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High-affinity kainate and domoate receptors in rat brain

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Mammalian brain expresses receptors which bind the potent neurotoxins, kainate and domoate, with high affinity, and which form a subclass of ionotropic glutamate receptors. A new member of these receptors, expressed in both adult and embryonic CNS is compared in its ligand binding properties to its closely sequence-related homologs.

Glutamate receptor; High-affinity kainate binding; Domoate

1. INTRODUCTION

In the mammalian brain, excitatory amino acid (EAA) transmitters, such as glutamate, act on ionotropic receptors encoded by a superfamily of sequence-related genes [1–3]. Thus within the non-NMDA receptor grouping, both AMPA and kainate can act on the same receptor class (a 'high affinity AMPA/low affinity kainate' type) encoded by the GluR-A to -D (GluR1–4) series [4–8]. The affinity of this class of receptors for kainate is in the μM range but kainate activation of GluR-A to -D channels produces large non-desensitizing current responses resembling those found in vivo [9]. The distribution of high-affinity kainate sites (dissociation constants (K_d's) of 5 and 50 nM) in rodent brain does not resemble that of the AMPA/low affinity kainate class [10]. Such observations have been resolved by the discovery of the KA-1 and -2 [11–13], and the GluR-5 and -6 [14–16] subfamily of genes. These encode subtypes of high-affinity kainate receptors, with a ten- fold higher affinity shown by KA-1 and -2 (K_d ~5 nM) than by GluR-5 and -6 (K_d ~50 nM). However, GluR- 5 and -6 exhibit a higher affinity for the agonist, domoate, than KA-1 and -2.

We now report the characterization of GluR-7, a polypeptide that is closely related in sequence to the GluR-5 and -6 subunits. The expression pattern of GluR-7 mRNA in the brain matches in part the distribution of high-affinity [^{3}H]kainic acid sites. Furthermore, GluR-7 exhibits a unique excitatory amino acid pharmacology which sets it apart from the GluR-5 and -6 receptor subunits.

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2. MATERIALS AND METHODS

2.1. *Isolation of cloned GluR-7 cDNA*

A partial GluR-7 sequence was obtained from rat brain cDNA using the polymerase chain reaction (PCR). Oligonucleotide primers and reaction conditions for PCR were as described [5]. A 1.9 kb cDNA clone was isolated from a rat brain cerebellum library constructed in λgt10, using the cloned PCR fragment as a probe. This clone encoded ~330 amino acids of the carboxy-terminal half of the GluR-7 subunit. To obtain the amino-terminal sequence, three oligonucleotides: 5'-GCCAATGATGCGGGTAGACAGA-3', complementary to a region that encodes LSTRIIG (subunit residues 633–639); 5'-GCGGTACCCACATCTTCTCAAAGGTGGAG-3', complementary to a region encoding STFEKMWA (residues 702–709); and 5'-GCGGTACCTGGAGATCTTGGATTTCTTG-3', complementary to a sequence encoding KKSKIST (residues 696–703) were used to prime cDNA from rat cortical mRNA. This cDNA was then used to construct a λgt10 library which was screened with a synthetic 46mer oligonucleotide (5'-ACTGGGGTTGGTGCCATTGGGTTTTCGATATAAGATGCTCACTCCG-3'), complementary to GluR-7 codons for subunit residues 539–553. A full 5' end-containing clone was isolated. A 2.9 kb *Eco*RI–*Acc*I fragment encoding the 634 amino-terminal residues of GluR-7 and a 1.3 kb *Acc*I–*Hin*dIII fragment encoding the remaining 249 carboxy-terminal residues were ligated into a eukaryotic expression vector [17].

2.2. *Ligand binding studies*

Supercoiled plasmid DNA of the GluR-7 expression vector was used to transfect human embryonic kidney 293 cells, and membrane was prepared for binding as described previously [16]. Binding assays using [^{3}H]kainate (58 Ci mmol^{-1}, NEN) were carried out in a total volume of 0.5 ml for 60 min at 0°C with non-specific binding defined in the presence of 1 mM L-glutamate. Competition studies were performed in the presence of 50 nM [^{3}H]kainate.

