# Expression of Kynurenine Aminotransferase in the Subplate of the Rat and Its Possible Role in the Regulation of Programmed Cell Death

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**The neurons of the transient subplate zone, considered important for the prenatal development of the cerebral cortex, were shown here to express kynurenine aminotransferase (KAT)-I from embryonic day (E) 16 until postnatal day (P) 7 in the rat. No other cells of brain tissue exerted KAT-I immunoreactivity during this period. From P3 on, the neurons of the subplate gave rise to KAT-I immunoreactive, varicose axons, which entered the thalamus and terminated around thalamic nerve cells that are devoid of KAT-I immunoreactivity. Other subplate markers displayed a different expression pattern during development. Thus, subplate neurons displayed parvalbumin (PV) immunoreactivity from E16 to P10 and an intense NPY immunoreaction from P7 to P1. They also exhibited nitric oxide synthase immunoreactivity from E16 to P10, whereas on the surface of the subplate neurons, the α7 subunit of the nicotinic acetylcholine receptor (nAChR) was present from P1 to P10. The cells of Cajal–Retzius were nAChR-immunoreactive during this period. Between P1 and P7, the perikarya of subplate neurons also showed an intense immunoreaction with the *N*-methyl-D-aspartate (NMDA) receptor subtype R2A. After the first postnatal week, many of the KAT-I positive subplate neurons display a gradual decrease of immunoreactivity and undergo programmed cell death. Since KAT-I persists in the subplate through the period E16–P7, we conclude that KAT-I is a useful and reliable subplate marker in the rat. Since it is assumed that migration of nerve cells is regulated by NMDA receptors, and since kynurenic acid — the only naturally occurring NMDA receptor antagonist — is synthesized by KAT, we suggest that a temporary breakdown of the delicate equilibrium between NMDA and KAT might induce abnormal neuronal migration, giving rise to developmental abnormalities.**

## Introduction

The subplate layer is a transient embryonic zone that plays an important role in the ontogeny and phylogeny of the cerebral cortex (Kostovic and Rakic, 1990). Postmitotic neurons, migrating from the ventricular zone up to the superficial layer of the cerebral wall, divide the early preplate into a superficial superplate and a deep subplate (Sidman and Rakic, 1973; Kostovic and Rakic, 1980). Transient neurons of the subplate are considered to be instrumental in the development of the cortex and in the establisment of corticothalamic and thalamocortical connections (see Discussion for details). While, in most mammalian species, including primates, the majority of subplate neurons are lost in the development of the cortex (Kostovic and Rakic, 1980), the subplate persists into adulthood in rodents (Woo *et al*., 1991). Subplate dysfunction in the developing brain may give rise to diseases such as epilepsy and schizophrenia by disturbing neuronal migration and generating abnormal function of circuits (Rakic, 1988; Akbarian *et al*., 1993, 1996; Bloom, 1993).

As the only known endogenous *N*-methyl-D-aspartate (NMDA) receptor antagonist, kynurenic acid displays a neuroprotective effect in neurodegenerative diseases (Du *et al*., 1992; Nozaki and Beal, 1992; Knyihár-Csillik *et al.,* 1999). Kynurenic acid is known to be produced by the activity of the enzyme kynurenin aminotransferase (KAT). According to the studies of Guidetti *et al.* (Guidetti *et al*., 1997), there are two subtypes of this enzyme — KAT-I and KAT-II — with distinctive properties. KAT-I, identical with glutamine transaminase K, has an optimal pH of 9.5, prefers pyruvate as a cosubstrate and is inhibited by glutamine. KAT-II, identical with L-α-aminoadipate transaminase, has an optimal pH of 7.0, shows no preference for pyruvate and is essentially insensitive to inhibition by glutamine. While the presence of KAT-II mRNA in the brain has been identified with Northern blot analysis (Okuno *et al*., 1991), to date, antibodies for immunohistochemical localization are only available against KAT-I (Knyihár-Csillik *et al*., 1999). Therefore, in the present study we examined KAT-I immunoreactivity in the cells of the subplate and its remnants in rat embryos, newborn rats and rat pups, at the light and electron microscopic level. Since migration of nerve cells has been proposed to be regulated by NMDA receptors (Komuro and Rakic, 1993), we also studied the expression of the NMDA receptor subtype R2A, and its relation to KAT-I expression in the cells of the subplate. At the same time, we compared the existence of KAT-I immunoreactivity to those of several known markers of the subplate, such as parvalbumin (PV), neuropeptide Y (NPY), nitric oxide synthase (NOS) and the α7-subunit of the nicotinic acetylcholine receptor (nAChR).

## Materials and Methods

Investigations were performed on Wistar and Sprague–Dawley rats: 20 rat embryos (embryonic days E14–E21), 9 newborns and 22 rat pups of ages 1–14 days of both sexes. Experiments were carried out in accordance with the European Communities Council Directive (24 November 1986; 86/609/EEC). Rats were anesthetized with ether and subjected to transcardial perfusion, first with 10 ml of 0.1 M PBS (pH 7.4) followed by 200 ml of fixative containing paraformaldehyde, lysine and periodate (PLP) (McLean and Nakane, 1974). Perfusion was carried out at a rate of 10 ml/min. Brains were removed and postfixed in the same fixative for 3 h at 4°C. Vibratome sections, 50 μm thick, were obtained from the parietal cortical area immediately after postfixation. Alternatively, after incubation in PBS containing 15% sucrose for 48 h, the samples were processed in a graded series of sucrose (10%, 20%, 30%) dissolved in PBS, placed in Tissue-Tek embedding medium (Miles, Diagnostic Devision Elkhart, IN) and frozen in a chamber cryostat at –20°C. In this case, freezing was immediately followed by sectioning. Serial, 40 μm thick cryostat sections, were obtained from the paramedian sagittal plane of the whole brain. Following pretreatment with 2% $H_2O_2$, incubation with primary antiserum was performed either at 4°C for 3 days or overnight at room temperature, both on free-floating sections and on sections mounted on gelatine-pretreated slides. Primary antiserum used was an anti-KAT polyclonal antibody raised in rabbit against rat kidney KAT-I (Okuno *et al*., 1990), diluted to 1:1500 in 0.01 M PBS (pH 7.4) containing 1% bovine serum albumin and 5% normal goat serum (NGS). Following a 90 min incubation in biotinylated anti-rabbit secondary antibody, the sections were processed according to the avidin–biotin system (Vectastain ABC Elite, Vector Laboratories, Burlingame, CA).

Essentially the same technique was used for the visualization of PV,

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NOS and NPY immunoreactivity. The PV antibody raised in mouse (Sigma-Aldrich, St Louis, MO) was diluted to 1:20 000; the bNOS antibody raised in mouse (Sigma-Aldrich) was diluted to 1:3000; the NPY antibody raised in rabbit (Sigma-Aldrich) was diluted to 1:2000.

For the detection of the α7-subunit of nAChR, biotinylated α-bungarotoxin (Molecular Probes, Eugene, OR) was used at a dilution of 1:2000.

