Molecular Brain Research 92 (2001) 1–11

MOLECULAR BRAIN RESEARCH

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Research report

Differential expression patterns of three glutamate transporters (GLAST, GLT1 and EAAC1) in the rat main olfactory bulb

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Accepted 20 March 2001

Abstract

Glutamate is the main neurotransmitter in the olfactory bulb. Therefore, glutamate transporters, which regulate the concentration of extracellular glutamate, might play pivotal roles in odor processing. In this study, we examined expressions of three glutamate transporters (GLAST, GLT1 and EAAC1) in the olfactory bulb using in situ hybridization and immunohistochemistry. EAAC1 mRNA was expressed in neurons, such as periglomerular cells, tufted cells, mitral cells and granule cells as shown before in other brain areas. In contrast, GLAST and GLT1 were found in glial cells throughout the olfactory bulb, with intenser expressions in the glomerular layer, external plexiform layer and internal plexiform layer where glutamatergic synapses are concentrated. In addition, using double staining immunohistochemistry we clearly showed that GLAST and GLT1 were expressed in astrocytes. Furthermore, we found that GLAST was also intensely expressed in the subependymal layer where precursor cells exist. These results suggest each glutamate transporter plays its unique role not only in glutamatergic neurotransmission but also in cell differentiation and migration in the olfactory bulb.

Theme: Neurotransmitters, modulators, transporters, and receptors

Topic: Excitatory amino acids: anatomy and physiology

Keywords: Glutamate; Uptake; Localization; Metabolism

1. Introduction

Glutamate transporters play central roles in regulating extracellular glutamate concentrations in the brain. Glutamate uptake from the synaptic cleft is important for terminating synaptic transmission and preventing the accumulation of excessive glutamate in the synapse which leads to neuronal cell death [16]. In addition, glutamate transporters presumably participate in the regulation of cell differentiation, migration and synaptogenesis in the central nervous system [17,23]. To date, five different sodium-dependent glutamate transporters have been cloned: GLAST (EAAT1) [22,24], GLT1 (EAAT2) [15], EAAC1 (EAAT3) [8], EAAT4 [5] and EAAT5 [2]. The regional and cellular localizations of these transporter subtypes have been investigated at both protein and mRNA levels [2,5,8,15,22,24] and it was demonstrated that the first three were widely distributed in the brain, whereas the localizations of EAAT4 and EAAT5 were mostly restricted to the cerebellum and retina, respectively [2,5,8,15,22,24]. Each of GLAST, GLT1 and EAAC1 had a unique map of expression in the brain and they were distributed differentially within neurons and astroglias [4].

The olfactory bulb shows widespread immunoreactivity for glutamate [11]. Within the olfactory bulb, the processing of odorant information takes place in the glomerular layer and external plexiform layer, where glutamate plays a pivotal role in the synaptic transmissions [27]. The odor processing is carried out using intrinsic neurons specific to each level, namely periglomerular and granular cells. These intrinsic neurons receive glutamatergic inputs from mitral and tufted cells (M/T cells). Besides, olfactory receptor cells are likely to project glutamatergic axons to M/T cells and periglomerular cells. To maintain such complicated glutamatergic networks in the olfactory bulb, glutamate transporters must be indispensable. In addition,

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PII: S0169-328X(01)00098-5

as shown in previous reports [17,23], glutamate transporters may be also involved in the neurogenetic process of intrinsic neurons of the bulb, which continuously occur all through life. Despite the importance of glutamate transporters in the olfactory bulb, little information is available about their distribution patterns there. This study was, thus, designed to clarify the organization of glutamate uptake systems in the olfactory bulb. We investigated the detailed expression of GLAST, GLT1 and EAAC1 in the olfactory bulb at mRNA and protein levels.

2. Materials and methods

2.1. In situ hybridization histochemistry

Three male Wistar rats weighing about 150 g (6 weeks old) were deeply anesthetized with diethyl ether and perfused with 4% paraformaldehyde in 0.1 M phosphate-buffered saline. After perfusion, olfactory bulbs were removed. After overnight postfixation, they were dehydrated in 30% sucrose in 0.1 M phosphate buffer (PB). Coronal sections (20 μm) were cut on a cryostat, thaw-mounted onto PLL-coated slides (Matsunami Glass, Japan) and stored at −80°C until use.

After being warmed to room temperature, slide-mounted sections were fixed in 4% paraformaldehyde in 0.1 M PB for 20 min, washed in PB (twice for 5 min), treated with 10 μg/ml proteinase-K in 50 mM Tris–HCl and 5 mM EDTA for 30 s and then fixed again. After washing in distilled water, the sections were acetylated with 0.25% acetic anhydrate in 0.1 M triethanolamine, rinsed with PB, dehydrated in ascending ethanol series (70%, 80%, 90% and 100% for 3 min each), defatted in chloroform for 6 min, immersed in 100% ethanol (twice for 3 min each time) and air dried. Rat GLAST, GLT1 and EAAC1 cDNA fragments were obtained by using polymerase chain reaction (PCR), and the PCR products were subcloned into pGEM-T vector (Promega, USA). These constructs contained the following cDNA fragments; 1021–1547 of rat GLAST cDNA, 320–802 of rat GLT1 cDNA, 1163–1573 of rat EAAC1 cDNA (starting codon is 1). Antisense RNA probes were generated by in vitro transcription using SP6 RNA polymerase (GLAST, GLT1), T7 RNA polymerase (EAAC1) and labeled with [α-^{35}S]UTP. As controls for hybridization specificity, sense probes were transcribed from the same plasmids using T7 RNA polymerase (GLAST, GLT1) and SP6 RNA polymerase (EAAC1). The labeled probes ($5–10\times10^6$ cpm/ml) in hybridization buffer (50% deionized formamide, 0.3 M NaCl, 20 mM Tris–HCl, 5 mM EDTA, 10 mM PB, 10% dextran sulfate, 1× Denhardt's solution, 0.2% sarcosyl, 500 μg/ml yeast transfer RNA and 200 μg/ml salmon sperm DNA) were denatured for 2 min at 80°C, quenched on ice and placed on the sections. Hybridization was performed in a humid chamber overnight at 55°C. After hybridization, the slides were soaked in 5× SSC, 1% 2-mercaptoethanol at 55°C, washed in 50% deionized formamide, 2× SSC and 10% 2-mercaptoethanol (high stringency buffer) for 30 min at 65°C. After rinsing the sections in RNase buffer (0.5 M NaCl, 10 mM Tris–HCl, and 1 mM EDTA) three times for 10 min each time, they were treated with 1 μg/ml RNase-A in RNase buffer for 10 min at 37°C and washed in RNase buffer for 10 min. After washing for 30 min at 65°C in high stringency buffer, sections were soaked with 2× SSC and 0.1× SSC for 10 min each at room temperature, dehydrated in an ascending ethanol series, and air dried. The sections were then exposed to X-ray film for 5 days, whereafter they were coated with Kodak NBT-2 emulsion diluted 1:1 with water. Sections were then exposed at 4°C for 3 weeks in a tightly sealed dark box. After being developed in D-19 developer (Kodak), fixed with photographic fixer and washed with tap water, the sections were counterstained with thionin solution to allow morphological identification.