2.3. *In situ hybridization*

Oligonucleotides (45mers) were 3' end-labelled with [^{35}S]dATP (1,200 Ci/mmol). In situ hybridization was performed as described [18]. The atlasses of Paxinos and Watson [19] and Paxinos et al. [20] were used to identify and confirm rat brain structures. For GluR-7, two oligonucleotides were used; (i) a 46mer, identical to the probe used

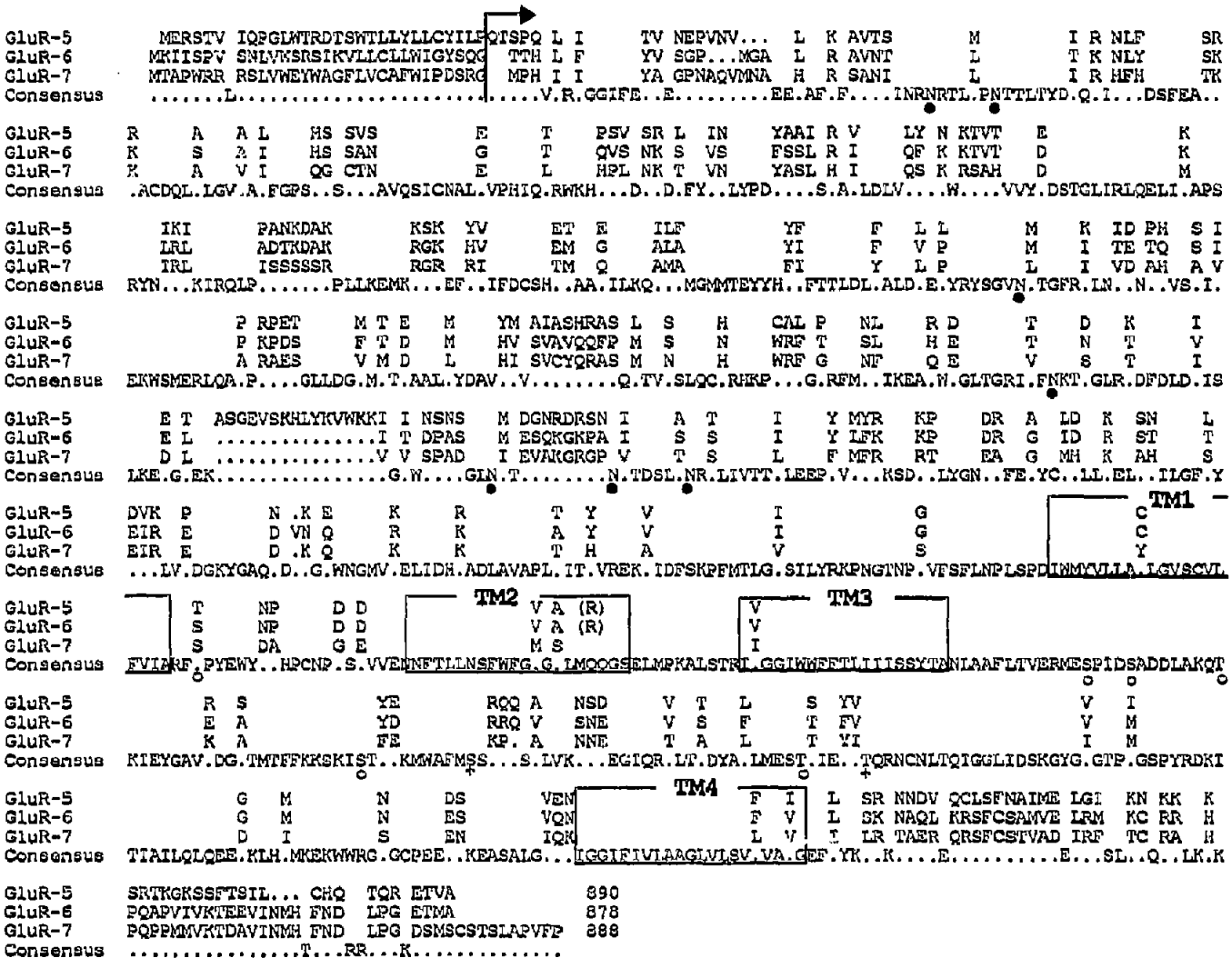

Fig. 1. Polypeptide sequence alignment of the rat glutamate receptor subunits GluR-5, GluR-6 and GluR-7. Sequences are numbered starting with their predicted mature N-termini, and signal sequences are shown left of the arrow. A consensus sequence shows amino acids identical in the three proteins. The putative transmembrane regions, TM1 to TM4, are boxed. *N*-Linked glycosylation sites (NxS/T, x = P) in the predicted extracellular domain of the three subunits are indicated by filled circles; +, consensus phosphorylation sites for protein kinase C (S/T-(x)-R/K); ○, consensus phosphorylation sites for Ca^{2+}-calmodulin-dependent protein kinase type II (S/T-(x-x)-D/E).

