 hr after plating and up to 3–4 d in culture (Lerma et al., 1993). This response most clearly matched GluR-6(Q) homomeric responses. With neurons kept for longer periods, the AMPA/low-affinity kainate (nondesensitizing) response predominated (Lerma et al., 1993). The *in situ* hybridization and immunocytochemistry studies predict that most types of hippocampal neurons (e.g., pyramidal cells) would express kainate-preferring receptors, again suggesting that the architectural features of many neurons might preclude the electrophysiological detection of kainate receptors.

In the developing brain, a dendritic location of kainate receptors might be used to elicit very local cytoarchitectural changes

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Figure 15. Emulsion autoradiographs illustrating cellular resolution of GluR-5-expressing cells in the stratum oriens of the developing hippocampus. *Left column* shows the Nissl stain (bright-field) and *right column* shows the corresponding dark-field images. *Arrowheads* indicate examples of labeled cells. Many cells are labeled at P0, whereas only a few cells are labeled in the stratum oriens of the adult (*g, h*). See appendix for abbreviations. Scale bar, 400 μ m.

of the dendritic tree. Growth rates of developing dendrites can be slowed down by focal application of physiological concentrations of glutamate or kainate to individual growth cones (Mattson et al., 1988a). These regressive effects of kainate are mediated by ionotropic receptors located on the growth cone (Mattson et al., 1988a,b) and would be in keeping with dendritic locations seen in the adult (Huntley et al., 1993; Vickers et al., 1993). In a study using cultured cerebellar granule cells, kainate (60 μM) enhanced neurite development (both elongation and sprouting), whereas AMPA (20 μM) had no effect (Pizzi et al., 1994). This observation might further support the idea of kainate-selective receptors that are present on dendrites or axons. Immunocytochemistry with an antibody that cross-reacts with the GluR-5, GluR-6, and GluR-7 subunits shows that these subunits are particularly enriched on dendritic shafts (Huntley et al., 1993; Vickers et al., 1993), although this has not been so far examined in the developing CNS.

Kainate receptor configurations in development

The electrophysiology of recombinant subunits and immunoprecipitation studies from native brain membranes suggest that some kainate-preferring receptors probably exist *in vivo* as heteromeric assemblies of different types of subunit, for example, GluR-6/KA-2 (Herb et al., 1992; Sakimura et al., 1992; Wenthold et al., 1993). GluR-5 and GluR-6 can also function in homomeric configurations *in vitro* (Egebjerg et al., 1991; Sommer et al., 1992), but whether they exist like this *in vivo* is unknown. Thus, a specific aim was to examine possible partnership relations among different high-affinity kainate receptor subunits during development. Generally, as far as can be seen by *in situ* hybridization, and with the exception of GluR-5 (see below), these are probably the same as in the adult brain (see Wisden and Seeburg, 1993b), although there are likely to be subtle differences (see Results). However, the intensification/exaggeration of the adult patterns seen in the early postnatal period may indicate an importance of these receptors in perinatal plasticity. Alternatively, the postnatal intensification of the adult pattern might represent peaks of cell populations, many cells of which are then eliminated during the normal course of neurogenesis (see below).

High-affinity kainate binding sites as markers of subunit composition?

There are two high-affinity kainate binding sites found on brain membranes of K_D of 5 and 50 nM, respectively (Coyle, 1983; Hampson et al., 1987), and these have essentially the same distribution as determined by autoradiography (Monaghan and Cotman, 1982; Unnerstall and Wamsley 1983; Miller et al., 1990). The relative proportion of these two sites in some brain areas changes during development, although as in the adult, the qualitative distribution pattern of the two sites is more or less identical (Miller et al., 1990). At first sight, these two sites are reflected in the recombinant kainate receptor subunits: homomeric KA-subunits have $K_D \sim 5\text{--}10$ nM (Werner et al., 1991; Herb et al., 1992), while homomeric configurations of GluR-5, -6, and -7 average $\sim 50\text{--}100$ nM K_D for kainate (Bettler et al., 1992; Lomeli et al., 1992; Sommer et al., 1992). However, since homomeric cloned KA subunits do not form functional ion channels, but assuming that they do so *in vivo* as partners with the GluR subunits, it is difficult to know how to precisely relate the binding to the recombinant data. For example, taking the results of Miller et al. (1990), the affinity (K_D) of kainate sites