2.2. Immunohistochemistry

Three male Wistar rats weighing about 150 g (6 weeks old) were deeply anesthetized with diethyl ether and perfused with 4% paraformaldehyde in 0.1 M phosphate-buffered saline. After perfusion, olfactory bulbs and cerebellums were removed. After overnight postfixation, they were dehydrated in 30% sucrose in 0.1 M phosphate buffer (PB). Coronal sections (16 μm) were divided into four groups. The first and second groups were incubated with antisera against GLAST and GLT1, respectively, followed by biotinylated goat anti-guinea pig IgG, and finally visualized by avidin–biotin–peroxidase complex method (Vector Lab). The third and fourth groups were processed by indirect immunofluorescence method. Sections in each group were incubated with the mixture of antisera against GLAST and GFAP or GLT1 and GFAP. GLAST- and GLT1-like immunoreactivities were visualized by staining with horse anti-guinea pig IgG conjugated FITC and GFAP-like immunoreactivity was visualized by staining with anti-mouse IgG conjugated Texas Red. Positive immunofluorescence for GLAST and GLT1 was observed under a B2 mirror filter and that for GFAP was observed under a G mirror filter. The detail of the immunohistochemical methods was described in our previous report [19]. Incubation of the sections was carried out at the following dilutions of the antisera with phosphate-buffered saline (pH 7.4): antisera against GLAST (Chemicon), GLT1 (Chemicon) and GFAP (Chemicon) 1:2000; biotinylated goat anti-guinea pig IgG (Vecter Lab) 1:250; horse anti-guinea pig IgG conjugated FITC (Chemicon) 1:250; and horse anti-mouse IgG conjugated Texas Red (Vector) 1:250.

3. Results

3.1. In situ hybridization analysis

To investigate the specificity of cRNA probes for detection of GLAST, GLT1 and EAAC1 mRNAs, in situ hybridization histochemistry on coronal sections from the rat olfactory bulb was performed with the antisense cRNA probe (AS) and the corresponding sense cRNA probe (S) for each mRNA (Fig. 1). No hybridization signal for GLAST, GLT1 and EAAC1 mRNAs was found in the olfactory bulb when the sense cRNA probes were used (Fig. 1D–F), indicating that the antisense cRNA probes can specifically recognize the corresponding transcripts.

To know the general expression patterns of GLAST, GLT1 and EAAC1 mRNAs in the olfactory bulb, we first performed low-magnification dark-field observation (Fig. 1). Although all three mRNAs were abundantly expressed in the bulb, their expression patterns varied a lot. GLAST mRNAs were strongly expressed in all layers of the bulb including the subependymal layer (arrow in Fig. 1A), with the highest intensity in the glomerular layer and external plexiform layer (Fig. 1A). Very strong GLT1 mRNA expression was also seen in the glomerular layer and external plexiform layer (Fig. 1B), in addition its moderate expression was observed in the other layers except the subependymal layer (arrow in Fig. 1B). EAAC1 mRNA was strongly expressed in the mitral cell layer and granular

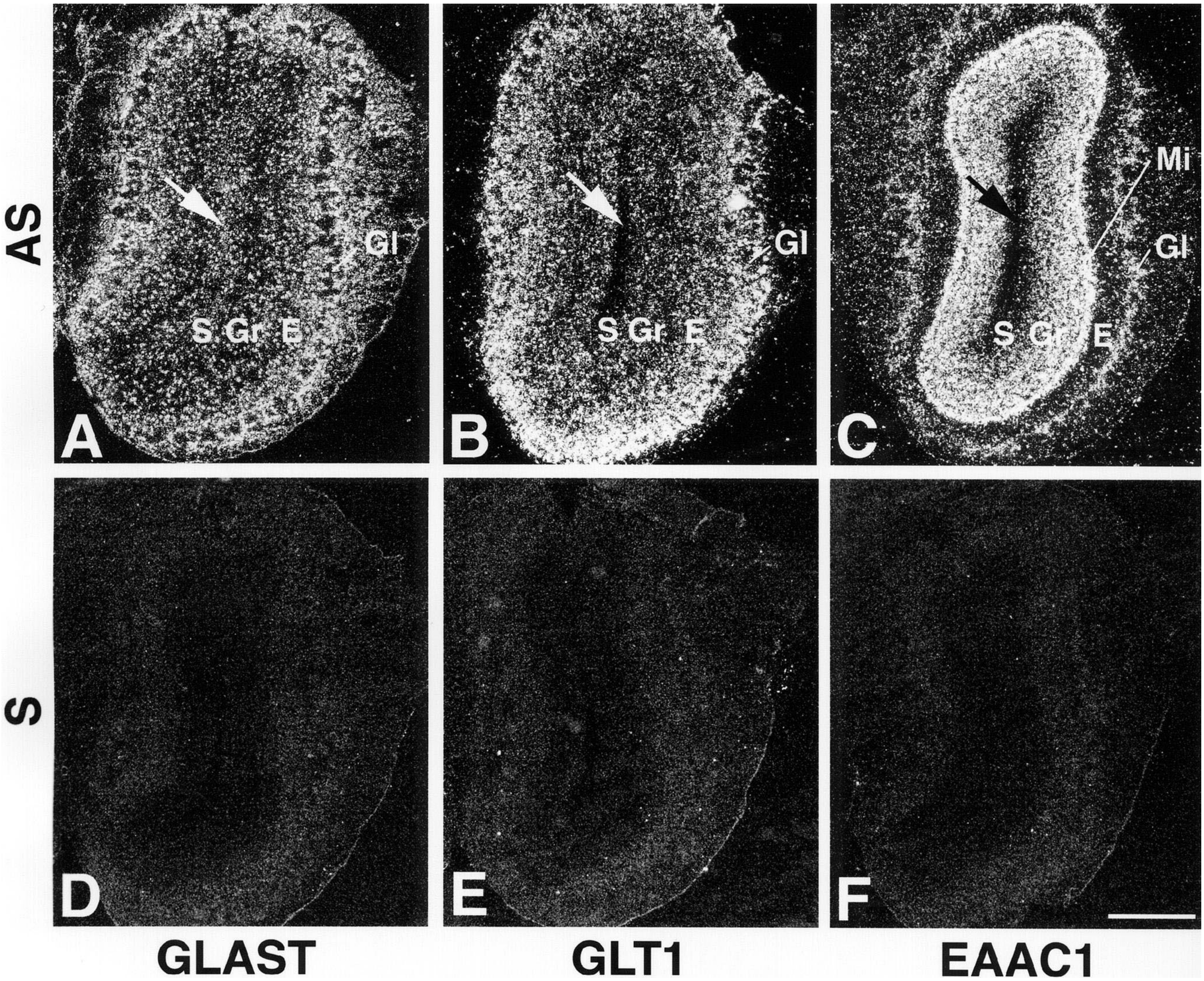

Fig. 1. Dark-field photomicrographs showing laminar distributions of GLAST (A), GLT1 (B) and EAAC1 (C) mRNAs in the olfactory bulb. Note that the expression pattern of EAAC1 mRNA was quite distinct from those of GLAST and GLT1 mRNAs. Note differential expressions of GLAST, GLT1 and EAAC1 mRNAs in the subependymal layer where progenitor cells are compactly distributed (arrows). No hybridization signal for GLAST, GLT1 and EAAC1 was found in the olfactory bulb when single-stranded sense RNA probes were used in the control experiments (D–F). Gl, glomerular layer; E, external plexiform layer; Mi, mitral cell layer; Gr, granule cell layer; S, subependymal layer. Scale bar=500 μm.

cell layer, and its moderate expression was detected in the glomerular layer and external plexiform layer (Fig. 1C). However, the subependymal layer was devoid of EAAC1 mRNA expression (arrow in Fig. 1C).