KAT-I, PV, NOS, NPY and nAChR immunoreactivity was visualized with diaminobenzidine to which hydrogen peroxide had been added (3 μl of 30% $H_2O_2$ to 10 ml of 0.05% diaminobenzidine). Sections were dehydrated in a graded series of ethanol and processed in carbol–xylene. Slides were coverslipped with Permount (Fisher, Fair Lawn, NJ).

The specificity of the immunohistochemical reaction was assessed by means of one of the following treatments: (i) Omission of the first (specific) antiserum. (ii) Use of normal rabbit serum instead of the specific antiserum. (iii) Treatment according to the avidin–biotin complex method, from which one of the steps had been omitted. (iv) Preabsorbtion of the specific antibody with pure rat kidney KAT-I (Okuno *et al.*, 1990) at 4°C for 24 h. None of these specimens showed any immunoreactivity.

#### *Immunocytochemical Localization of the R2A Subunit of the NMDA Receptor*

Fixation, preparation and sectioning of the brain was carried out exactly as previously described, except that postfixation in PLP was performed only for 1 h at 4°C. Microscopic analysis of NMDA-R2A immunolabeling showed that the best NR2A labeling with minimal background could be achieved by fixation with periodate–lysine–paraformaldehyde for 1 h. After sectioning either with a vibrotome or with a cryostat, primary antibody incubation with anti-NR2A serum (RBI Research Biochemicals International, Natick, MA) diluted to 1:200 in Tris-buffered saline (TBS) containing 2% NGS, was carried out overnight at 4°C, followed by 1 h incubation at room temperature. The samples were washed three times for 5 min in TBS (pH 8.2) containing 1% NGS. For immunogold labeling, sections were treated with ~1 nm gold-labeled goat anti-rabbit IgG (H+L) 1 ml gelatine (IGSS) quality (Amersham Life Sciences, UK) diluted to 1:20 in TBS (pH 8.2) containing 0.4% lysine, 0.1% gelatine and 1% NGS, for 90 min at room temperature. After incubation, sections were rinsed 3 × 15 min in TBS (pH 8.2) containing 1% NGS, and 3 × 5 min in distilled water. This was followed by 10–20 min silver intensification. The silver enhancement solution was prepared by mixing equal parts of Enhancer and Initiator of the IntenSE M kit (Amersham Life Sciences). After the silver enhancement process, which was monitored under a microscope with bright-field illumination, specimens were rinsed 3 × 5 min in excess distilled water. Sections were dehydrated in a graded series of ethanol and processed in carbol–xylene. Slides were coverslipped with Permount (Fisher).

#### *Combined KAT-I Immunohistochemistry and Immunogold–Silver Staining Method on the Same Section*

Sections of the subplate were double labeled for KAT-I and NMDA-R2A. Sections were labeled for NMDA-R2A with the immunogold–silver staining method as described above, then they were immunolabeled for KAT-I with the ABC method. Sections were photographed on Leitz Diaplan and Nikon Nomarski microscopes.

#### *Double Staining of KAT-I and Parvalbumin Immunoreactivity*

Cryostat sections (30 μm thick) of the subplate were incubated in the cocktail medium containing KAT-I antiserum (dilution 1:1500) and parvalbumin antiserum (dilution 1:20 000). After 48 h incubation at 4°C KAT-I was visualized with diaminobenzidine (DAB) using the ABC technique and PV was visualized with Nickel–DAB, using the PAP method.

#### *Electron Microscopy*

For electron microscopic immunohistochemistry, KAT-I and NMDA R2A were visualized in Vibratome sections (50 μm), which were treated in the same manner as the light microscopic specimens. After 30 min of osmic acid fixation followed by dehydration, the sections were flat-embedded on liquid release-pretreated slides in Durcupan ACM. Relevant areas were excised with a razor blade under a microscope, remounted to pre-polymerized blocks and sectioned with a diamond knife on a Reichert Ultrotome. Serial sections of silver interference colour were collected on copper slot grids and stained with lead citrate and uranyl acetate. Sections were examined and photographed using a Zeiss Opton 902 electron microscope.

## Results

At the light microscopic level it is evident that neurons and glial cells of the embryonic and early postnatal brain did not stain for KAT-I except for the subplate neurons (Fig. 1*a*,*d*–*f*) which displayed an intense KAT-I immunoreaction. The KAT-I-immunopositive cells of the subplate were initially apolar, and later acquired a unipolar morphology. Based on electron microscopy, KAT-I in subplate neurons was bound to ribosomes of the rough endoplasmatic reticulum (Fig. 1*b*). Immunopositive dendrites of embryonic subplate cells were in synaptic contact with immunonegative axons (Fig. 1*b*). Distribution of the KAT-I-immunopositive neurons in the subplate layer was not homogeneous, especially at the beginning of extrauterine life. In some places they were arranged in columns or tended to form cell groups consisting of two or three cells attached to each other (Fig. 2*a*). Later in development, subplate neurons gave rise to KAT-I-immunopositive varicose axons (Fig. 2*b*). Synapses of KAT-I-immunonegative axons were formed both on the bodies and on the dendrites of subplate cells (Fig. 2*c*). In both the axo-somatic and the axo-dendritic synapses, the presynaptic elements were KAT-I-immunonegative axons that revealed an immature structure. It should be emphasized that the KAT-I immunoreactivity was present only in postsynaptic localizations. Axons of KAT-I-immunopositive subplate cells were never found to establish synapses with any of the KAT-I-immunopositive cells.

From the second postnatal week on, an increasing number of KAT-I-immunopositive subplate cells exhibited signs of cell death. The apoptotic cells were characterized by a shrinkage of the nucleus and an increased density of the cytoplasm (Fig. 4*b*,*c*). However, the subplate itself, i.e. the layer between the sub-ventricular zone and the cortical plate, still persisted.

In the dorsal thalamus, KAT-I-immunopositive axons approaching thalamic cells were varicose; the varicosities of the axons surrounding the cell surfaces of the thalamic cells were loaded with the end-product of the KAT-I immunoreaction. KAT-I-immunopositive axons established pericellular nests around thalamic cells (Fig. 2*d*). Under the electron microscope it could clearly be seen that KAT-I-immunopositive axons establish synapses both with somata and dendrites of thalamic cells (Fig. 2*e*).

The cells of the subplate expressed the R2A subunit of the NMDA receptor (Fig. 3*a*). Using the gold–silver labeling method, the grains representing the receptor were found to be present in the cytoplasm as well as on the surface of subplate cells. It could readily be demonstrated, especially if using the Nomarski optics, that neither the nucleus nor the nucleolus contained any silver grains; they were restricted partly to the cytoplasm and partly to the surface of the cells (Figs. 3*b*,*c*). In between the cells presenting NMDA receptors on their surfaces, non-reactive cells could also be seen.

Double staining with the NMDA-R2A receptor antibody and KAT-I antiserum revealed that NMDA receptors were present both in the cytoplasm (Fig. 4*a*) and in dendritic processes of KAT-I-immunopositive cells.

