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Differential expression of AMPA-type glutamate receptor subunits during development of the chick optic tectum

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Abstract

Glutamate receptors have been often associated with developmental processes. We used immunohistochemical techniques to evaluate the expression of the AMPA-type glutamate receptor (GluR) subunits in the chick optic tectum (TeO). Chick embryos from the 5th through the 20th embryonic day (E5-E20) and one-day-old (P1) chicks were used. The three types of immunoreactivity evaluated (GluR1, GluR2/3, and GluR4) had different temporal and spatial expression patterns in the several layers of the TeO. The GluR1 subunit first appeared as moderate staining on E7 and then increased on E9. The mature GluR1 pattern included intense staining only in layer 5 of the TeO. The GluR2/3 subunits presented low expression on E5, which became intense on E7. The staining for GluR2/3 changed to very intense on E14 in tectal layer 13. Staining of layer 13 neurons is the most prominent feature of GluR immunoreactivity in the adult TeO. The GluR4 subunit generally presented the lowest expression starting on E7, which was similar to the adult pattern. Some instances of transient expression of GluR subunits were observed in specific cell populations from E9 through E20. These results demonstrate a differential expression of the GluR subunits in the embryonic TeO, adding information about their possible functions in the developmental processes of the visual system.

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Glutamate receptors (GluRs) are present in most neurons of the vertebrate brain. They appear to be involved in diverse brain functions, such as learning, memory, synaptic plasticity, and developmental processes (1,2). The ionotropic branch of the GluRs includes three groups of receptors classified according to their agonist selectivity: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainate and N-methyl-D-aspartate (NMDA). The functional characteristics of these GluRs result from the contribution of the four subunits that constitute the functional receptor (3). For instance, AMPA receptors that present at least one GluR2 subunit show low permeability to Ca^{2+}, while the AMPA receptors formed by the other subunits allow a significant Ca^{2+} influx (4). This finding may have important implications during development, when Ca^{2+} probably plays a major role in the control of cell migration, differentiation, neuritogenesis, and

cell death (5,6). A model that could be especially useful to study these questions is the chick optic tectum (TeO). The avian TeO, in addition to expressing several types of glutamate receptors (7,8), exhibits a clear lamination pattern and a well-characterized pattern of cell proliferation, migration, and differentiation (9). In the present study we determined the immunoreactivity of the AMPA-type GluR subunits during development of the chick TeO.

Seventy-one chick embryos (*Gallus gallus*) were used ranging in age from embryonic day 5 (E5) through the first post-eclosion day (P1). The embryos older than E12 were deeply anesthetized with ketamine and xylazine and perfused through the heart with phosphate-buffered saline and 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). Younger embryos were quickly decapitated. All brains were removed and kept in fixative for 12 h and then transferred to a 30% sucrose solution in PB for cryoprotection. Coronal sections (14-16 µm) of the frozen brains were cut on a cryostat and the sections including the TeO were selected for immunohistochemistry. Commercial antibodies (Chemicon, Temecula, CA, USA) against the GluR1, GluR2/3, and GluR4 AMPA receptor subunits were diluted 1:500 in PB containing 0.3% Triton X-100. Sections were incubated with the primary antibodies for 14-18 h at room temperature (ca. 22°C), and then with a biotinylated goat anti-rabbit antiserum (Vector Labs., Burlingame, CA, USA) for 60 min at room temperature. The sections were finally incubated for 1 h at room temperature with the avidin-biotin complex (ABC Elite, Vector Labs.) for 90 min. The sections were thoroughly washed with PB between steps. The immunoreaction was visualized using 0.05% diaminobenzidine and 0.01% hydrogen peroxide in PB. The intensity of staining for the GluR subunits was subjectively estimated. Nevertheless, a five-value scale was established to obtain a semiquantitative evaluation of immunoreactivity for each antibody. No attempt was made to compare the intensity of staining between the subunits tested because of probable differences in the properties of the antibodies. For embryonic stages E5 through E18 we used a specific embryonic chick nomenclature to identify tectal layers (10). The identification of these layers after E18 was performed according to the stereotaxic atlas of the chick brain (11) using Cajal's nomenclature (12). Controls for immunostaining included the omission of the primary antibodies or their replacement with normal rabbit serum. The staining was completely eliminated in both situations. It should be added that these antibodies have been extensively characterized (7,8).

Differential patterns of expression were observed for the GluR subunits during development of the chick TeO. These data are described below and are summarized in Figures 1 and 2.