To examine the cellular localizations, we further investigated their expression patterns by bright-field observation. GLAST and GLT1 mRNAs were observed in glial cells in the olfactory nerve layer (arrows in Fig. 2A and C), while we could not detect EAAC1 mRNA there. EAAC1 mRNA was detected in cells forming the walls of glomeruli, in

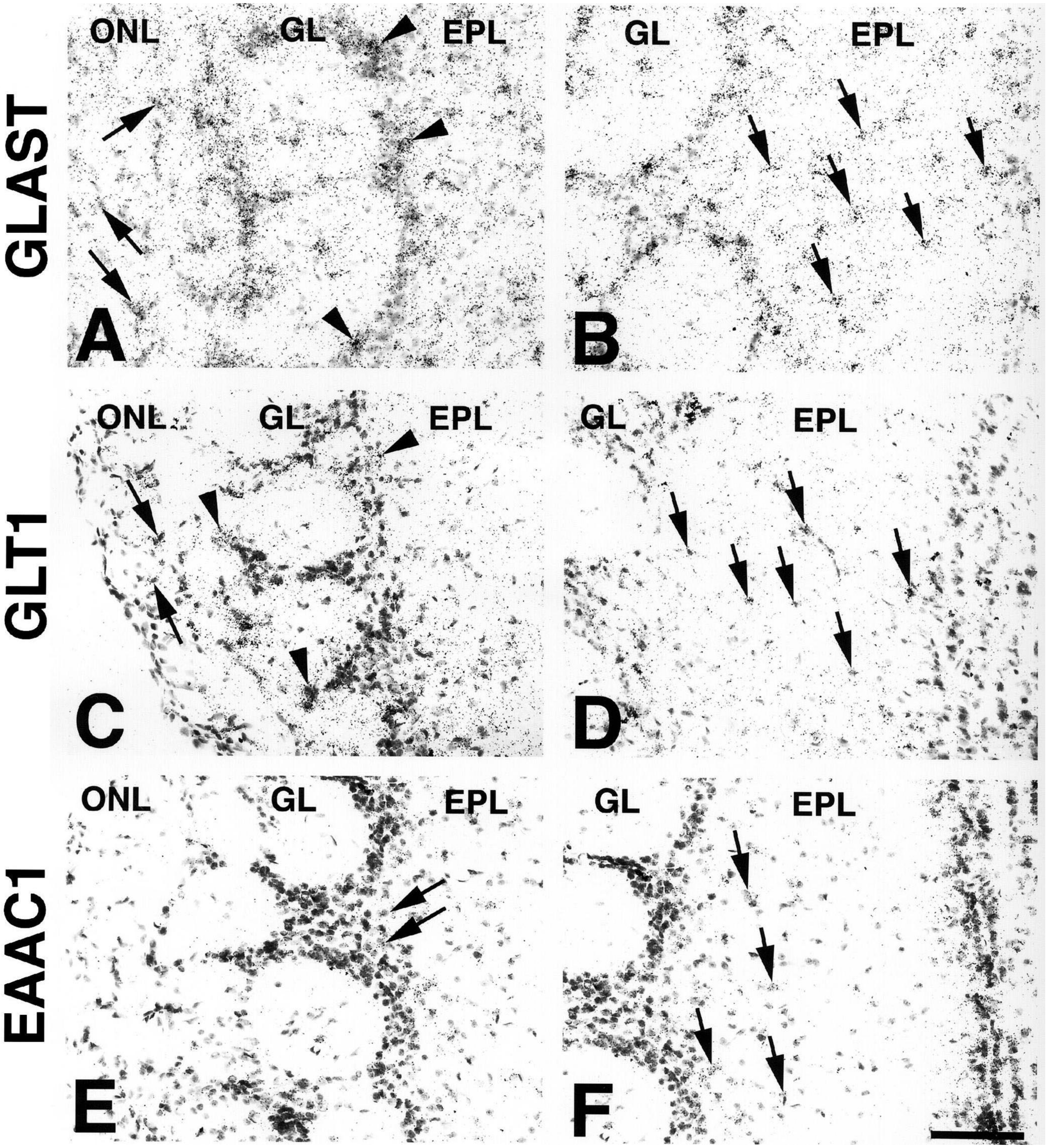

Fig. 2. Expressions of GLAST, GLT1 and EAAC1 mRNAs in the glomerular layer (A, C, E) and external plexiform layer (B, D, F). Arrows and arrowheads in A, B, C and D indicate the accumulation of hybridization signals in glial cells, whereas arrows in E and F indicate the accumulation of hybridization signals in neuronal cells. ONL, olfactory nerve layer; GL, glomerular layer; EPL, external plexiform layer. Scale bar=100 μm.

which appeared periglomerular neurons (arrows in Fig. 2E). In contrast, GLAST and GLT1 mRNA positive cells were scattered around glomeruli, localized nearby periglomerular neurons, indicating glial expression of GLAST and GLT1 mRNAs (arrowheads in Fig. 2A and C).

In the external plexiform layer, GLAST and GLT1 mRNA positive cells were scattered with regular spacing (arrows in Fig. 2B and D). This distinctive feature of the cell distributions was reminiscent of that of GFAP positive cells in the layer [3], suggesting that GLAST and GLT1 mRNAs are expressed in astrocytes. EAAC1 mRNA was mainly detected in relatively large-sized cells in the outer part of the layer (arrows in Fig. 2F), suggesting that tufted cells or van Gehuchten cells express it [13,20].

EAAC1 mRNA was abundantly expressed in mitral neurons and granule neurons (arrowheads and arrows in Fig. 3E). In contrast, GLAST and GLT1 mRNAs were detected in glial cells in both layers (arrowheads and arrows in Fig. 3A and C).

Many cells in the subependymal layer expressed GLAST mRNA (arrows in Fig. 3B), while neither GLT1 mRNA nor EAAC1 mRNA was detected there (Fig. 3D and F).