The presence of KAT-I immunoreactivity characterized the subplate during the embryonic period of the developing rat cortex from E16 to E21 and during the postnatal (P) period P1–P7. PV immunoreactivity and NOS were present in sub-

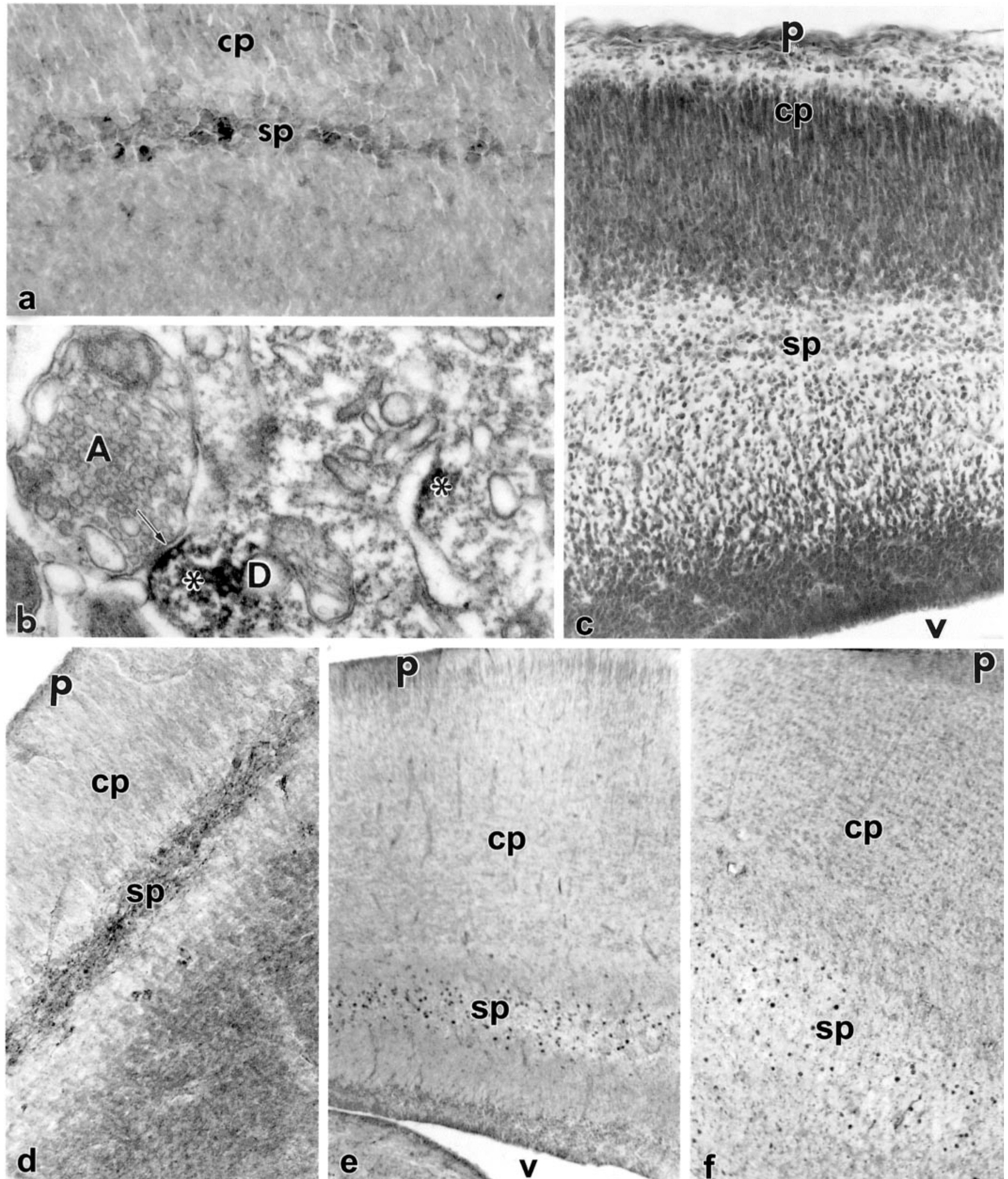



**Figure 1.** Localization of KAT-I in the developing cerebral cortex of the rat. (*a*) KAT-I immunopositivity of the subplate (sp) at E16. cp, cortical plate (×400). (*b*) Axodendritic synapse (arrow) in the subplate at E16. KAT-I immunoreactivity (asterisk) is located in the dendrite (D) of a subplate cell. The immature axon (A) does not exert any immunoreactivity. (*c*) Nissl-stained section, adjacent to (*a*), representing the whole thickness of the cortex for orientation purposes. p, pial surface; cp, cortical plate; sp, subplate; V, part of lateral ventricle. Vertical section (×100). (*d*) KAT-I immunopositivity of the subplate (sp) at E17. p, pial surface; cp, cortical plate (×250). (*e*) At P1, none of the cortical cells (cx) express KAT-I except for those of the subplate (sp) which are characterized by intense KAT-I immunoreaction. p, pial surface; V, lateral ventricle (×150). (*f*) KAT-I immunopositivity of the subplate (sp) at P6. p, pial surface; cp, cortical plate; sp, subplate (×150).