The GluR1 subunit started to be detected on E7. The immunoreactivity for this subunit first appeared in a single layer and was rated as moderate. This layer appeared to correspond to the outermost part of the first tectal transient cellular compartment (10). On E8, this stained layer apparently divided, with the outermost portion still presenting labeling for GluR1. The new innermost layer, which in the adult animal corresponds to Cajal's layer 13, was not labeled on E8 or thereafter. On E9, there was an apparent increased labeling in the outer layer (second cellular transient compartment), just before this layer further divided in two, originating the presumptive superficial and intermediate tectal layers. Staining for GluR1 was present in both of the new sublayers, with different levels of intensity. In general, staining in the superficial layers was more intense than in the intermediate layers. The most distinctive feature of GluR1 staining in the chick tectum was clearly the labeling in layer 5.

The GluR2/3 subunits were the first to

appear during TeO embryonic development, starting on E5. On this day, the staining for GluR2/3 was weak and restricted to only one layer, i.e., the germinative zone. On E7, however, immunoreactivity was already intense in most of that layer, which corresponds at this stage to the first transient cellular compartment. Moderate staining for GluR2/3 also appeared in an external part of this transient compartment, which appears to be the same area that expresses GluR1 at this stage. After a subdivision on E8, both branches exhibited staining for GluR2/3. However, the innermost branch presented in general more intense staining from E8 through P1. The outermost portion also presented staining for GluR2/3 which, despite some variations, only attained a slight level in our subjective scale. The neurons in layer 13 were by far those most intensely stained for any of the antibodies used in this study.

The GluR4 subunit began to be expressed on E7, with a weak intensity of labeling that was almost constant throughout development. Only one change was noted, which occurred between E11 and E16. During this period, staining for GluR4 appeared to increase transiently between E11 and E16 in a cellular compartment that generates some of the superficial tectal layers. Staining was only slight on P1, and the most distinctive feature of GluR4 was its presence in some large cells of layer 13, which did not label with any other antibody used here.