3.2. Immunohistochemical observation

In situ hybridization analyses clearly showed that EAAC1 mRNA was expressed in neurons, such as periglomerular neurons, tufted cells, mitral cells, and granule cells. However, GLAST and GLT1 mRNA expression patterns were almost similar, and glial expression of these mRNAs was suggested. We, thus, performed immunohistochemical analyses for GLAST and GLT1 to further characterize differences between them.

Although the specificity of the antibodies against GLAST and GLT1 had been confirmed by the company, we further investigated the specificity. No immunoreaction product was seen in the control sections of the olfactory bulb in which the primary antibodies were omitted (Fig. 4C and D). In addition, we performed immunohistochemistry for GLAST and GLT1 in the cerebellum. Strong GLAST-like immunoreactivity (GLAST-IR) was observed in the molecular layer, and moderate GLT1-like immunoreactivity (GLT1-IR) was seen in the cerebellar cortex (Fig. 4E and F), however Purkinje cells were devoid of immunoreaction for both antibodies. These appearances were well coincident with those reported previously [10,28], indicating that the antibodies can specifically recognize the corresponding proteins also under our experimental condition.

GLAST-IR was observed throughout the olfactory bulb, with relatively stronger expression in the olfactory nerve layer, glomerular layer and external plexiform layer (Figs. 4A and 5A). GLT1-IR was also seen in most layers except the subependymal layer (asterisk in Figs. 4B and 5B).

In the olfactory nerve layer, GLAST-IR and GLT1-IR were observed in astrocyte-like cells (arrows in Fig. 5C and D). In the glomerular layer, GLAST-IR was evenly distributed throughout glomeruli (Fig. 5E), while GLT1-IR was observed mainly in the shells of glomeruli (arrows in Fig. 5F). In the external plexiform layer, abundant GLAST-IR and GLT1-IR were observed. GLAST-IR exhibited a homogenous appearance (Fig. 6A), while GLT1-IR showed a dotted and patchy appearance in this layer (Fig. 6B). In the mitral cell layer and granule cell layer, both mitral cells (arrows in Fig. 6C and D) and granule cells (asterisks in Fig. 6C and D) were devoid of GLAST-IR and GLT1-IR, indicating again glial expressions of GLAST and GLT1 in this layer. A striking difference was observed in the subependymal layer, where GLAST-IR was observed abundantly (Fig. 6E), but GLT1-IR was not detected (asterisks in Fig. 6F).

3.3. Localization of GLAST and GLT1 in astrocytes

The above-mentioned results strongly suggest glial expression of GLAST and GLT1. To examine which types of glial cells express them, we carried out double labeling immunohistochemistry using an antiserum against GFAP, a marker for astrocyte, and antiserums for GLAST and GLT1. Fig. 7A and B show GLAST- and GLT1-IR in the olfactory nerve layer visualized by FITC. Fig. 7C and D are the same fields as Fig. 7A and B, respectively, showing the cells with GFAP-IR visualized by TR. Both GLAST-IR positive cells and GLT1-IR positive cells simultaneously expressed GFAP-IR in this layer. In the other layers, we observed that most of GLAST-IR positive cells and GLT1-IR positive cells also expressed GFAP-IR (data not shown). These findings indicated that GLAST and GLT1 were mainly localized in astrocytes in the olfactory bulb.

4. Discussion

Previous reports have indicated that GLAST and GLT1 are mainly localized in glial cells and EAAC1 in neurons [25,26]. The present report clearly showed the same tendency also in the olfactory bulb. In addition, we further provided direct evidence that GLAST and GLT1 were expressed in astrocytes in the olfactory bulb using double labeling immunohistochemistry.

We found EAAC1 mRNA in periglomerular cells, mitral cells and granule cells. All these EAAC1 positive neurons receive glutamatergic innervation [27]. In addition, ultrastructural observation revealed that EAAC1-like immunoreactivity was generally concentrated in neuronal cell bodies and postsynaptic elements, but barely found in presynaptic nerve terminals [18]. Thus, EAAC1 is possibly involved in postsynaptic uptake of released glutamate in these bulbar neurons.

Recently, Isaacson demonstrated simultaneous activation of neighboring mitral cells by the spread of released

