Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 8879–8883, October 1992
Neurobiology

Early regional specification for a molecular neuronal phenotype in the rat neocortex

(monoclonal antibody/immunohistochemistry/organotypic culture/cell culture/neurogenesis)

YASUYOSHI ARIMATSU*, MAMI MIYAMOTO*, ITSUKO NIHONMATSU*, KANAKO HIRATA*, YOSHIHIKO URATANI†, YUMIKO HATANAKA*, AND KEIKO TAKIGUCHI-HAYASHI*

Laboratories of *Neuromorphology and †Cellular Molecular Biology, Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194, Japan

Communicated by Torsten N. Wiesel, June 16, 1992 (received for review November 27, 1991)

ABSTRACT **The timing of neocortical regional specification was examined using a monoclonal antibody, designated PC3.1, that binds a 29-kDa polypeptide and recognizes a neuronal subpopulation located in the lateral but not dorsomedial neocortex in the rat. When lateral cortical tissue fragments at embryonic days 12 and 16 were maintained in an organotypic culture system, a substantial number of neurons became PC3.1-immunopositive. In marked contrast, considerably fewer, if any, PC3.1-positive neurons were observed in cultures of dorsal cortical tissue. The selective appearance of PC3.1-immunopositive neurons was also observed in dissociated cultures derived from the lateral, but not dorsal, cortical primordium at embryonic day 13 and later. In light of previous reports showing that the interactions between developing neocortical neurons and cortical afferents begin at embryonic day 14 or later, our findings imply that some regional specification occurs well before these interactions and suggest the importance of elements intrinsic to the neocortex in establishing neocortical regional specificity. Furthermore, [^{3}H]thymidine birth-dating experiments revealed that the majority of presumptive PC3.1-immunopositive neurons underwent their final mitosis around embryonic day 15, suggesting that the regional specification events for these neurons occur before their neurogenesis.**

The adult mammalian neocortex is subdivided into functional areas having distinct cytoarchitectonic, connectional, and molecular characteristics (1–3). The mechanisms of regional specification, however, remain to be elucidated. To clarify the factors that regulate specification, it is important to first know the timing of various aspects of specification during the course of development. Such information would allow a better understanding of the extent to which neocortical heterogeneity is established through mechanisms intrinsic to the neocortex or through interactions of cortical cells with extracortical environments.

It has been shown that some area-specific patterns of afferents and efferents can form in heterotopically transplanted rat neocortical tissue, even that derived from fetuses at relatively late stages, in accordance with their location in the host tissue (4, 5). Furthermore, the area-specific cytoarchitectonic characteristics of the rat primary somatosensory area (barrels) could be detected only after thalamocortical innervation (6), and presumptive visual cortex at embryonic day 17 or 18 (E17/E18) formed barrels when transplanted into the parietal region (7). These findings have led to the view that the neocortical neuroepithelium generates more or less homogeneous cortex across its extent, which only later diversifies into distinct areas through epigenetic mechanisms (8–10). It is not likely, however, that the interaction between cortical and extracortical structures is the sole factor that determines cortical regional identity (8, 11, 12). Although not fully proven, some evidence suggests that certain area-specific features of the neocortex are established early by a developmental process specified prior to the arrival of thalamocortical axons (12–15).

Previous studies on neocortical specification have focused primarily on morphological features, such as cytoarchitecture and patterns of afferent and efferent connections (4–7, 11–13). Although considerable molecular heterogeneity of the neocortex has been described (16–19), it is still unclear to what extent unique molecular features are present exclusively in particular regions. In the present study, we generated a monoclonal antibody, designated PC3.1, that selectively labels a unique neuronal subpopulation in lateral neocortical areas in developing and adult rats. Since PC3.1 antibody recognizes distinct cellular features expressed exclusively in the lateral cortical regions, we could determine the timing of regional specification events for this phenotype by examining the expression of PC3.1 immunoreactivity in separate cultures of lateral and dorsal cortical primordia from rats at various developmental stages. Our findings indicate that some specification events occur prior to interactions between neocortical and extracortical structures.

MATERIALS AND METHODS

Experimental Animals. Rats of Wistar strain were used. Pregnant rats were obtained by overnight mating. The day when a vaginal plug was observed in the morning was designated E0. Birth usually occurred late on E21 (= postnatal day 0, P0).