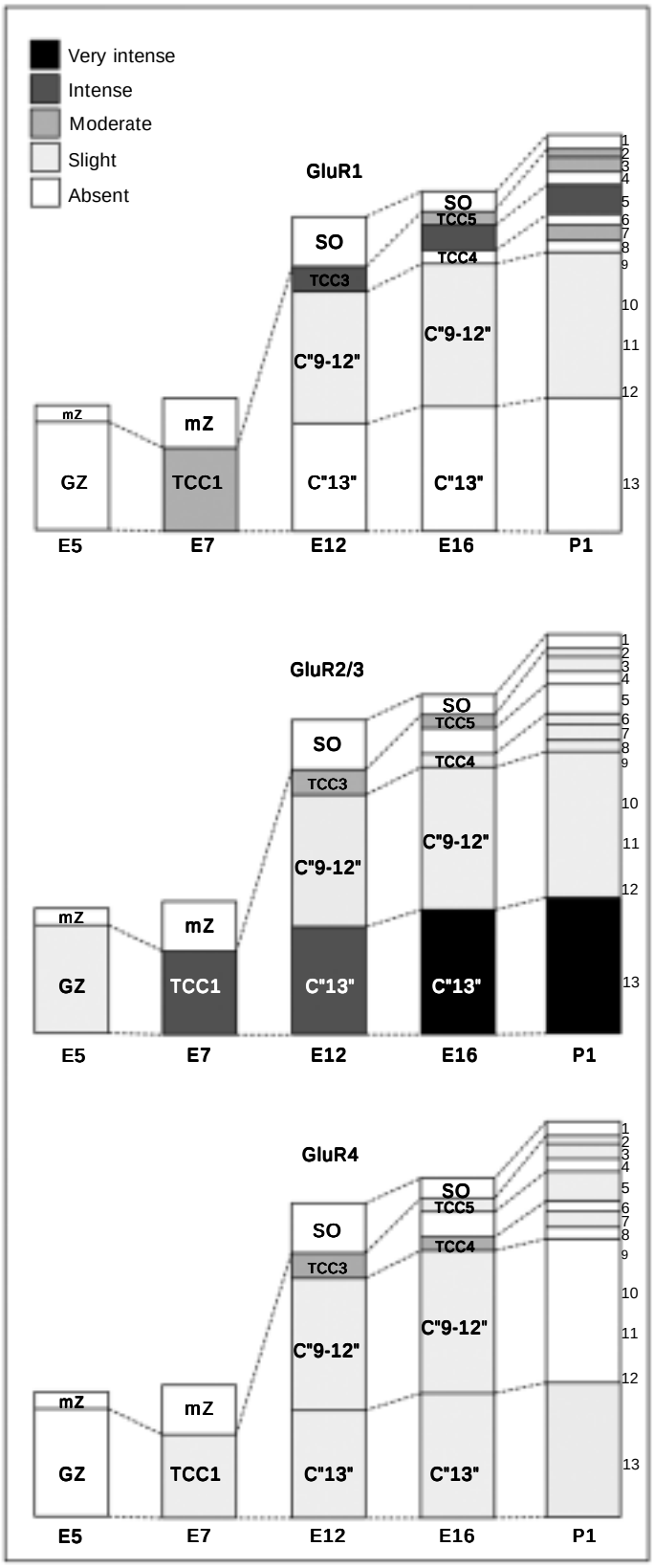

Figure 1. The temporal evolution of immunoreactivity for the glutamate receptor subunits GluR1, GluR2/3, and GluR4 during tectal development in the chick. The subjective scale for the semiquantitative analysis is shown at the top left. Dashed lines indicate the spatial changes of the different cellular compartments. GZ, germinative zone; mZ, marginal zone; TCC, transient cellular compartment; SO, stratum opticum; C"9-12", compartment that generates the adult layers 9-12; C"13", compartment that generates the adult tectal layer 13. The numbers on the right of each diagram indicate the tectal layers according to Cajal's nomenclature (12). The nomenclature used here was modified from Scicolone et al. (10).

The results of this study indicate that there are different spatial and temporal patterns of expression of AMPA GluR1, GluR2/3, and GluR4 subunits in the chick TeO. Unfortunately, we have not been able to obtain independent, specific labeling for the GluR2 and GluR3 subunits. However, there is evidence that the mRNA coding for GluR3 is expressed at low levels in the vertebrate brain (7,8). Therefore, we consider that most of the staining that we have obtained in the TeO with the antibody against the GluR2/3 subunits might have been due to the presence of large amounts of the GluR2 subunit. Accordingly, the discussion that follows mainly involves the GluR2 subunit when referring to the GluR2/3 data.

Possible associations between the differential expression of various GluR subunits and developmental processes of the TeO may exist. For example, the early expression of all subunits tested here suggests some function in neurogenesis and migration since these processes occur at least until E8 in the