to screen the specifically primed library (see above), and (ii) a 45mer, 5'-ATTCTCCACCACCTCAGAGCCGGGGTTGCAGGGGTGG-GCATCATA-3', complementary to codons for the mature GluR-7 residues 561–573. The specificity of the GluR-7 signal was confirmed with the two independently employed oligonucleotides and by competition with unlabelled oligonucleotide.

3. RESULTS AND DISCUSSION

Subunits of glutamate-gated cation channels form a large gene family within which the three subunits, GluR-5, -6 and -7, comprise a closely sequence-related subfamily (Fig. 1). RNA editing determines the presence of an arginine residue in a key functional position

Table I

Ligand-binding properties of homomeric kainate receptors

	GluR-5	GluR-6	GluR-7
[3H]Kainate	73 ± 19	36 ± 4.7	62.5 ± 18
Domoate	2.1 ± 0.8	8.6 ± 1	36.6 ± 4.7
Quisqualate	280 ± 52	403 ± 38	7,022 ± 179
Glutamate	290 ± 156	1,080 ± 100	2,194 ± 108
CNQX	2,000 ± 500	3,150 ± 510	820 ± 185
AMPA	3,000 ± 1,000	>10,000	>10,000

Values represent mean ± S.E.M. of three independent experiments. Numbers for [3H]kainate represent *Kd* values (in nM), all other numbers represent *Ki* (nM) values.