Generation of Monoclonal Antibodies. Fresh neocortical tissue (parietal and temporal regions) from 3-week-old rats was homogenized in phosphate-buffered saline (pH 7.4). BALB/c mice were immunized with several subcutaneous injections of the homogenate emulsified in Freund's complete (first injection only) or Freund's incomplete adjuvant. Four days after an intravenous booster injection of the cortical antigen, the spleen cells from the mice were harvested and fused with P3-NS1/1-Ag4-1 plasmacytoma cells. Selection of the hybridoma cell lines was accomplished by indirect immunofluorescence assay on cryostat sections of paraformaldehyde-fixed cerebral cortex (20). One of the cell lines secretes the monoclonal antibody PC3.1 and another produces monoclonal antibody PC3.2. The immunoglobulin subclass of them is IgG1.

Immunohistochemistry. Rats were anesthetized with ether and fixed by cardiac perfusion with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Brains were removed and fixed further by immersion in the same fixative

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: E, embryonic day; P, postnatal day.

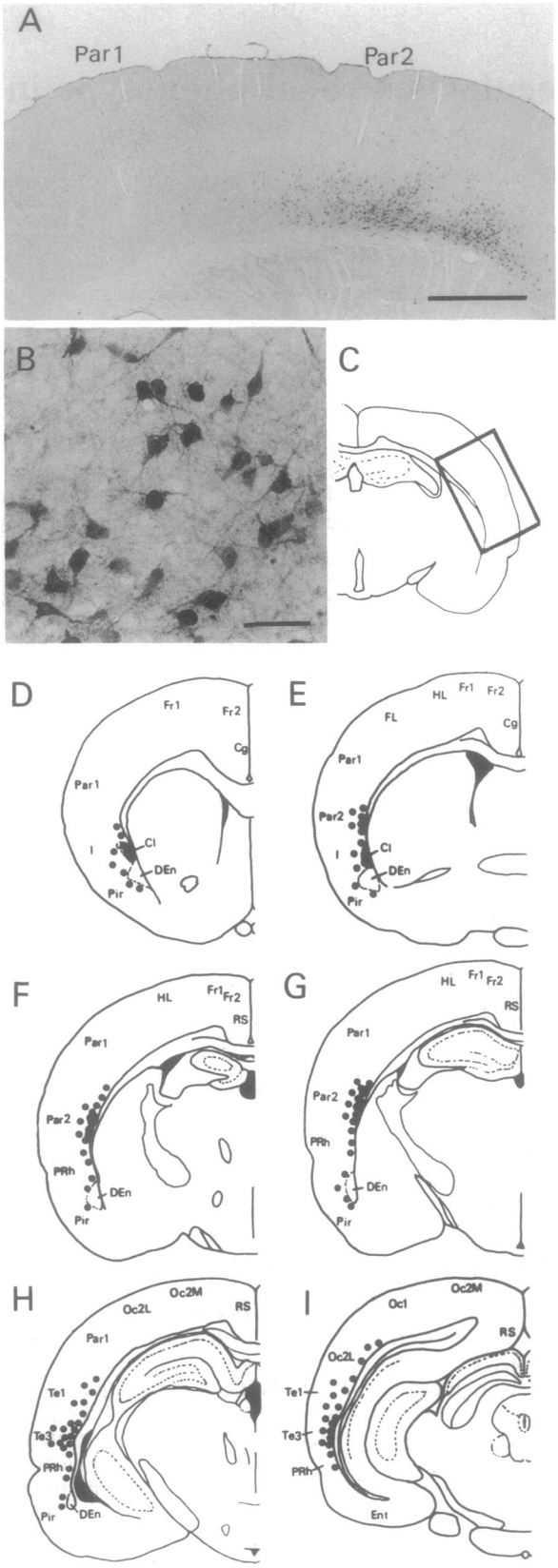

FIG. 1. Distribution of neuronal subpopulation detected by monoclonal antibody PC3.1. (*A*) Frontal section of an adult rat brain immunostained with PC3.1 antibody. Immunoreactivity is seen within neurons in the deep layers of the secondary somatosensory area (Par2) but not in the primary somatosensory area (Par1). (Bar = 1 mm.) (*B*) Higher magnification of layer VI of the secondary somatosensory area shown in *A*. (Bar = 50 μm.) (*C*) Schematic drawing of the section shown in *A*. The field seen in *A* is indicated by the rectangle. (*D–I*) Schematic representation, in six frontal planes, of the distribution of PC3.1-immunopositive neurons (filled circles). Boundaries of cortical areas were determined according to Paxinos and Watson (22). Cg, cingulate cortex; Cl, claustrum; DEn, dorsal endopiriform nucleus; Ent, entorhinal cortex; FL, forelimb area of cortex; Fr1, frontal cortex, area 1; Fr2, frontal cortex, area 2; HL, hindlimb area of cortex; I, insular cortex; Oc1, occipital cortex, area 1; Oc2L, occipital cortex, area 2, lateral; Oc2M, occipital cortex, area 2, medial; Par1, parietal cortex, area 1; Par2, parietal cortex, area 2; Pir, piriform cortex; PRh, perirhinal cortex; RS, retrosplenial cortex; Te1, temporal cortex, area 1; Te3, temporal cortex, area 3.

(4°C, 7 hr). The block of brain was equilibrated in 30% sucrose in phosphate-buffered saline and frozen in OCT compound (Lab-Tek); 15-μm-thick sections were cut on a cryostat. The sections were mounted onto gelatin/chrome alum-subbed glass slides and incubated sequentially with 5% normal goat serum in phosphate-buffered saline containing 0.1% Triton X-100 (20 min), PC3.1 monoclonal antibody (13 μg/ml, 2 hr), goat anti-mouse IgG (Cappel Laboratories; 1:50, 2 hr), and mouse peroxidase-antiperoxidase (The Jackson Laboratory; 1:500, 1 hr). The bound antibody was visualized by incubating the sections with 0.02% 3,3′-diaminobenzidine/0.01% H_2O_2 in 50 mM Tris·HCl at pH 7.6 (20 min).