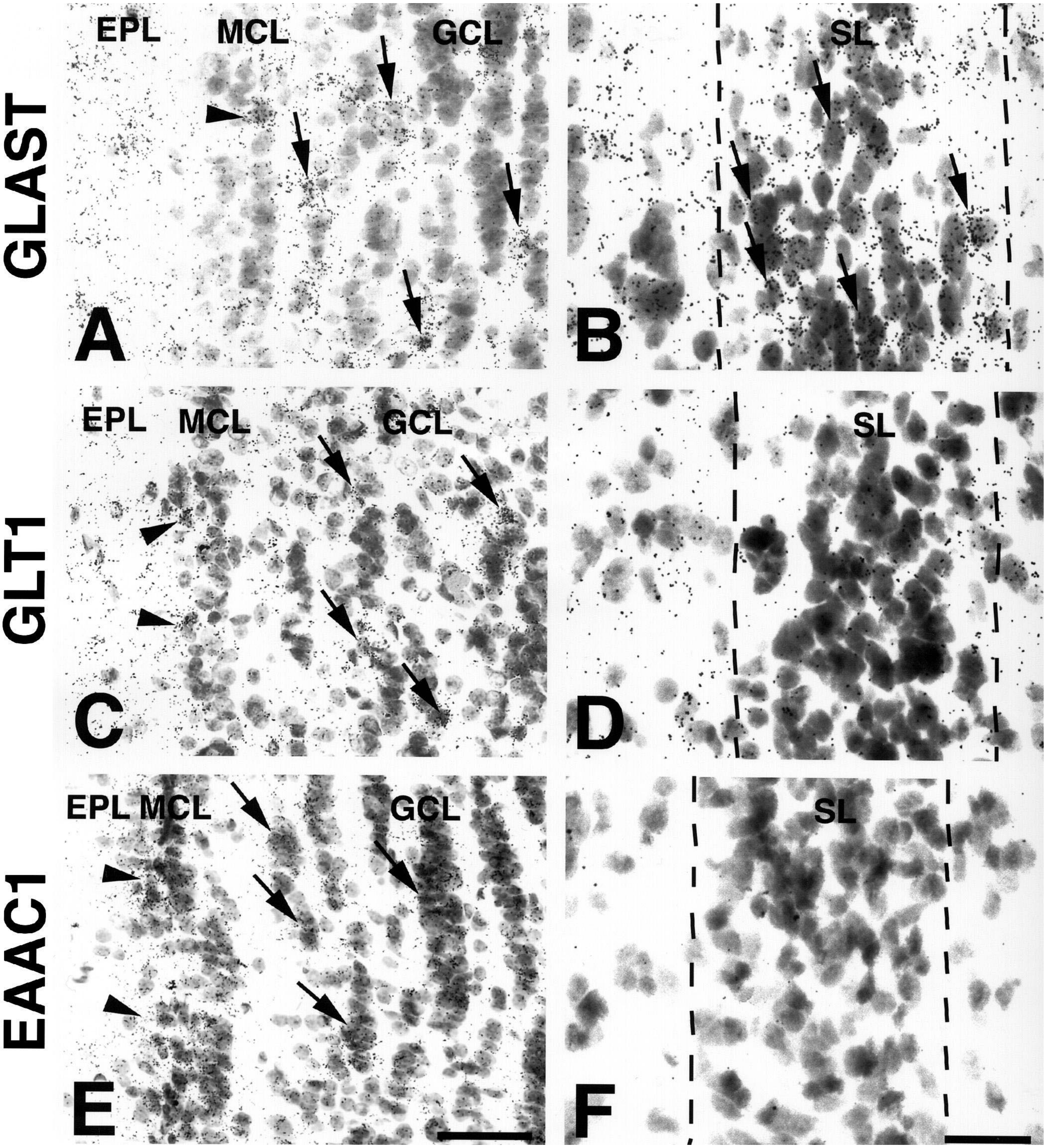

Fig. 3. Expressions of GLAST, GLT1 and EAAC1 mRNAs in the mitral cell layer, granule cell layer (A, C, E) and subependymal layer (B, D, F). Arrows and arrowheads in A and C indicate the accumulation of hybridization signals in glial cells. In contrast, arrows and arrowheads in E indicate the accumulation of hybridization signals in neuronal cells. Note that many cells in the subependymal layer expressed GLAST mRNA (arrows in B), while neither GLT1 mRNA nor EAAC1 mRNA was detected (D, F). EPL, external plexiform layer; MCL, mitral cell layer; GCL, granule cell layer; SL, subependymal layer. Scale bar=50 μm (A, C, E) and 30 μm (B, D, F).

glutamate [7]. This neurotransmission was enhanced by blockade of glutamate uptake, suggesting that such paracrine-like effects of glutamate are regulated by glutamate transporters. Interestingly, we found intense GLAST- and GLT1-IR in the neuropils of the glomerular layer and external plexiform layer, where mitral cells make gluta-

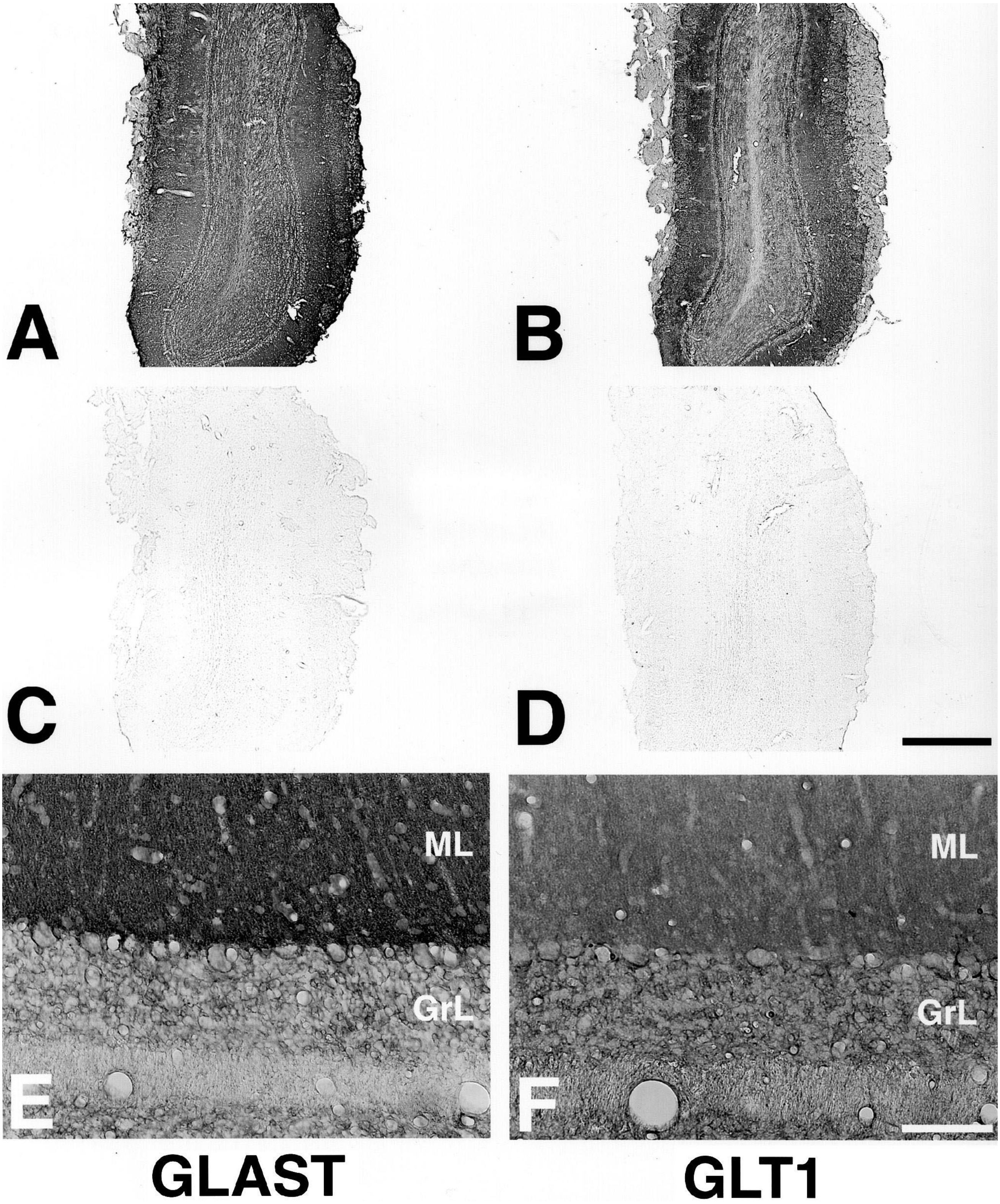

Fig. 4. Control studies for evaluating the specificity of antisera against GLAST and GLT1. GLAST- and GLT1-IR were observed throughout the olfactory bulb (A, B), however no immunoreactive product was obtained when the primary antibodies were omitted (C, D). We found GLAST- and GLT1-IR also in the cerebellar cortex (E, F). ML, molecular layer; GrL, granular layer. Scale bar=600 μm (A–D) and 60 μm (E, F).