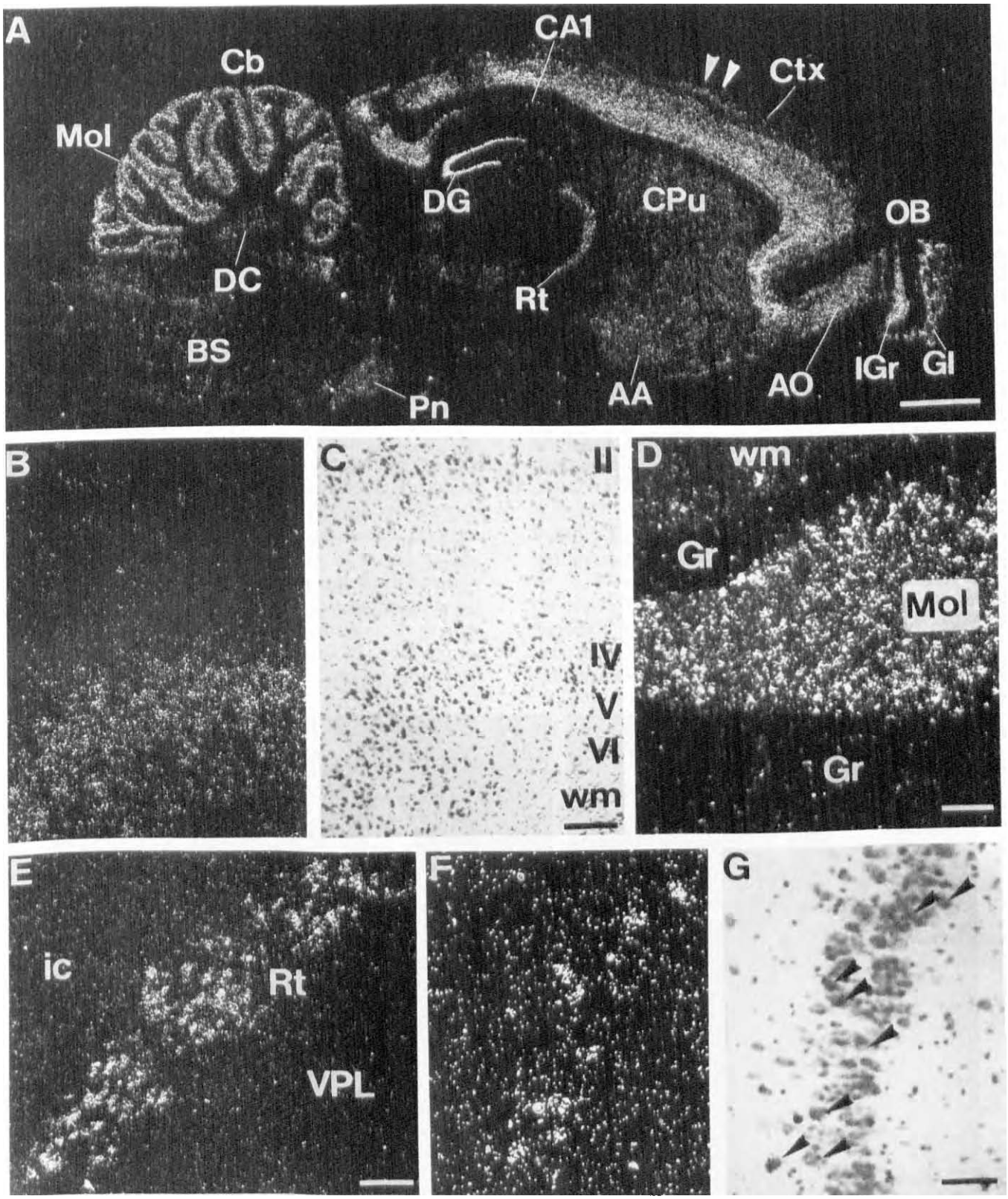

Fig. 2. Distribution of GluR-7 transcripts in the adult rat brain as assessed by in situ hybridization. (A) X-ray film autoradiograph of GluR-7 mRNA in saggital section, bar = 2 mm; (B,C) darkfield and brightfield photomicrographs of GluR-7 mRNA in neocortex, bar = 500 μm; (D) darkfield view of GluR-7 mRNA in cerebellum, bar = 500 μm; (E) GluR-7 mRNA in the reticular thalamus, bar = 200 μm; (F,G) darkfield and brightfield views of occasional cells (arrowheads) in the CA1 pyramidal cell layer expressing the GluR-7 gene, bar = 80 μm. AA, anterior amygdaloid area; AO, anterior olfactory nucleus; BS, brainstem; Cb, cerebellum; Cpu, caudate-putamen; Ctx, neocortex (II, IV, V, VI, denote cortical layers); DC, deep cerebellar nuclei; DG, dentate granule cells; Gl, glomerular layer of olfactory bulb; Gr, cerebellar granule cells; ic, internal capsule; IGr, granule cell layer of the olfactory bulb; Mol, molecular layer of cerebellum; OB, olfactory bulb; Rt, reticular thalamic nucleus; WM, white matter; VPL, ventroposterior lateral thalamic nucleus.

of the putative channel forming segment TMII of GluR-5, -6 and GluR-B [21]. The isolated cDNA clones of the GluR-7 subunit encoded a glutamine in this region, which corresponds to the unedited TMII form. A PCR analysis of adult brain-derived cDNA did not reveal GluR-7 sequences with an arginine codon in TMII (not shown), suggesting that GluR-7 mRNA, at least in the adult brain, may not undergo editing.