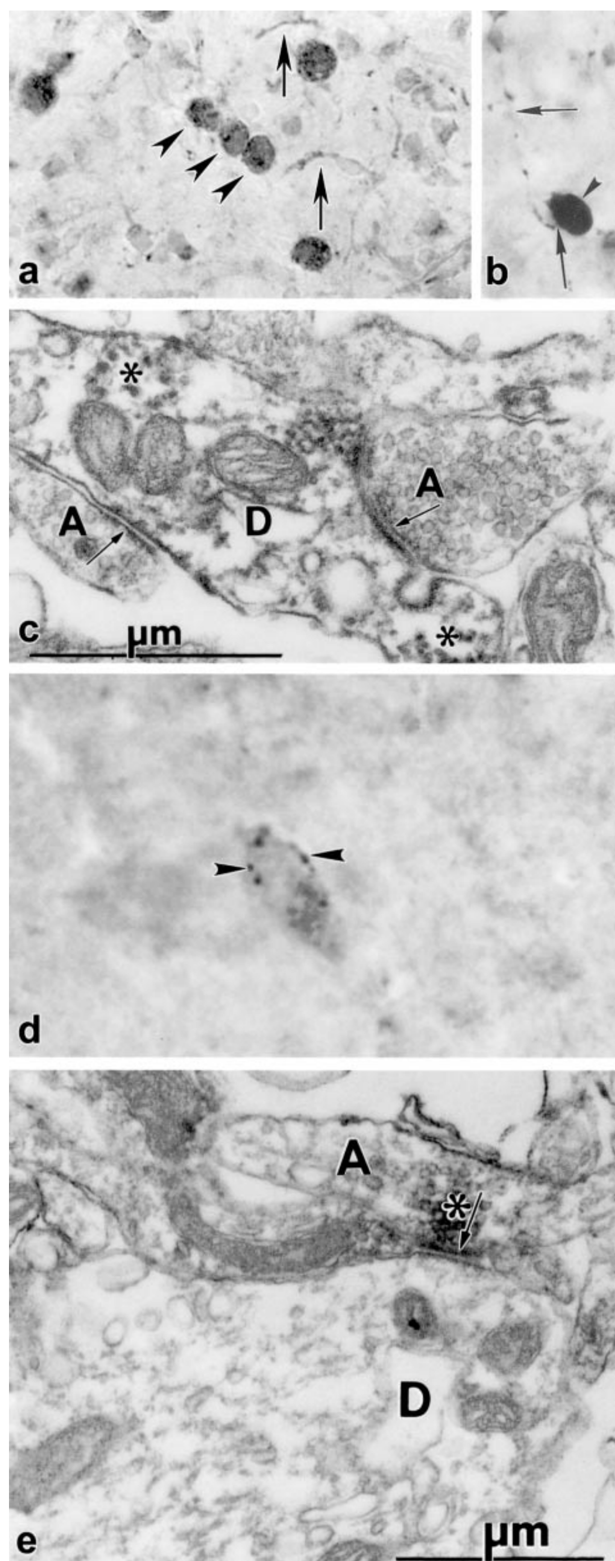



**Figure 2.** (*a*) Nerve cells of the subplate (P3 rat) emit long, varicose processes (arrow). The cells are often localized in groups (arrowheads); perikarya and axonal processes are characterized by KAT-I enzyme expression (×1200). (*b*) KAT-I-immunopositive nerve cell (arrowhead) with an elongated process (arrow), in the subplate of a P6 rat (×1200). (*c*) Axo-dendritic synapses in the subplate of a P6 rat. The immunonegative axons (A) establish synapses (arrows) with a KAT-I-expressing dendrite (D). (*d*) KAT-I immunopositivity in the dorsal thalamus at P6: KAT-I-immunopositive axon (arrowheads) terminating around a nerve cell of the thalamus (×1500). (*e*) Electron micrograph of an axodendritic synapse (arrow) in the thalamus. The axon (A) is KAT-immunopositive (asterisk); the dendrite (D) is free of any KAT-I immunoreactivity.

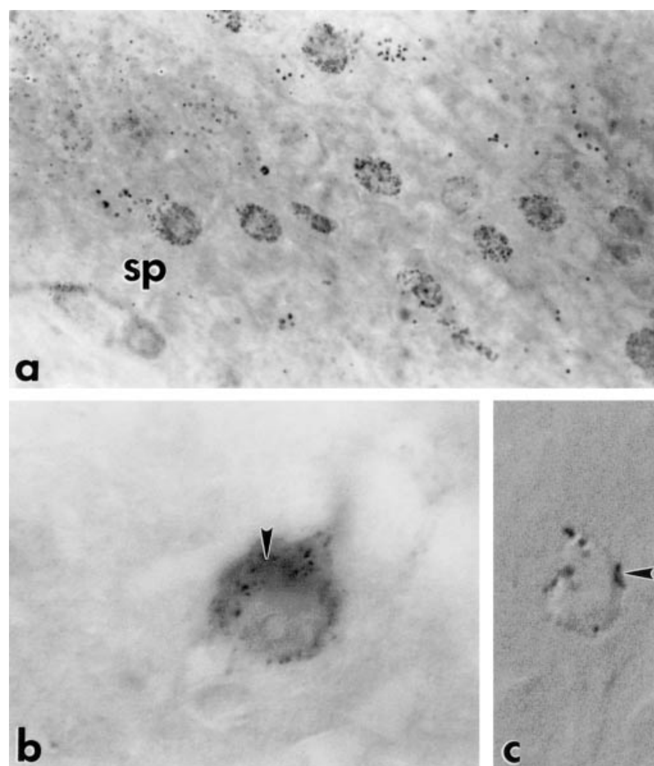
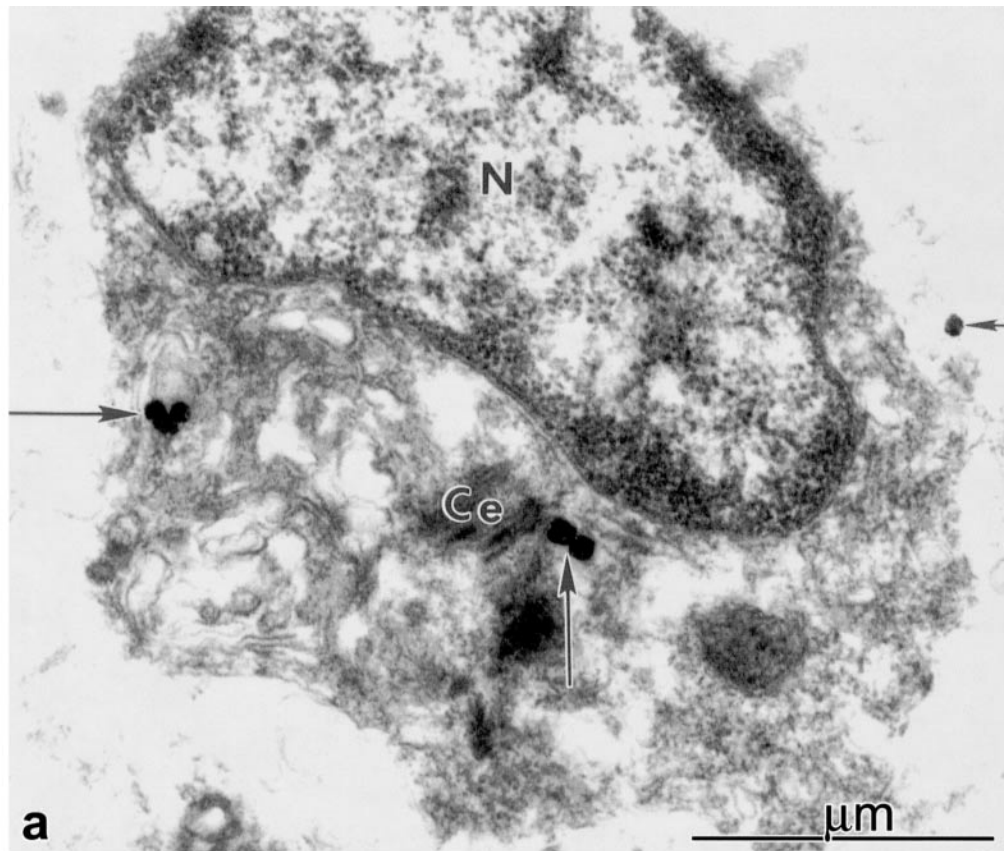


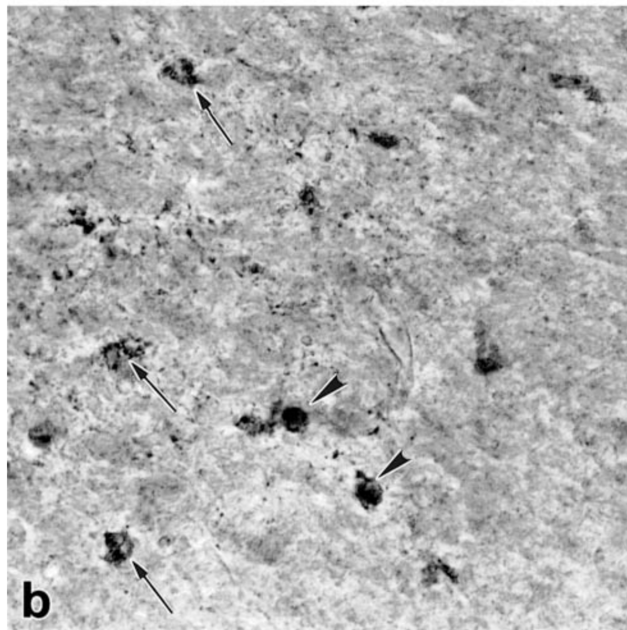

**Figure 3.** NMDA-R2A reaction in the subplate at P4. (*a*) Distribution of the NMDA receptors at low power (×200). (*b*,*c*) NMDA-receptors on the surface and in the cytoplasm of subplate cells (arrowheads). Nomarski optics, high power (×1500).