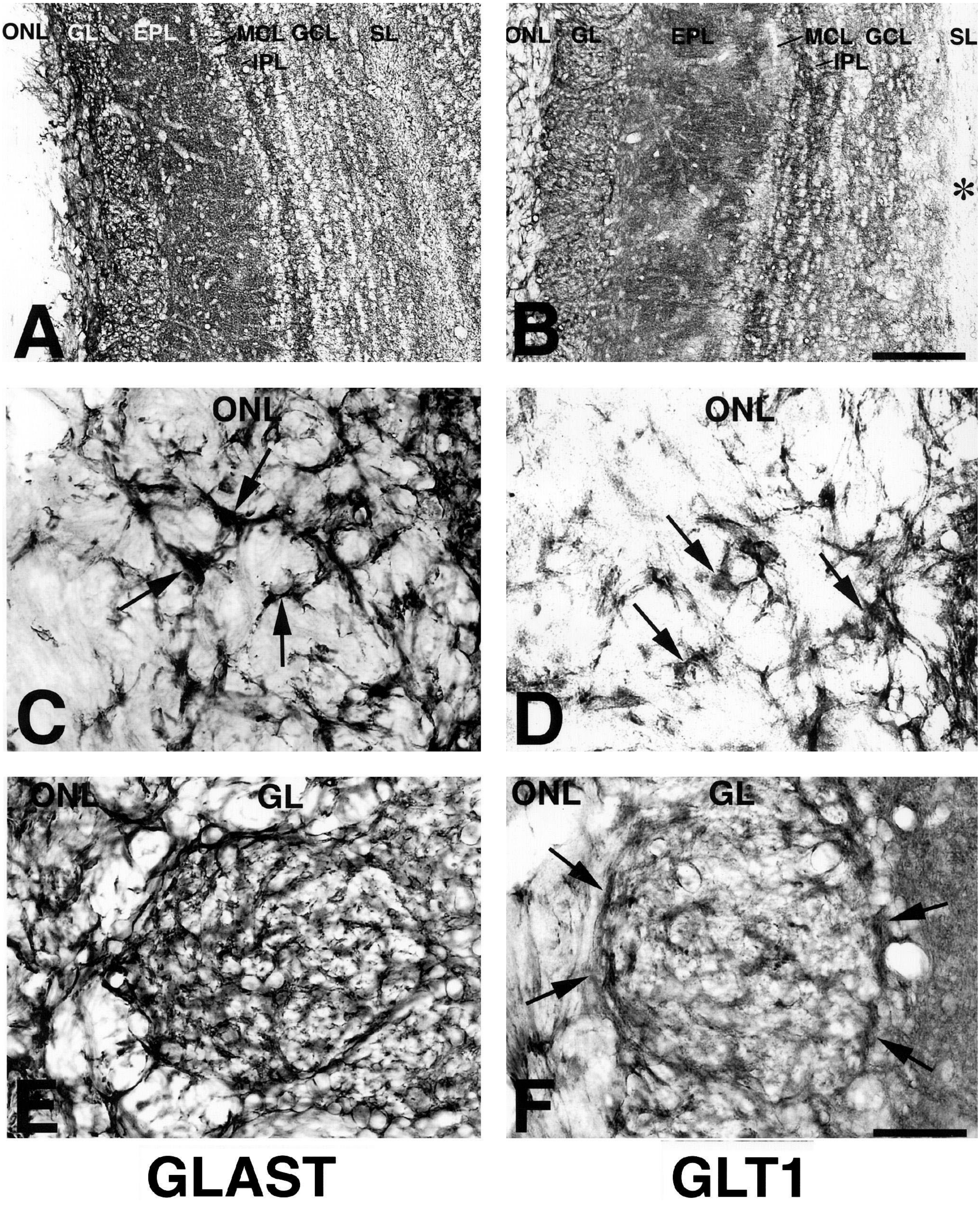

Fig. 5. Photomicrographs showing localization of GLAST- and GLT1-IR in the rat olfactory bulb (A, B), olfactory nerve layer (C, D) and glomerular layer (E, F). An asterisk in B indicates that subependymal layer was devoid of GLT1-IR. In the olfactory nerve layer, GLAST-IR and GLT1-IR were observed in astrocyte-like cells (arrows in C and D). Note that GLAST-IR was evenly distributed throughout glomeruli (E), while GLT1-IR was observed mainly in the shells of glomeruli (arrows in F). ONL, olfactory nerve layer; GL, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; IPL, internal plexiform layer; GCL, granule cell layer; SL, subependymal layer. Scale bar=200 μm (A, B) and 50 μm (C–F).