Organotypic Culture. At E12 and E16, fetal rats were killed by decapitation, and dorsal and lateral portions of the mid-anterior cortical primordium were dissected out (see Fig. 2 *A* and *B*). The tissue fragments were placed on collagen-coated microporous membranes of Millicell CM cups (PICM03050; Millipore) inserted in wells of six-well plates (Costar). They were cultured at 37°C in 5% CO_2/95% air with a serum-containing medium [44.5% Dulbecco's modified Eagle's medium (GIBCO), 44.5% Ham's F12 medium (GIBCO), 5% heat-inactivated horse serum (GIBCO), 5% newborn calf serum (Mitsubishi Kasei), and 1% heat-inactivated rat serum] that filled the well under the membrane. At 21 days (for E12 tissue) or 19 days (for E16 tissue) *in vitro* (i.e., at culture days equivalent to P12 and P14 *in vivo*, respectively), the cultures were fixed with 4% paraformaldehyde (90 min), sectioned (10 μm), and stained for PC3.1 immunoreactivity by the peroxidase-antiperoxidase method. The total number of PC3.1-positive neurons in every other section was counted, and the mean number of positive neurons per unit area was calculated for each tissue fragment.

Dissociated Culture. Dorsal and lateral portions of the cortical primordium were dissected out of E13, E16, and P4 rats as described above. The tissue fragments were treated with papain, and the dissociated cells were plated at a concentration of 8×10^4 to 4×10^5 cells per well (depending on the rat's age) on astroglial monolayer in wells of tissue culture chamber/slides (79 mm^2 per well; Lab-Tek). The cells were cultured with the serum-containing medium described above at 37°C in 5% CO_2/95% air. To eliminate dividing nonneuronal cells, 2.5 μM cytosine arabinonucleoside was added to the culture medium for 24 hr at 8–9 and 5–6 days *in vitro* for E13 and E16 cultures, respectively (each at culture days equivalent to P0–P1 *in vivo*). At various days *in vitro*, the cells were fixed with 4% paraformaldehyde/0.1% glutaraldehyde/0.2% picric acid in 0.1 M sodium phosphate buffer at pH 7.4 (5 min), after which PC3.1 immunoreactivity was examined by the indirect immunofluorescence technique. The number of PC3.1-positive neurons was determined by counting stained cells in all microscopic fields of the well. The total neuronal number was determined from counts of microtubule-associated protein 2 (MAP2)-immunopositive cells stained with anti-MAP2 monoclonal antibody (Amersham) in 10% of the microscopic fields. Procedures for tissue culture and immunocytochemistry of cultured cells have been described in detail previously (21).