plate cells from E16 to P10. However, the localization and morphological appearance of PV- and NOS-immunoreactive cells differed considerably from that of KAT-I-immunoreactive cell populations. The fusiform PV- and NOS-immunopositive cells were located above KAT-I cells and exhibited a rich arborization of neuronal processes directed against the cortical plate (Fig. 5*a*–*d*). Double staining clearly delineated the KAT-I- and PV-immunoreactive sublayers of the subplate (Fig. 5*g*), which proves that different cell populations express KAT-I and PV without any trace of coexistence. On the other hand, NPY immunoreactivity was characteristic of the subplate only during the postnatal period from P7 to P10 (Fig. 5*e*). We found expression of the α7-subunit of nAChR in the spherical (mostly unipolar) subplate cells from E16 until postnatal day P10 (Fig. 5*f*). The α7-subunit of nAChR was also present in embryonic Cajal– Retzius cells.

## Discussion

By the time neurons originating in the ventricular and subventricular zone begin to migrate outwards to establish the initial cortical plate below the pia (Hicks and D'Amato, 1964; Rakic, 1974), there already exists a sizable network of monoaminergic fibers, the primordial plexiform layer (Marin-Padilla, 1971, 1972, 1978). The function of the earliest generated neurons that form the subplate zone is not fully understood, but several hypotheses have been suggested that underscore their further development of the cerebral cortex. Thus, it has been shown that the developing thalamocortical axons establish temporary synapses with neurons of the subplate, in a so-called ‘waiting (holding) compartment’, until their target cells in the cortical plate acquire the necessary degree of maturation (Rakic,



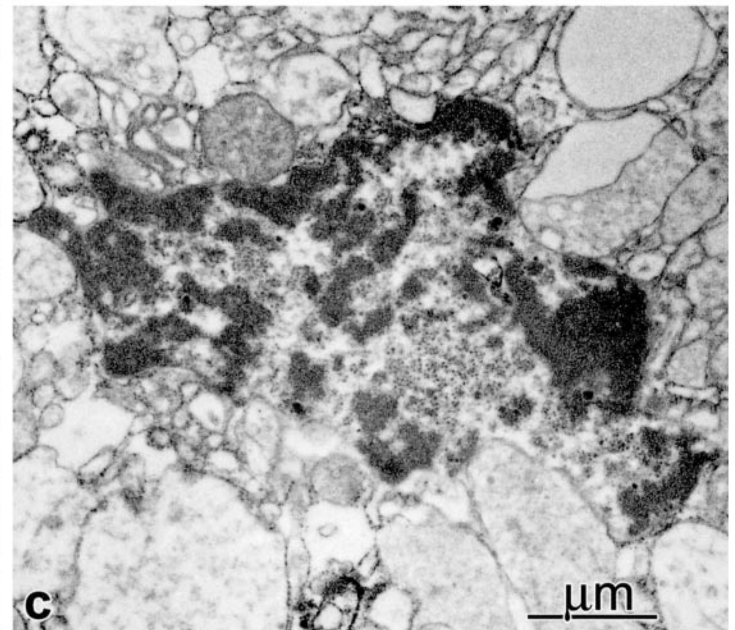




**Figure 4.** (*a*) Localization of NMDA-R2A receptor subunits (arrows) in the cytoplasm of a KAT-I-immunoreactive (asterisk) subplate cell of a P2 rat. N, nucleus; ce, centriolum. (*b*) The beginning of disintegration of KAT-I-immunopositive cells in the subplate at P8 (arrows). Only a few KAT-I-immunopositive cells (arrowheads) remain unaffected at this age (×400). (*c*) Electron microscopic equivalent of cell death in the subplate zone (P9).

1976, 1977, 1982, 1988; Kostovic and Rakic, 1980, 1984, 1990; Ghosh *et al.*, 1990; Molnar and Blakemore, 1995; Molnar *et al.*, 1998), similar to callosal axons (Goldman-Rakic, 1982). Neurons of the subplate zone also emit so-called 'pioneer axons' which serve as itineraries for developing corticothalamic fibers (McConnell *et al.*, 1989; Allendoerfer and Shatz, 1994). Thus, subplate neurons pioneer the first axonal pathways from the cerebral cortex. According to Molnar (Molnar, 2000), the interactions of the thalamocortical projections with the early generated, largely transient cells of the subplate play a crucial