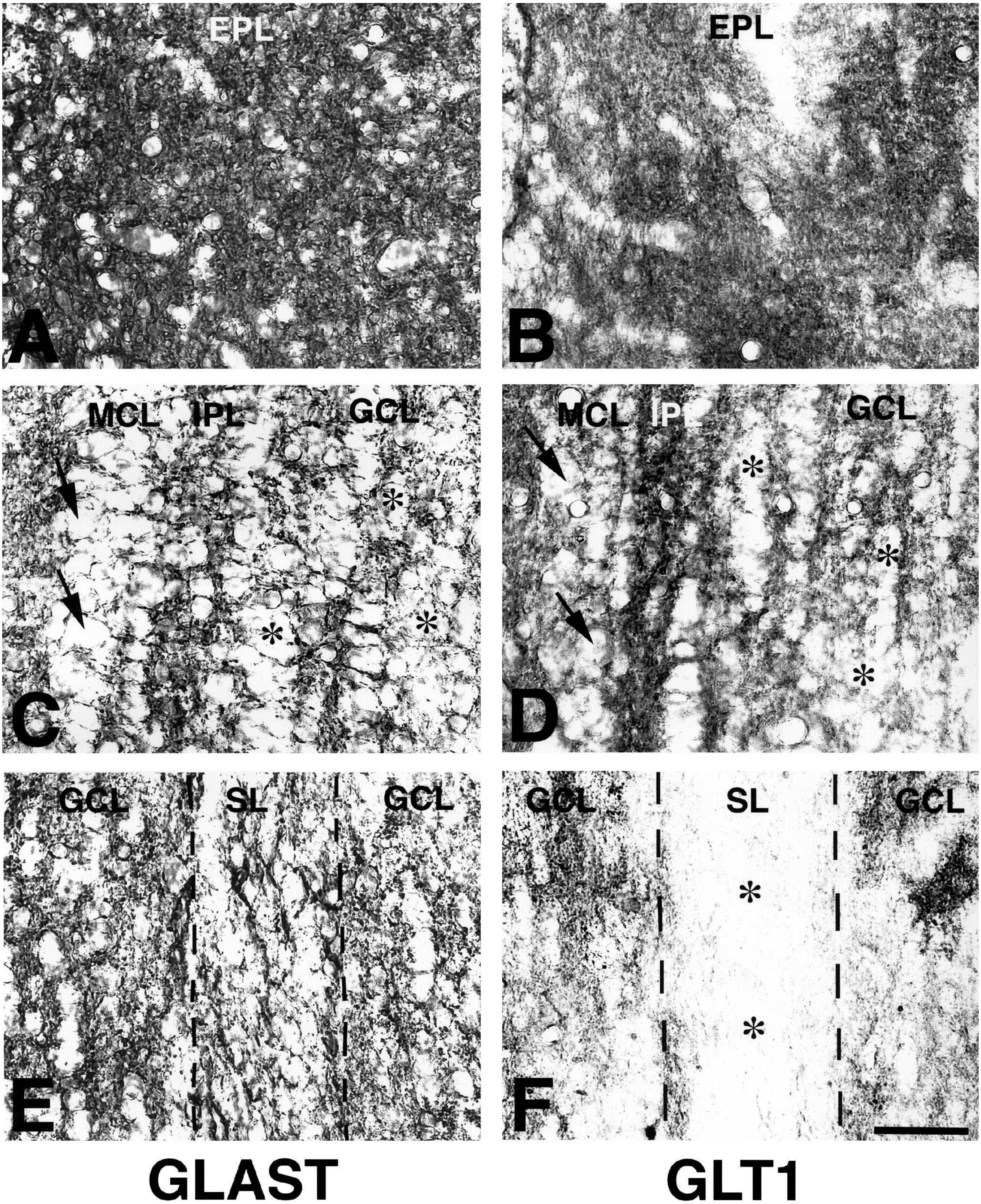

Fig. 6. Photomicrographs showing localization of GLAST- and GLT1-IR in the external plexiform layer (A, B), mitral cell layer, internal plexiform layer, granule cell layer (C, D) and subependymal layer (E, F). Arrows in C and D indicate that mitral cells were devoid of GLAST-IR and GLT1-IR. Asterisks in C and D indicate that granule cells were devoid of GLAST-IR and GLT1-IR. Note that GLAST-IR was abundantly observed in the subependymal layer (E), whereas GLT1-IR was not detected (asterisks in F). EPL, external plexiform layer; MCL, mitral cell layer; IPL, internal plexiform layer; GCL, granule cell layer; SL, subependymal layer. Scale bar $=50$ μm.

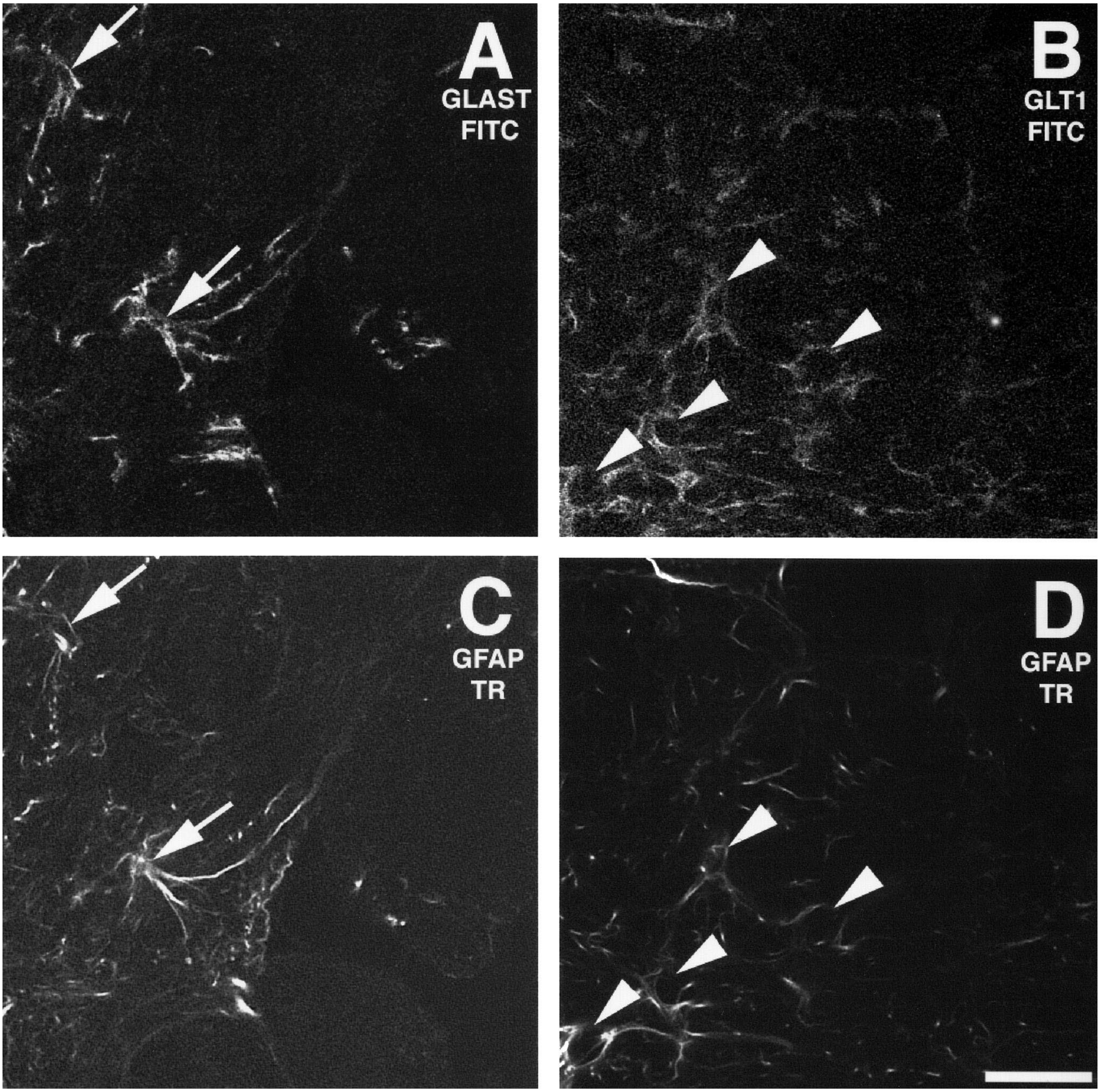

Fig. 7. Astroglial localization of GLAST and GLT1 in the olfactory bulb. Sections were double labeled with GLAST and GFAP antisera (A, C) or GLT1 and GFAP antisera (B, D). Arrows and arrowheads indicate some examples of double labeled soma and fibers. Scale bar=20 μm.

matergic dendrodendritic synapses with periglomerular cells or granule cells. Thus, it is likely that a functional unit of each mitral cell might be bordered by astrocytes expressing GLAST and GLT1, which regulate spilled glutamate from the nearby units. In addition, olfactory nerve axons also make glutamatergic synapses with mitral cell dendrites within glomeruli. It was shown that these axodendritic synapses and the dendrodendritic synapses between mitral cells and periglomerular cells are segregated into compartments by glial processes within a glomerulus [9]. It is, thus, noteworthy that we found differential expression patterns of GLAST- and GLT1-IR in the glomerular layer; GLAST-IR was intensely observed in the cores of glomeruli, in contrast, GLT1-IR was mainly seen in the sheath of each glomerulus. These findings suggest that GLAST is mainly localized in the astrocytes wrapping intraglomerular compartments to avoid the mingling of glutamate released from different origins. On the other hand, GLT1 expressed in the sheath of each glomerulus may have a role to isolate the glomerulus from spilled glutamate.

GLAST and GLT1 are also considered to be involved in the metabolism of glutamate [21]. Released glutamate is taken up by the transporters and converted to glutamine by glutamine synthetase in astrocytes. This glutamine is then released and taken up by presynaptic terminals and reconverted to glutamate [21]. As glutamine synthetase immunoreactivity is observed in neuropils of all layers in the olfactory bulb like GLAST- and GLT1-IR [14], we speculate that astrocytes in the olfactory bulb express glutamine synthetase and glutamate transporters simultaneously and play roles in the glutamate–glutamine recycling mechanism.