Determination of Neuronal Birthday. Timed pregnant rats were injected intraperitoneally with [^{3}H]thymidine (NEN; 6.7 Ci/mmol; 0.5 mCi/100 g of body weight; 1 Ci = 37 GBq). The delivered pups were sacrificed on P42, and cryostat sections of the brains were processed for immunohistochemical visualization of PC3.1 antigen with peroxidase-antiperoxidase as described above. The sections were then dipped into autoradiographic emulsion NR-M2 (Konica), exposed in a desiccating box (4°C, 8 weeks), developed with Konicadol-X (20°C, 4 min), and fixed with Konicafix (20°C, 5 min). The autoradiographic silver grains on the nuclei of the PC3.1-immunopositive neurons were counted under a microscope equipped with a 63× objective. The number of silver grains over individual neurons varied from 0 to 120, and those with ≥40 grains were regarded as being heavily labeled.

Immunoaffinity Purification. Lateral neocortical tissue from adult rats was homogenized in 3% Triton X-100/1 M NaCl in 50 mM Tris·HCl buffer (pH 7.6) containing proteinase inhibitor cocktail (1 mM phenylmethylsulfonyl fluoride/1 mM 1,10-phenanthroline/1 μg of pepstatin A per ml/1 μg of leupeptin per ml/1 μg of antipain per ml) and centrifuged at 100,000 × *g* for 1 hr. The supernatant was incubated with Affi-Gel-protein A beads (Bio-Rad) coupled with PC3.1 antibody or control monoclonal antibodies, PC3.2 and VC5.1 (IgG1; ref 20). PC3.2 antibody recognizes a rare neuronal subpopulation scattered in various cortical regions of the rat (data not shown). VC5.1 antibody recognizes a neuronal subpopulation in the cat cerebral cortex (20) but does not detect any structures in the rat cortex (unpublished observations). The bound components were eluted with 100 mM glycine (pH 2.5) and then neutralized with sodium phosphate buffer (pH 8.0); aliquots of the eluates were subjected to SDS/PAGE (12.5%). The proteins were visualized by silver staining.

RESULTS

Restricted Localization of PC3.1-Immunopositive Neurons. In the cerebral cortex of the adult rat, monoclonal antibody PC3.1 selectively recognized a unique neuronal subpopulation located in restricted lateral neocortical regions (Fig. 1). These include the secondary somatosensory (Par2), primary and secondary auditory (Te1 and Te3, respectively), and secondary visual (Oc2L) areas. Additionally, immunopositive neurons were found in the adjacent lateral periallocortex (mesocortex), including insular (I) and perirhinal (PRh) areas, in the claustrum (Cl), and just outside the dorsal endopiriform nucleus (DEn). No immunopositive neurons were observed in the dorsomedial neocortex, including motor (Fr1 and Fr2), primary somatosensory (Par1), and primary visual (Oc1) areas, or in the medial periallocortex, including anterior cingulate (Cg) and retrosplenial (RS) areas. Within the lateral neocortex, most PC3.1-positive neurons were located in layer VI. These neurons could first be recognized at P6 (data not shown).

Selective Appearance of PC3.1-Positive Neurons in Organotypic and Dissociated Cultures. To clarify the timing of the cortical regional specification for PC3.1-immunopositive neurons, we examined the expression of immunoreactivity using different culture systems. First, tissue fragments from the dorsal and lateral portions of the developing cortex of E12 and E16 rat fetuses were maintained in an organotypic culture system. The tissue was placed on collagen-coated microporous membrane and cultured for 19–21 days. As shown in Fig. 2, a substantial number of PC3.1-positive neurons was observed in cultures of the lateral cortical primordium from either E12 (27.5 ± 7.2 neurons per mm^2, n = 14) or E16 (39.5 ± 8.0 neurons per mm^2, n = 11) fetuses. This is in marked contrast to the detection of considerably fewer, if any, immunopositive neurons in cultures of the dorsal cortical primordium (E12, 1.0 ± 0.3 neuron per mm^2, n = 16; E16, 2.9 ± 0.5 neurons per mm^2, n = 8).