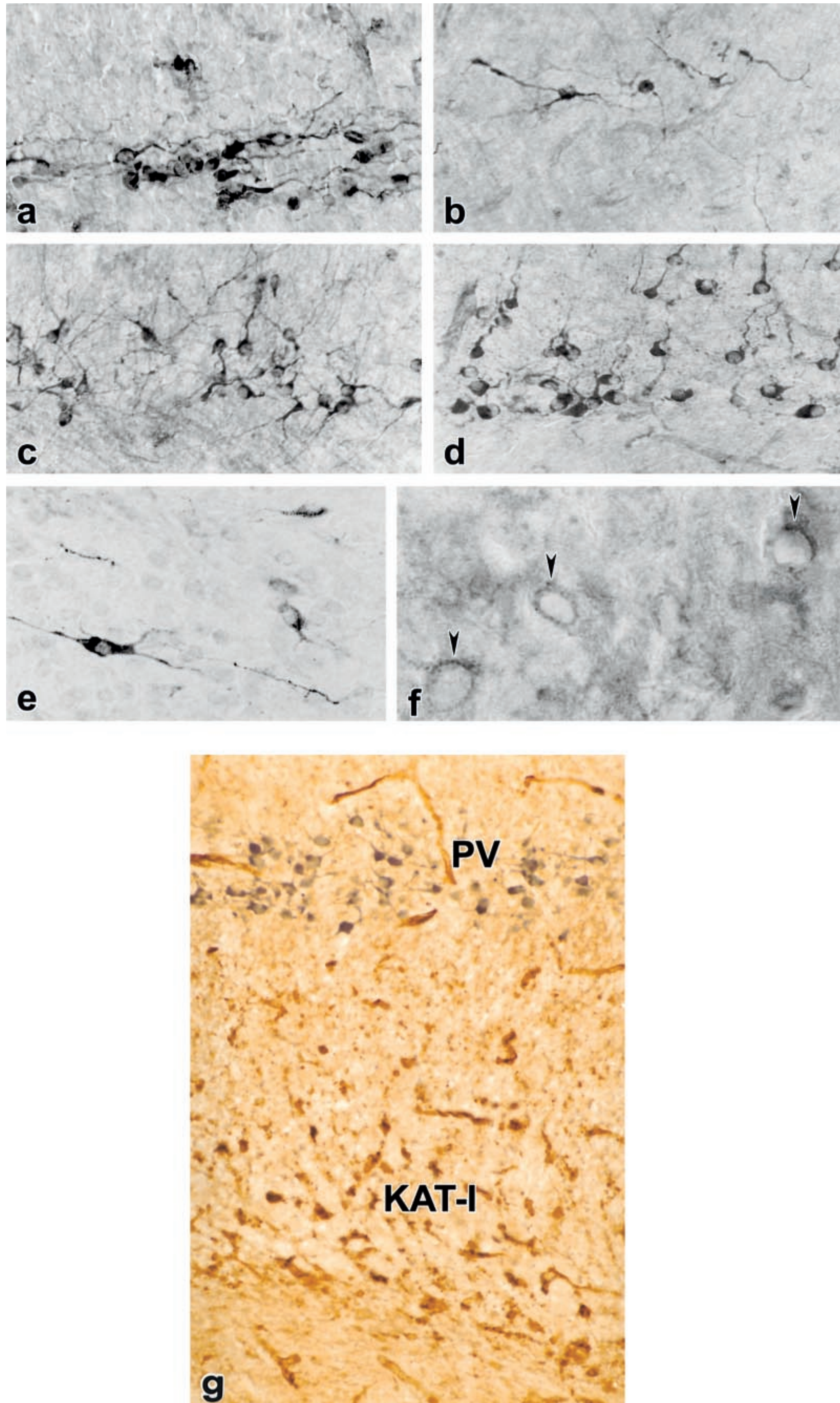



**Figure 5.** Localization of various markers in the subplate: (*a*) parvalbumin immunoreactivity at E16 (×200); (*b*) NOS immunoreactivity at E16 (×200); (*c*) parvalbumin immunoreactivity at P7 (×200); (*d*) NOS immunoreactivity at P7 (×200); (*e*) NPY immunoreactivity at P7 (×200); (*f*) nAChR immunoreactivity (arrowheads) at P7 (×1500); (*g*) double staining of KAT-I and PV at P7 (×200).

role in their development. Our observation that axons of subplate cells, containing the enzyme KAT-I and expressing NMDA-R2A, establish close contact with developing neurons of the thalamus, is in accord with this notion. Cells of the subplate regulate neuronal migration in the developing cortex through a process controlled by NMDA receptors (Komuro and Rakic, 1993; Feldmeyer and Cull-Candy, 1996). According to Hanganu *et al.* (Hanganu *et al.*, 2001) subplate neurons receive functional synaptic inputs mediated, *inter alia*, by NMDA receptors. The density of NMDA receptors is low at birth and increases in the postnatal period (Smith and Thompson, 1999). In this context, it may be significant that migration of nerve cells is regulated by NMDA receptors (Komuro and Rakic, 1993). Double staining proves that the enzyme KAT-I and the R2A subunits of the NMDA receptor are, for the most part, co-localized in nerve cells of the subplate. Other studies suggested the importance of GABA and GABA receptors in cell proliferation and development of the subplate (Meinecke and Rakic, 1992; Haydar *et al.*, 0,0,0,02000). Notwithstanding the above roles, blockade of activity in the subplate produces misguided thalamic connections (Catalano and Shatz, 1998). In this respect, it should be emphasized that not only the presence, but also the neuronal activity of subplate neurons may be factors which play an important role in cortical development (Yuste and Sur, 1999). A spontaneous correlated activity among spatially separated neurons found in the marginal zone of intact hemisphere preparations (Schwartz *et al.*, 1998) might, with due extrapolation, reflect the importance of spontaneous functional activity of the subplate neurons (Hanganu *et al.*, 2001). Therefore, the notion that neurotrophins and activity-dependent development of the cerebral cortex are inseparable entities seems to be a logical consequence of the above premises (Katz and Shatz, 1996), despite recent studies (Crowley and Katz, 1999; Verhage *et al.*, 2000) suggesting that neural activity may not be essential for the establishment of cortical networks. Further evidence against activity-dependent cortical development is the observation that neocortical regionalization can develop in the absence of thalamic innervation (Miyashita-Lin *et al.*, 1999), perhaps due to the effects of local concentrations of regulatory genes (Bishop *et al.*, 2000). In spite of such reservations, it still appears that the subplate may play important roles in the establishment of corticothalamic and thalamocortical pathways as well (Molnar *et al.*, 1998; Molnar, 2000).

Due to the importance of the subplate for normal development of the cerebral cortex, several attempts have been made to find specific markers and morphoregulatory molecules that characterize its function. A fibronectin-like molecule was found both in the developing cerebral cortex and in the subplate zone (Chun and Shatz, 1988), and the antigen SP1 could be successfully used to analyze the fate of the cells constituting the subplate (Wahle *et al.*, 1994). Also, cells of the subplate zone were shown to exhibit strong, transient immunoreactivity of the nerve growth factor-inducible gene *VGF* (Lombardo *et al.*, 1995), which reflects the presence of the antigen in axon terminals originating from thalamic neurons. Since the transmitter nitric oxide also characterizes the subplate (Derer and Derer, 1993; Luth *et al.*, 1995; Yan and Ribak, 1997; Fatemi *et al.*, 1998; Judas *et al.*, 1999; Downen *et al.*, 1999), attempts were made to designate NOS, an enzyme readily accessible for immunohistochemical studies, as the specific marker substance of the subplate. Finally, transient immunoglobulin-like molecules (Dunn *et al.*, 1995) and the sulfoglucuronyl carbohydrate-binding protein-1 (Nair *et al.*, 1998) was observed in immature neurons of the subplate. Also, calcium-binding proteins like PV (Hogan and Berman, 1994; Honig *et al.*, 1996) and calbindin-D (Berger *et al.,* 1993; Hogan and Berman, 1994) were shown to be present in the subplate. The PV-immunoreactive interneurons follow an inside-out pattern of maturation of cortical laminae (Hogan and Berman, 1994). According to Honig *et al.* (Honig *et al.*, 1996) molecular markers are expressed in spatial and temporal patterns that characterize humans, non-human primates, carnivores and rodents. According to Finney *et al.* (Finney *et al.*, 1998), subplate neurons provide a major glutamatergic synaptic input to the cortical plate. Remnants of the subplate neuronal population comprise the interstitial cells of adult cortical white matter (Dunn *et al.*, 1995).

In our present studies, we found that KAT-I is not only a novel marker for the localization of the subplate, but also a marker that seems to have important functional aspects, since kynurenic acid, the product of kynurenine aminotransferase (KAT-I) is, as far as is currently known, the only endogenous antagonist of NMDA receptors. In this capacity, KAT-I contributes to neuronal protection against the cytotoxic effect of endogenous excitotoxins (Schwarcz *et al.*, 1984, 1992).

According to our present studies, the morphological entity of the subplate does not represent a homogeneous cell population. This is especially striking in double-stained specimens where the PV- and the KAT-I-expressing cell populations appear in different colours (Fig. 5*g*). Cells expressing PV, NOS and NPY appear to migrate to the cortical plate. At the same time, cells displaying KAT-I and the nicotinic AChR seem rather to stay *in loco* and support the guiding of corticothalamic and thalamocortical pathways.