Although GLAST and GLT1 were widely expressed with a similar pattern, a significant difference was noted in the subependymal layer. GLAST mRNA and protein were abundantly expressed in the subependymal layer, but GLT1 mRNA and protein were not detected there. In the SL, most spindle-shaped cells with strong thionin staining exhibited GLAST. The anterior part of the subventricular zone constitutes a source of progenitor cells which migrate into the olfactory bulb [1,12]. Therefore, we speculate that GLAST is expressed in the migratory cells originating from the subventricular zone. As the proliferation and differentiation of the migratory cells are known to be regulated by glutamate [6], the GLAST expressed in the subependymal layer might be indispensable for keeping an adequate local glutamate level.

References

[1] J. Altman, Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb, J. Comp. Neurol. 137 (1966) 433–458.

[2] J.L. Arriza, S. Eliasof, M.P. Kavanaugh, S.G. Amara, Excitatory amino acid transporter 5, retinal glutamate transporter coupled to a chloride conductance, Proc. Natl. Acad. Sci. USA 94 (1997) 4155–4160.

[3] M.S. Bailey, M.T. Shipley, Astrocyte subtypes in the rat olfactory bulb: morphological heterogeneity and differential laminar distribution, J. Comp. Neurol. 328 (1993) 501–526.

[4] N.C. Danbolt, F.A. Chaudhry, Y. Dehnes, K.P. Lehre, L.M. Levy, K. Ullensvang, J. Storm-Mathisen, Properties and localization of glutamate transporters, Prog. Brain Res. 116 (1998) 23–43.

[5] W.A. Fairman, R.J. Vandenberg, J.L. Arriza, M.P. Kavanaugh, S.G. Amara, An excitatory amino-acid transporter with properties of a ligand-gated chloride channel, Nature 375 (1995) 599–603.

[6] V. Gallo, J.M. Zhou, C.J. McBain, P. Wright, P.L. Knutson, R.C. Armstrong, Oligodendrocyte progenitor cell proliferation and lineage progression are regulated by glutamate receptor-mediated K^+ channel block, J. Neurosci. 16 (1996) 2659–2670.

[7] J.S. Isaacson, Glutamate spillover mediated excitatory transmission in the rat olfactory bulb, Neuron 23 (1999) 377–384.

[8] Y. Kanai, M.A. Hediger, Primary structure and functional characterization of a high-affinity glutamate transporter, Nature 360 (1992) 467–471.

[9] H.J. Kasowski, H. Kim, C.A. Greer, Compartmental organization of the olfactory bulb glomerulus, J. Comp. Neurol. 407 (1999) 261–274.

[10] K.P. Lehre, L.M. Levy, O.P. Ottersen, J. Storm-Mathisen, N.C. Danbolt, Differential expression of two glial glutamate transporters in the rat brain: quantitative and immunocytochemical observations, J. Neurosci. 15 (1995) 1835–1853.

[11] C.J. Liu, P. Grandes, C. Matute, M. Cuenod, P. Streit, Glutamate-like immunoreactivity revealed in rat olfactory bulb, hippocampus and cerebellum by monoclonal antibody and sensitive staining method, Histochemistry 19 (1989) 267–273.

[12] M.B. Luskin, Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone, Neuron 11 (1993) 173–189.

[13] F. Macrides, S.P. Schneider, Laminar organization of mitral and tufted cells in the main olfactory bulb of the adult hamster, J. Comp. Neurol. 208 (1982) 419–430.

[14] C.O. Okere, H. Kaba, Region-specific localization of glutamine synthetase immunoreactivity in the mouse olfactory bulb: implications for neuron–glia interaction in bulbar synaptic plasticity, Brain Res. 857 (1–2) (2000) 308–312.

[15] G. Pines, N.C. Danbolt, M. Bjørås, Y. Zhang, A. Bendahan, L. Eide, H. Koepsell, J. Storm-Mathisen, E. Seeberg, B.I. Kanner, Cloning and expression of a rat brain L-glutamate transporter, Nature 360 (1992) 464–467.

[16] M.B. Robinson, L.A. Dowd, Heterogeneity and functional properties of subtypes of sodium-dependent glutamate transporters in the mammalian central nervous system, Adv. Pharmacol. 37 (1997) 69–115.

[17] D.J. Rossi, N.T. Slater, The developmental onset of NMDA receptor-channel activity during neuronal migration, Neuropharmacology 32 (1993) 1239–1248.

[18] J.D. Rothstein, L. Martin, A.I. Levey, M. Dykeshoberg, L. Jin, D. Wu, N. Nash, R.W. Kuncl, Localization of neuronal and glial glutamate transporters, Neuron 13 (1994) 713–725.

[19] K. Sato, K. Yoshida, K. Fujiwar, K. Tada, M. Tohyama, Glycine cleavage system in astrocyte, Brain Res. 567 (1991) 64–70.

[20] S.P. Schneider, F. Macrides, Laminar distributions of interneurons in the main olfactory bulb of the adult hamster, Brain Res. Bull. 3 (1978) 73–82.

[21] A. Schousboe, Transport and metabolism of glutamate and GABA in neurons are glial cells, Int. Rev. Neurobiol. 22 (1981) 1–45.

[22] T. Storck, S. Schultz, K. Hofmann, W. Stoffel, Structure, expression and functional analysis of a Na^+-dependent glutamate/aspartate transporter from rat brain, Proc. Natl. Acad. Sci. USA 89 (1992) 10955–10959.

[23] M.L. Sutherland, T.A. Delaney, J.L. Noebels, Glutamate transporter mRNA expression in proliferative zones of the developing and adult murine CNS, J. Neurosci. 16 (1996) 2191–2207.

[24] K. Tanaka, Expression cloning of a rat glutamate transporter, Neurosci. Res. 16 (1993) 149–153.

[25] R. Torp, N.C. Danbolt, E. Babaie, M. Bjørås, E. Seeberg, J. Storm-Mathisen, O.P. Ottersen, Differential expression of two glial glutamate transporters in the rat brain: an in situ hybridization study, Eur. J. Neurosci. 6 (1994) 936–942.

[26] R. Torp, F. Hoover, N.C. Danbolt, J. Storm-Mathisen, O.P. Ottersen, Differential distribution of the glutamate transporters GLT1 and rEAAC1 in rat cerebral cortex and thalamus: an in situ hybridization analysis, Anat. Embryol. 195 (1997) 317–326.

[27] P.Q. Trombley, G.M. Shepherd, Synaptic transmission and modulation in the olfactory bulb, Curr. Opin. Neurobiol. 3 (1993) 540–547.

[28] K. Ullensvang, K.P. Lehre, J. Storm-Mathisen, N.C. Danbolt, Differential developmental expression of the two rat brain glutamate transporter proteins GLAST and GLT, Eur. J. Neurosci. 9 (1997) 1646–1655.