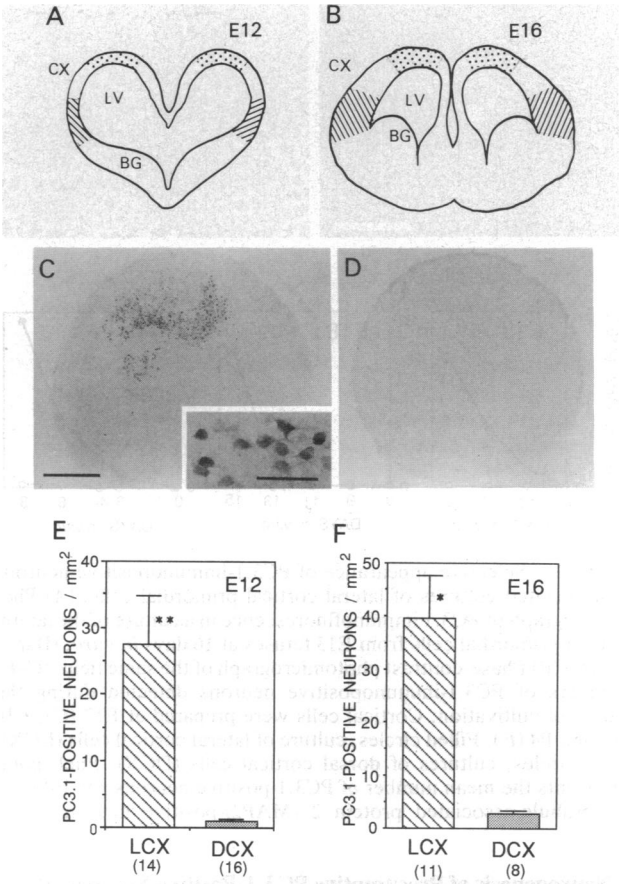

FIG. 2. Selective appearance of PC3.1-immunopositive neurons in organotypic cultures of lateral cortical primordial tissue. (*A* and *B*) Schematic illustration of the rat brain at E12 (*A*) and E16 (*B*) in the frontal plane. Dorsal (dotted) and lateral (shaded) portions of the cortical primordium (CX) were taken for culture. Analogous portions of the cortical primordium were also taken at different developmental stages for dissociated culture. BG, basal ganglia; LV, lateral ventricle. (*C*) Section of an organotypic culture (at 21 days *in vitro*) derived from the lateral cortical primordium at E12 stained for PC3.1 immunoreactivity. (Bar = 500 μm.) (*Inset*) Higher magnification of the same section. (Bar = 50 μm.) (*D*) Section of a culture from the dorsal cortical primordium at E12 fixed and immunostained for PC3.1 immunoreactivity at the same time as the section presented in *C*. Note that a number of immunopositive neurons are seen in *C* but not in *D*. (*E* and *F*) Numbers of PC3.1-immunopositive neurons detected in the organotypic cultures derived from E12 (*E*) and E16 (*F*). Shaded columns, cultures of lateral cortical primordium (LCX); dotted columns, cultures of dorsal cortical primordium (DCX). Each value represents the mean number (±SEM) of PC3.1-positive neurons per mm^2. Numbers of cortical primordia analyzed are shown in parentheses. *, **, Significantly greater [$P < 0.002$, $P < 0.001$, respectively; Student's t test] than dorsal cortical culture.

We also examined expression of PC3.1 immunoreactivity in dissociated cultures derived from the dorsal and lateral cortical primordium at E12, E16, and P4. The tissue fragments were dissociated, and the dispersed cells from each portion were cultured for 3–15 days (Fig. 3 *A* and *B*). As was the case in organotypic cultures, a substantial portion (0.4–1.6%) of neurons in all of the lateral cortical cultures examined became immunopositive (Fig. 3 *C–E*). Interestingly, the timing of the first appearance of immunopositive neurons *in vitro* (at culture days that would be the equivalent of P2–P8) was in good agreement with that *in vivo* (at P6). In contrast, <0.02% of the neurons were PC3.1-immunopositive in cultures of dorsal cortical cells.