The role of KAT-I is transient, since later, during the course of development, as the subplate cells enter the process of programmed cell death (Kostovic and Rakic, 1980; Al-Ghoul and Miller, 1989; Ferrer *et al.*, 1990), this enzyme disappears. This again is in complete harmony with the role of KAT-I as proposed earlier. According to recent investigations, the neurodevelopmental origin of schizophrenia once again seems to hold its ground (Bloom, 1993), as follows from the studies of Akbarian *et al.* (Akbarian *et al.*, 1993, 1996). Dysfunctions of the NMDA receptors seem to play a crucial role in the development of numerous neurological and psychiatric diseases, including epilepsy, Parkinson's disease, Alzheimer's disease, depression, post-traumatic stress disorder, alcoholism and schizophrenia (Heresco-Levy and Javitt, 1998), while those of calcium-binding proteins are supposed to be involved in the development of hydrocephalus (Ulfig *et al.*, 2001). Further investigations may shed light on the role of dysfunctioning and surviving KAT-I-containing subplate neurons in structural and functional irregularities of the cerebral cortex. In this respect, it seems to be of major importance that kynurenic acid, the product of the enzyme KAT, inhibits α7-nicotinic receptor activity (Hilmas *et al.*, 2001), since the α7-nicotinic receptor, a ligand-gated ion channel that admits calcium ions into cells, has various developmental roles (Freedman *et al.*, 2000). It has to be assumed that in the course of normal development, α7-nicotinic receptor and kynurenic acid are in a delicate equilibrium. Our observation that the α7-nicotinic receptor is present in the subplate and in Cajal–Retzius cells (Meyer *et al.*, 1999) seems to be closely related to the developmental role of α7-nicotinic receptor, the malfunction of which has recently been implicated in the developmental pathogenesis of schizophrenia (Freedman *et al.*, 2000). Recent studies in this laboratory, performed in collaboration with gynecologists and psychiatrists on human embryos, may prove or disprove the validity of this assumption.

## Notes

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## References

Akbarian S, Bunneyt WE Jr, Potkin SG, Wigal SB, Hagman JO, Sandman CA, Jones EG (1993) Altered distribution of nicotinamide–adenine dinucleotide phosphate–diaphorase cells in frontal lobe of schizophrenics implies disturbances of cortical development. Arch Gen Psychiatry 50:227–230.

Akbarian S, Kim JJ, Potkin SG, Hetrick WP, Bunney WE, Jones EG (1996) Maldistribution of interstitial neurons in prefrontal white matter of the brains of schizophrenic patients. Arch Gen Psychiatry 53:425–436.

Al-Ghoul WM, Miller MW (1989) Transient expression of Alz-50 immunoreactivity in developing rat neocortex: a marker for naturally occurring neuronal death? Brain Res 481:361–367.

Allendoerfer KL, Shatz CJ (1994) The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. Annu Rec Neurosci 17:185–218.

Berger B, Alvarez C, Goldman-Rakic PS (1993) Neurochemical development of the hippocampal region in the fetal rhesus monkey. I. Early appearance of peptides, calcium-binding proteins, DARPP-32, and monoamine innervation in the entorhinal cortex during the first half of gestation (E47 to E90). Hippocampus 3:279–305.

Bishop KM, Goudreau G, O'Leary DD (2000) Regulation of area identity in the mammalian neocortex by Emx2 and Pax6. Science 288: 344–349.

Bloom FE (1993) Advancing a neurodevelopmental origin for schizophrenia. Arch Gen Psychiatry 50:224–227.

Catalano SM, Shatz CJ (1998) Activity-dependent cortical target selection by thalamic axons. Science 281:559–562.

Chun JJM, Shatz CJ (1988) A fibronectin-like molecule is present in the developig cat cerebral cortex and is correlated with subplate neurons. J Cell Biol 106:857–872.

Crowley JC, Katz LV (1999) Development of ocular dominance columns in the absence of retinal input. Nature Neurosci 2:1125–1130.

Derer P, Derer M (1993) Ontogenesis of NADPH-diaphorase neurons in the mouse forebrain. Neurosci Lett 152:21–25.

Downen M, Zhao ML, Lee P, Weidenheim KM, Dickson DW, Lee SC (1999) Neuronal nitric oxide synthase expression in developing and adult human CNS. J Neuropathol Exp Neurol 58:12–21.

Du F, Schmidt W, Okuno E, Kido R, Köhler C, Schwarcz R (1992) Localization of kynurenine aminotransferase immunoreactivity in the rat hippocampus. J. Comp. Neurol. 321:477–487.

Dunn JA, Kirsch JD, Naegele JR (1995) Transient immunoglobulin-like molecules are present in the subplate zone and cerebral cortex during postnatal development. Cereb Cortex 5:494–505.

Fatemi SH, Sidwell R, Akhter P, Sedgewick J, Thuras P, Bailey K, Kist D (1998) Human influenza viral infection *in utero* increases nNOS expression in hippocampi of neonatal mice. Synapse 29:84–88.

Feldmeyer D, Cull-Candy S (1996) Functional consequences of changes in NMDA receptor subunit expression during development. J Neurocytol 25:857–867.

Ferrer I, Bernet E, Soriano E, Del Rio T, Fonseca M (1990) Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. Neuroscience 39:451–458.

Finney EM, Stone JR, Shatz CJ (1998) Major glutamatergic projection from subplate into visual cortex during development. J Comp Neurol 398:105–118.

Freedman R, Adams CE, Leonard S (2000) The alpha7-nicotinic acetylcholine receptor and the pathology of hippocampal interneurons in schizophrenia. J Chem Neuroanat 20:299–306.

Ghosh A, Antonini A, McConnell SK, Shatz CJ (1990) Requirement for subplate neurons in the formation of thalamocortical connections. Nature 347:179–181.

Goldman-Rakic PS (1982) Neuronal development and plasticity of association cortex in primates. Neurosci Res Prog Bull 20:520–532.

Guidetti P, Okuno E, Schwarz R (1997) Characterization of rat brain kynurenine aminotransferase I and II. J Neurosci Res 50:457–465.

Hanganu IL, Kilb W, Luhmann HJ (2001) Spontaneous synaptic activity of subplate neurons in neonatal rat somatosensory cortex. Cereb Cortex 11:400–410.

Haydar TF, Wang F, Schwartz ML, Rakic P (2000) Differential modulation of proliferation in the neocortical ventricular and subventricular zones. J Neurosci 20:5764–5774.

Heresco-Levy U, Javitt DC (1998) The role of *N*-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission in the pathophysiology and therapeutics of psychiatric syndromes. Eur Neuropsychopharmacology 8:141–152.

Hicks SP, D'Amato CJ (1968) Cell migrations to the isocortex in the rat. Anat Rec 160:619–634.

Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarz R, Albuquerque EX (2001) J Neurosci 21:7463–7473.

Hogan D, Berman NE (1994) The development of parvalbumin and calbindin D28k immunoreactive interneurons in kitten visual cortex. Brain Res Dev Brain Res 77:1–21.

Honig LS, Herrmann K, Shatz CJ (1996) Developmental changes revealed by immunohistochemical markers in human cerebral cortex. Cereb Cortex 6:794–806.