in the reticular thalamic nucleus is 14.7 at P14 and 6.3 in the adult. However, in the adult (and largely throughout development) the only kainate subunit abundantly detectable by *in situ* hybridization is that of GluR-7, with some low levels of KA-2 and GluR-6 mRNA also present (Wisden and Seeburg, 1993b). Yet the recombinant form of GluR-7 has a K_D for kainate of ~ 70 nM (Bettler et al., 1992; Lomeli et al., 1992), so it is hard to see how the reported K_D of 6.3 for the adult reticular thalamic nucleus correlates with the subunit composition in this area. Similarly, the K_D for kainate in CA3 at 14 d is 7.8, and in the adult, 5.8 (Miller et al., 1990), yet this region expresses GluR-6, KA-1, and KA-2 throughout development. If CA3 pyramidal cells assemble functional KA-1/KA-2/GluR-6 channels, the K_D would be predicted to be between 50 and 100 nM based on expressing the cloned subunits on human embryonic kidney or HeLa cells (Bettler et al., 1992; Herb et al., 1992). In any case, even if the CA3 K_D is due to homomeric KA-subunits, GluR-6 mRNA (and probably protein) is abundant in these cells (Egebjerg et al., 1991; Wisden and Seeburg, 1993b). Alternatively, some of the binding sites in CA3 stratum lucidum could be presynaptic, resulting from kainate receptors on mossy fiber terminals derived from the dentate granule cells (Represa et al., 1987). However, even in this instance, based on mRNA expression profiles in the dentate granule cells, we might still expect the GluR-6 and GluR-7 subunits in addition to those of KA-1 and KA-2 to be present. In order to explain the K_D values, one would have to argue for a selective sorting of just the KA-subunits into the mossy fibers. The use of subunit-specific antibodies should resolve this point. It is also possible that in neuronal membranes, rather than in the epithelial cells used for the recombinant studies, the subunits exhibit different affinities.

A special role for GluR-5 in the developing CNS?

In contrast to the other subunits, the GluR-5 gene undergoes many clear *qualitative* changes in its expression over time, as is particularly clear from the developing sensory cortex (see Fig. 11). In the sensory cortex, the GluR-5 subunit shows a distinct perinatal peak in layers II, III, and IV. The very intense signal in layer II appears as a homogeneous band on the x-ray autoradiographs, whereas the distribution in layers III and IV is more punctate. The time course of this GluR-5 expression peak is consistent with the period of greatest developmental plasticity in the somatosensory cortex, that is, formation of the "barrels," the somatotopic representation of the sensory vibrissae in rodents (Jacobson, 1991). These barrels are composed of small aggregates of layer IV neurons, innervated by clusters of thalamocortical afferents. Barrel formation takes place during the first 24 hr after birth (P0). Interestingly, Shlaggar et al. (1993) report that chronic blockade of ionotropic glutamate receptors during this time interferes with barrel formation in the developing rat. These observations may be suggestive of a role of the GluR-5 subunit in plasticity dependent processes. The changes in GluR-5 expression are specific to just one region of developing cortex.

A further indication of a possible "special" role of GluR-5 in development is that GluR-5 transcripts similarly peak in the septum, thalamus, CA1 interneurons of the hippocampus in the stratum oriens (Figs. 14, 15). Peaks of ^3H -kainic acid binding are seen especially in the thalamus, CA1 and subiculum (Miller et al., 1990; Garcia-Ladona and Gombos, 1993; present results). These binding peaks possibly correspond to GluR-5 expression, although there is no obvious binding peak in the somatosensory

cortex with respect to other areas of the cortex. In the hippocampus, a whole large population of GluR-5-expressing interneurons in the stratum oriens appears to switch off this gene (Fig. 15).