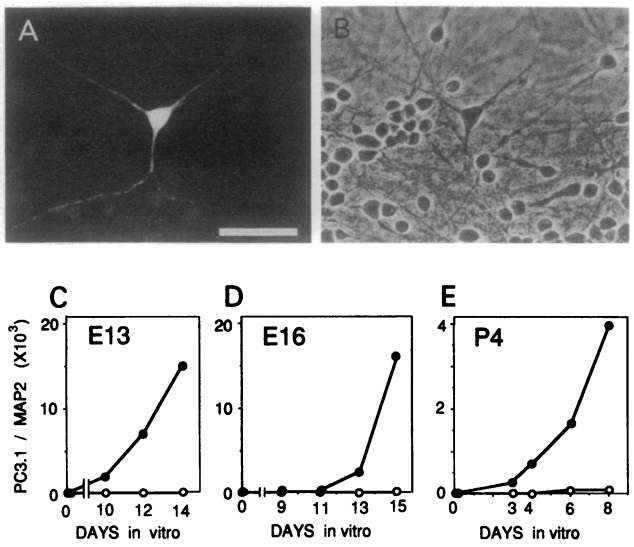

FIG. 3. Selective appearance of PC3.1-immunopositive neurons in dissociated cultures of lateral cortical primordial cells. (*A*) Photomicrograph of PC3.1 immunofluorescence in a culture of the lateral cortical primordial cells from E13 fetuses at 16 days *in vitro*. (Bar = 50 μm.) (*B*) Phase-contrast photomicrograph of the same field. (*C–E*) Numbers of PC3.1-immunopositive neurons detected during the course of cultivation. Cortical cells were prepared at E13 (*C*), E16 (*D*), and P4 (*E*). Filled circles, culture of lateral cortical cells (LCX). Open circles, cultures of dorsal cortical cells (DCX). Each point represents the mean number of PC3.1-positive neurons per 10^3 total microtubule-associated protein 2 (MAP2)-positive neurons (n = 2–4).

Neurogenesis of Presumptive PC3.1-Positive Neurons. It has been reported that most neurons in the rat neocortex are generated between E12 and E21 (15, 23–25). To directly compare the timing of the lateral cortical commitment to the production of PC3.1 neurons with that of the neurogenesis of these cells, we determined the date of their final mitosis (birthday). [^{3}H]Thymidine was administered to fetal rats at various embryonic days and, when the pups reached maturity, their cerebral cortices were processed for simultaneous detection of PC3.1 immunoreactivity and [^{3}H]thymidine incorporation. The percentage of PC3.1-immunopositive neurons with heavy [^{3}H]thymidine labeling in the secondary somatosensory area was at its highest level when [^{3}H]thymidine had been injected at E15 and at very low levels when [^{3}H]thymidine had been injected at E13 or before (Fig. 4). These results indicate that the majority of PC3.1-immunopositive neurons in this region undergo their final mitosis around E15 and that very few, if any, of them have stopped dividing at or before E13.

Immunoaffinity Purification Experiment. Because PC3.1 antigen was not detectable by immunoblot analysis, we characterized the molecules that bind PC3.1 antibody using immunopurification methods. A Triton X-100 extract of lateral cortical tissues of rats was incubated with protein A beads coupled with PC3.1 antibody. The components that bound to the beads were eluted and analyzed by SDS/PAGE followed by silver staining. Fig. 5 illustrates a band of 29-kDa polypeptide that is specific to PC3.1 antibody, suggesting that PC3.1 antibody recognizes the 29-kDa polypeptide or some component(s) that binds the polypeptide.

DISCUSSION

The monoclonal antibody PC3.1 generated in the present study recognized a neuronal subpopulation located in restricted regions of the neocortex. Previous studies have suggested that basic cellular constituents of the neocortex are similar from one region to another (26, 27). In fact, neurons