Judas M, Sestan N, Kostovic I (1999) Nitrinergic neurons in the developing and adult human telencephalon: transient and permanent patterns of expression in comparison to other mammals. Microsc Res Tech 45:401–419.

Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274:1133–1138.

Knyihár-Csillik E, Okuno E, Vécsei L (1999) Effects of *in vivo* sodium azide administration on the immunohistochemical localization of kynurenine aminotransferase in the rat brain. Neuroscience 94: 269–277.

Komuro H, Rakic P (1993) Modulation of neuronal migration by NMDA receptors. Science 260:95–97.

Kostovic I, Rakic P (1980) Cytology and time of origin of interstitial neurons in the white matter in infant and adult human and monkey telencephalon. J Neurocytology 9:219–242.

Kostovic I, Rakic P (1984) Development of prestriate visual projections in the monkey and human fetal cerebrum revealed by transient cholinesterase staining. J Neuroscience 4:25–42.

Kostovic I, Rakic P (1990) Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. J Comp Neurol 297:441–470.

Lombardo A, Rabacchi SA, Cremisi F, Pizzorusso T, Cenni MC, Possenti R, Barsacchi G, Maffei L (1995) A developmentally regulated nerve growth factor-induced gene, VGF, is expressed in geniculocortical afferents during synaptogenesis. Neuroscience 65:997–1008.

Luth HJ, Hedlich A, Hilbig H, Winkelmann E, Mayer B (1995) Postnatal development of NADPH-diaphorase/nitric oxide synthase positive nerve cells in the visual cortex of the rat. J Hirnforsch 36:313–328.

Marin-Padilla M (1971) Early prenatal ontogenesis of the cerebral cortex (neocortex) of the cat (*Felis domestica*). A Golgi study. I. The primordial neocortical organization. Z Anat Entwickl-Gesch 134: 117–145.

Marin-Padilla M (1972) Early prenatal ontogenesis of the cerebral cortex (neocortex) of the cat (*Felis domestica*). A Golgi study. II. Developmental differences and their significances. Z Anat Entwickl-Gesch 136:125–142.

Marin-Padilla M (1978) Dual origin of mammalian neocortex and evolution of the cortical plate. Anat Embryol 152:109–126.

McConnell SK, Gosh A, Shatz CJ (1989) Subplate neurons pioneer the first axon pathway from the cerebral cortex. Science 245: 978–982.

McLean IW, Nakane PK (1974) Periodate–lysine–paraformaldehyde fixative: a new fixative for immunoelectron microscopy. J Histochem Cytochem 22:1077–1983.

Meinecke DL, Rakic P (1992) Expression of GABA and GABA-A receptors by neurons of the subplate zone in developing primate occipital

cortex: evidence for transient local circuits. J Comp Neurol 317: 91–101.
Meyer G, Goffinet AM, Fairén A (1999) Feature article: What is a Cajal–Retzius cell? A reassessment of a classical cell type based on recent observations in the developing neocortex. Cereb Cortex 9:765–775.
Miyashita-Lin EM, Hevner R, Wassarman KM, Martinez S, Rubinstein JLR (1999) Early neocortical regionalization in the absence of thalamic innervation. Science 285:906–909.
Molnar Z (2000) Development and evolution of thalamocortical interactions. Eur J Morphol 38:313–320.
Molnar Z, Blakemore C (1995) How do thalamic axons find their way to the cortex? Trends Neurosci 18:389–397.
Molnar Z, Adams R, Blakemore C (1998) Mechanisms underlying the early establishment of thalamocortical connections in the rat. J Neurosci 18:5723–5745.
Nair SM, Zhao Z, Chou DK, Tobet SA, Jungalwala FB (1998) Expression of HNK-1 carbohydrate and its binding protein, SBP-1, in apposing cell surfaces in cerebral cortex and cerebeluum. Neuroscience 85:759–771.
Nozaki K, Beal MF (1992) Neuroprotective effect of L-kynurenine on hypoxia-ischemia and NMDA lesions in neonatal rats. J Cereb Blood Flow Metabolism 12:400–407.
Okuno E, Du F, Ishikawa M, Tsujimoto M, Nakamura M, Schwarcz R, Kido R (1990) Purification and characterization of kynurenine–pyruvate aminotransferase from rat kidney and brain. Brain Res 354:37–44.
Okuno E, Nakamura M, Schwarz R (1991) Two kynurenine aminotransferases in human brain. Brain Res 542:307–312.
Rakic P (1974) Neurons in the monkey visual cortex: systematic relation between time of origin and eventual disposition. Science 183: 425–427.
Rakic P (1976) Prenatal genesis of connections subserving ocular dominance in the Rhesus monkey. Nature 261:467–471.
Rakic P (1977) Prenatal development of the visual system in the Rhesus monkey. Phil Trans R Soc Lond (Biol) 278:245–260.
Rakic P (1982) Early developmental events: cell lineages, acquisition of neuronal positions and areal and laminar development. Neurosci Res Prog Bull 20:439–451.
Rakic P (1988) Specification of cerebral cortical areas. Science 241:170–176.
Schwarcz R, Foster AC, French ED, Whetsell WO Jr, Köhler C (1984) Excitotoxic models for neurodegenerative diseases. Life Sci 35:19–32.
Schwarcz R, Due F, Schmidt W, Turski WA, Gramsbergen JBP, Okuno E, Roberts RC (1992) Kynurenic acid: a potential pathogen in brain disorders. In Neurotoxins and neurodegenerative diseases (Langston JW, Young A, eds). Annals of the New York Academy of Sciences, vol. 648, pp. 140–153. New York: The New York Academy of Sciences:.
Schwartz T, Rabinovitz D, Uuni VK, Kumar VS, Smetters DK, Tsiola A, Yuste R (1998) Networks of coactive neurons in developing later I. Neuron 20:1271–1283.
Sidman RL, Rakic P (1973) Neuronal migration with special reference to developing human brain: a review. Brain Res 62:1–35.
Smith AL, Thompson ID (1999) Spatiotemporal patterning of glutamate receptors in developing ferret striate cortex. Eur J Neurosci 11:923–934.
Ulfig N, Szabo A, Bohl J (2001) Effect of fetal hydrocephalus on the distribution patterns of calcium-binding proteins in the human occipital cortex. Pediatr Neurosurg 34:20–32.
Verhage M, Maia AS, Plomp JJ, Brussard AB, Heeroma JH, Vermeer H, Toonen RT, Hammer RE, van den Berg TK, Missler M, Geuze HJ, Sudhof TC (2000) Synaptic assembly of the brain in the absence of neurotransmitter secretion. Science 287:864–869.
Wahle P, Lubke J, Naegele JR (1994) Inverted pyramidal neurons and interneurons in cat cortical subplate zone are labelled by monoclonal antibody SP1. Eur J Neurosci 6:1167–1178.
Woo TU, Beale JM, Finlay BI (1991) Dual fate of subplate neurons in a rodent. Cereb Cortex 1:433–443.
Yan XX, Ribak CE (1997) Prenatal development of nicotinamide adenin dinucleotide phosphate-diaphorase activity in the human hippocampal formation. Hippocampus 7:215–231.
Yuste R, Sur M (1999) Development and plasticity of the cerebral cortex: from molecules to maps. J Neurobiol 41:1–6.