An alternative for the GluR-5 expression peaks in these areas is that they represent a transient population of cells. In many parts of the developing vertebrate CNS, up to half of the neuronal population is eliminated in the cause of quantitative adjustments of interconnecting populations of neurons and in the elimination of aberrant projections (reviewed by Oppenheim, 1991). NMDA receptors are suggested to be one conduit used for an excitotoxic culling of cells, since NMDA receptor blockade with MK-801 during development results in increases in neuronal numbers in the adult (Mentis et al., 1993; Mount et al., 1993; O'Donoghue et al., 1993). So, rather than a gene regulation/switch, it is possible that the decline of GluR-5 expression (e.g., in the cortex, septum, and thalamus) is due to cell death of specific cell populations expressing GluR-5. The GluR-5 subunit might exert a direct excitotoxic effect on the cell in which it is expressed. A precedent is that overexpression of the related GluR-6 subunit in CA3 hippocampal neurons leads to cell death (Bergold et al., 1993).

The GluR-5 and GluR-6 subunits each exist in several versions, some of which are permeable to calcium, since critical amino acids determining ion selectivity in putative transmembrane domains can be substituted by RNA editing (Sommer et al., 1991, 1992; Egebjerg and Heinemann, 1993; Köhler et al., 1993). Editing the different forms of GluR-5 could occur at varying frequencies in the CNS during development (Sommer et al., 1991; Köhler et al., 1993). It would be interesting to examine by single cell PCR/patch-clamp methods (Lambolez et al., 1992; Mackler and Eberwine, 1993) the relative numbers of edited versions of GluR-5 mRNAs in cells taken from different ages of somatosensory cortex. It might also be worthwhile to look at the numbers of calcium permeable kainate receptors in this area of developing cortex using the cobalt histochemical assay (Pruss et al., 1991).

General changes in other ionotropic EAA receptors in development

Some NMDA, AMPA, and δ ionotropic glutamate receptor subunit genes also markedly change their expression during development. These changes are often found in similar brain regions to those of the high-affinity kainate receptors (Insel et al., 1990; Monyer et al., 1991, 1994; Pellegrini-Giampietro et al., 1991, 1992; Gallo et al., 1992; Watanabe et al., 1992; Lomeli et al., 1993). Any developmental effects of changes in high-affinity kainate receptor expression probably occur in concert with these other receptors. For example, the NR2D (ϵ 4) NMDA receptor subunit gene is highly expressed in the embryonic and early postnatal thalamus, but strongly downregulated in the adult (Watanabe et al., 1992; Monyer et al., 1994). This situation parallels the changes seen in GluR-5 gene expression in these regions during the same period of development and might represent a functionally related event.

Conclusions

Kainate-preferring receptors are expressed from an early age (E12/E14) in both peripheral and central neurons. Changes in the subunit composition of these receptors probably take place during brain development. In particular, the GluR-5 subunit gene is markedly regulated in the sensory cortex, thalamus, and

CA1 interneurons, and it might be worth focusing future efforts on understanding the exact role of this subunit during brain development.

Appendix

Abbreviations used in figures

| | |
|------|--|
| AT | anterior thalamus |
| ca | Cerebral aqueduct |
| CA1 | CA1 region of hippocampus |
| CA3 | CA3 region of hippocampus |
| Cb | Cerebellum |
| Cg | Cingulate gyrus |
| CNG | Cranial nerve ganglia |
| CPu | Corpus putamen |
| ct | Cartilage |
| Ctx | Cortex |
| DG | Dentate gyrus |
| DLG | Dorsal lateral geniculate nucleus |
| DRG | Dorsal root ganglia |
| E | Eye |
| ER | Entorhinal cortex |
| gr | Granule cell layer of cerebellum |
| H | Hippocampus |
| Hb | Habenula |
| ht | Heart |
| li | Liver |
| MG | Mural ganglia |
| mes | Mesencephalon |
| MLG | Medial lateral geniculate nucleus |
| mol | Molecular layer of cerebellum |
| OB | Olfactory bulb |
| OEp | Olfactory epithelium |
| P | Purkinje cell layer of cerebellum |
| Pn | Pons |
| R | Retina |
| rT | Reticular thalamus |
| S | Septum |
| Sb | Subiculum (including presubiculum and parasubiculum) |
| SC | Spinal cord |
| sCtx | Sensory cortex |
| Sk | Skull |
| St | Striatum |
| T | Thalamus |
| Te | Telencephalon |
| V | Follicles of vibrissae |
| VZ | Ventricular zone |

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