The properties of the GluR-7 subunit transiently expressed in 293 cells were analyzed. Ligand binding studies for kainate showed a single, high-affinity site for this ligand with a K_d of 62 nM. Other ligands of the excitatory amino acid receptor family were used as competitors for [^{3}H]kainate and exhibited a rank order of potency of domoate>CNQX>glutamate>quisqualate >AMPA. It is of interest to compare the ligand binding properties of GluR-7 with those of GluR-5 and -6 (Table I). All three receptors show similar affinities for [^{3}H]kainate (K_d's = 40–70 nM). GluR-5 exhibits a 20-fold higher affinity for domoate than GluR-7. At GluR-5 and -6, the rank order of competition for [^{3}H]kainate is domoate≫quisqualate=glutamate>CNQX≥AMPA, which differs from the order established for GluR-7. This difference may reflect the fact that the GluR-7 polypeptide sequence has slightly diverged from the closer related GluR-5 and -6 sequences (see Fig. 1). Despite the presence of a functional binding site, no glutamate- or kainate-activated currents were observed in cells engineered for GluR-7 expression (data not shown). This may indicate that GluR-7 does not assemble into functional homomeric channels. Alternatively, a fast time-course of desensitization ($\tau \leq 1$ ms) may preclude our registering of channel activity. In the GluR-5 to -7 family of glutamate receptor subunits, GluR-5 and -6 were observed to form functional channels by assembling with a KA-2 subunit [12]. However, no functional channels were found upon coexpression of GluR-7 with KA-1 or KA-2 (unpublished observations).

GluR-7 transcripts are present in restricted cell populations throughout the neuroaxis. The confined expression of the GluR-7 gene to the inner cortical layers (IV, V, VI) is a striking feature. Expression in layers II/III is minimal (Fig. 2A–C), however, parts of the cortex show a thin sublayer of cortical expression, possibly in layer III (indicated by arrowheads in Fig. 2A). The GluR-7 mRNA is found in the dentate granule cells of the hippocampus, anterior olfactory nucleus, caudate putamen, anterior amygdaloid area, reticular thalamic nucleus (Fig. 2A,E), and in putative stellate/basket cells of the cerebellar molecular layer (Fig. 2A,D). The globus pallidus contains relatively few hybridizing cells. In the olfactory bulb, the granule cell and external plexiform layers are also clearly labeled. In the hippocampal complex, labeling essentially stops at the subiculum, and at the level of X-ray film autoradiography, the pyramidal cell layers of the hippocampus carry no autoradiographic signal (Fig. 2A). Occasional scattered cells in the pyramidal cell layer contain GluR-7 transcripts (Fig. 2F,G). Cell populations that contain no detectable GluR-7 mRNA are the cerebellar granule