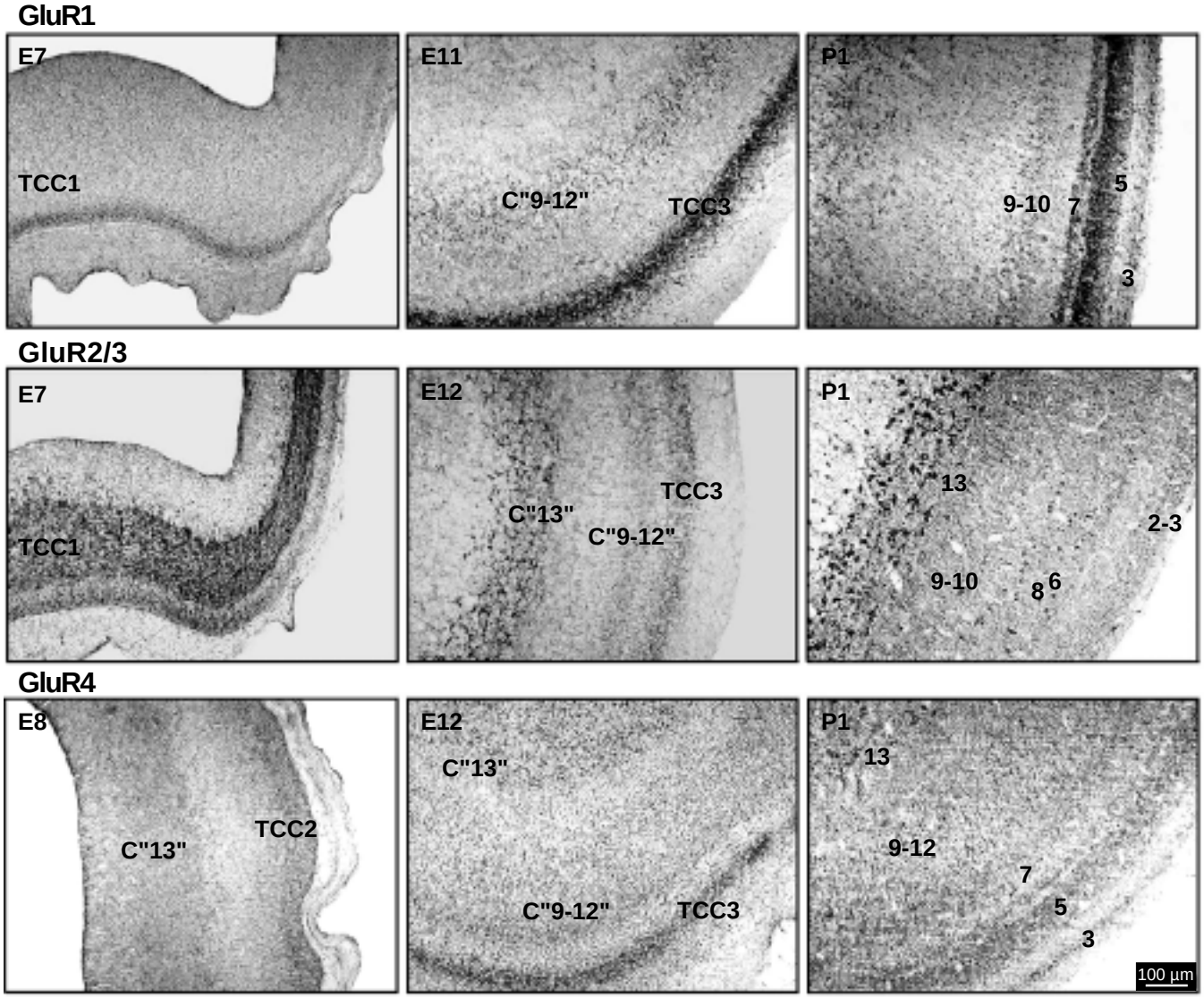

Figure 2. Digital images of the embryonic chick optic tectum showing some temporal changes of immunoreactivity for the glutamate receptor subunits GluR1, GluR2/3, and GluR4. Note the transiently increased expression of GluR4 around E12 and the more marked expression of GluR2/3 in early stages. Abbreviations as in Figure 1.

chick TeO. The especially precocious appearance of GluR2 subunits coincides approximately with the peak of mitotic activity (13). Another important aspect of the present findings is related to the retinal innervation of the TeO. Fibers from the contralateral retina start to innervate the chick TeO around E10 and these afferents, which represent the most prominent TeO innervation, continue to develop at least until E18 (9). Most of the changes in GluR expression were observed during this period, indicating a relationship between GluR expression and retinal afferentation. This is also known as a period of intense synaptogenesis in the chick tectum (13). A typical example of the correlation of GluR temporal expression and synaptogenesis appears to be the intense staining for GluR2 in the presumptive layer 13 of the TeO. A first increase of GluR2 expression in layer 13 neurons occurred on E10, followed by a marked peak on E14. Despite the fact that our data do not necessarily imply the occurrence of functional GluRs during development, it is noteworthy that calcium accumulation mediated by glutamate also peaks around E10 in the retina, decreasing as synaptogenesis progresses (14). A relation between the development of AMPA receptors and synaptogenesis has already been suggested for the development of the mouse cerebral cortex (15).

One of the most important aspects of the involvement of glutamate receptors in developmental functions is related to Ca^{2+} influx. Indeed, there is direct evidence that Ca^{2+} flow through AMPA-type GluRs regulates neurite outgrowth in developing retinal (16) and hippocampal (17) neurons. Since glutamate appears to be available early during development, activation of GluRs may have a strong impact on intracellular calcium and could therefore regulate a number of functions even before synaptogenesis (16). It is noteworthy that all subunits studied here may form GluRs with high calcium permeability, except for the edited form of GluR2 (18,19). The present data do not permit evaluation of the presence of the edited and non-edited GluR2 isoforms, and there is the possibility that the edited form of GluR2 may start to be expressed at some point during development, when the developmental functions of Ca^{2+} may compete with the possible deleterious effects of high intracellular Ca^{2+}. This possibility has yet to be analyzed by molecular biological/electrophysiological techniques.

The chick TeO appears to represent a suitable model to directly evaluate the role of glutamate in developmental processes. The AMPA receptors exhibit temporal expression changes during tectal development that are compatible with such a role for glutamate, which appears to also involve the NMDA receptors (1,20).

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