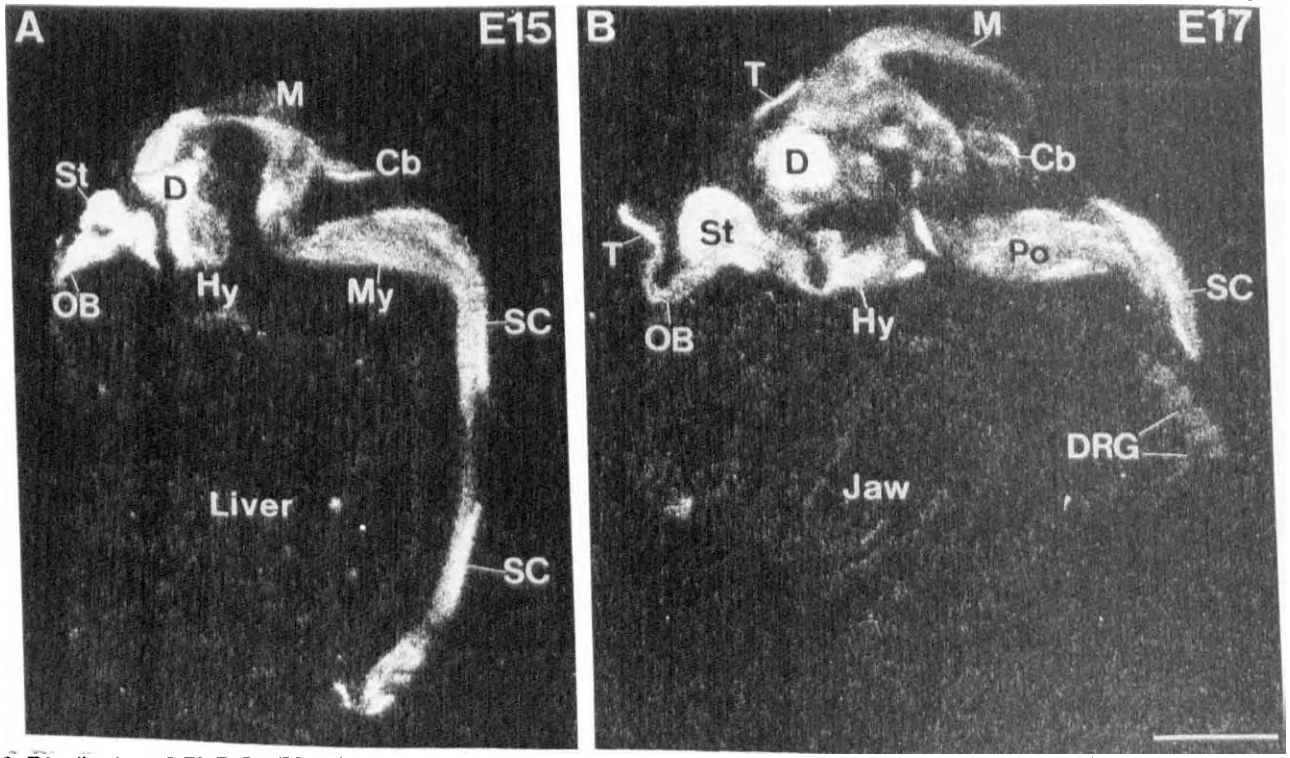

Fig. 3. Distribution of GluR-7 mRNA in the embryonic E15 (A) and E17 (B) rat brain as assessed by in situ hybridization. Cb, cerebellum; D, diencephalon; DRG, dorsal root ganglia; Hy, hypothalamus; M, mesencephalon; OB, olfactory bulb; Po, pons; SC, spinal cord; St, striatum; T, telencephalon. The positions of jaw and liver are marked. These areas contain no specific hybridization signal. Bar = 2 mm.

and Purkinje cells. GluR-7 transcripts were detected throughout the embryonic CNS including the spinal cord, from the earliest time point examined (E15) (Fig. 3A). There was considerable heterogeneity of GluR-7 expression in the E17 brain, with many small nuclei, particularly in caudal areas, containing heightened levels of GluR-7 mRNA. Expression was highest in putative thalamic structures, and in the striatum and telencephalon. There was low expression in E17 dorsal root ganglia (Fig. 3B). Around birth (postnatal day 1) the qualitative pattern of GluR-7 expression was that of the adult brain (not shown).

Key components of high-affinity [^{3}H]kainate binding are layer I and the inner laminae of the neo- and cingulate cortex, the superficial layers of the pyriform cortex, caudate putamen, external plexiform and granule cell layers of the olfactory bulb, CA3 sector of the hippocampus, reticular thalamic nucleus, hypothalamic median eminence and the granule cell layer of the cerebellum [23,25]. Clearly, the GluR-7 gene expression pattern corresponds with a subset of the autoradiographic patterns observed for high-affinity [^{3}H]kainate sites in the rat brain. This new member extends the family of those subunits which exhibit sites of approximate K_d's of 50 nM for [^{3}H]kainate. Another family, presently consisting of subunits KA-1 and KA-2 binds [^{3}H]kainate with a K_d of ≈5–10 nM [11,12]. Sites with comparable affinities for kainate have been described in brain membranes [10,22–24]. Whilst this manuscript was in preparation a similar report appeared elsewhere [26].

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