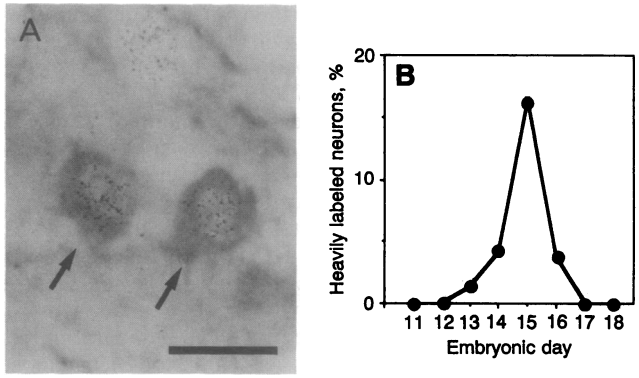

FIG. 4. Neurogenesis of presumptive PC3.1-immunopositive neurons as estimated by double-labeling experiments combining PC3.1 immunohistochemistry and [^{3}H]thymidine autoradiography. (*A*) Section through the secondary somatosensory cortex of a rat injected with [^{3}H]thymidine at E15. Arrows indicate PC3.1-immunopositive neurons with heavy labeling for [^{3}H]thymidine incorporation. (Bar = 20 μm.) (*B*) Percentage of heavily labeled PC3.1-positive neurons. Each point represents the mean value for four 1-mm-wide vertical strips of the secondary somatosensory cortex taken from two rats.

containing one of various neurotransmitters and neuropeptides or their synthesizing enzymes and receptors have been found in all neocortical areas (19, 28–31), although there are significant variations in their number in different areas (16). Thus, it is quite surprising that a neuronal population recognized by PC3.1 antibody was found only in limited cortical areas. The neuronal subset may represent unique anatomical substrates that contribute to specialized functions in these areas.

Although the molecular nature of the PC3.1 antigen has not yet been fully characterized, the result of the immunoaffinity purification experiment suggests that it is a 29-kDa polypeptide or related molecule(s). This assumption is supported by results from another experiment, in which PC3.1 antibody immunoprecipitated a polypeptide of 29 kDa in radioiodinated Triton X-100 extract of lateral cortical tissue (data not shown).

We have demonstrated in the present study, using the organotypic and dissociated culture systems, that the cortical primordium is already regionalized dorsolaterally for the capacity to produce PC3.1-positive neurons as early as E12–E13. Since previous investigations have shown that thalamic afferents begin to enter the neocortex at E16 or later (32–34) and that the corticothalamic axons do not reach the thalamic neuronal elements before E14 (34), it can be concluded that the regional specification for PC3.1 neurons is established prior to thalamocortical and corticothalamic interactions. Thus, our findings provide convincing evidence that certain regional specification is established early by

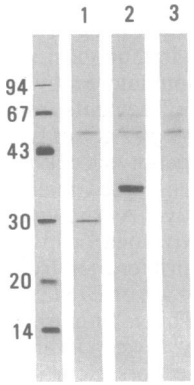

FIG. 5. Migration pattern in SDS/PAGE of components that bind protein A beads coupled with PC3.1 antibody (lane 1). Lane 2, components that bind control beads coupled with PC3.2 monoclonal antibody (IgG1). Lane 3, components that bind other control beads coupled with VC5.1 monoclonal antibody (IgG1). Note that the 29-kDa polypeptide can be seen in lane 1 but not in lanes 2 and 3. PC3.2 antibody-coupled protein A beads specifically bound 35-kDa polypeptide, but VC5.1 antibody-coupled protein A beads did not bind any polypeptides specifically.

elements intrinsic to the neocortex. It has been suggested that certain morphological features in some neocortical regions are not fully specified at the time of neurogenesis but can be induced during later stages by environmental cues, such as those from thalamocortical afferents (8, 9). It seems probable that neocortical regional specification involves a combination of mechanisms including early regionalization (e.g., for the molecular neuronal phenotype demonstrated in the present study) as well as certain connectional and cytoarchitectonic specifications during later stages.

Barbe and Levitt (35) have suggested that the capacity to produce (peri)allocortex-specific neurons is established in the lateral cortical primordium by E14 using a monoclonal antibody that recognizes limbic system-associated membrane protein (LAMP; ref. 36). They have further suggested that the relatively early restriction of the fate of the cortical primordium to express LAMP may reflect some phylogenetic difference between the (peri)allocortex and the neocortex. This conclusion was based on the fact that LAMP is widely but selectively distributed throughout phylogenetically "old" limbic structures. Our results, though agreeing with the concept of early specification, do not make such a distinction between the neocortex and periallocortex. We suggest that the specification occurs according to more general brain axes, although the exact nature of these is not yet known.

It has been demonstrated previously that the majority of layer VI neurons are born at E13–E15 (15, 23–25). In the present study, we directly demonstrated that a major portion of PC3.1-positive neurons (most of PC3.1 neurons are located in layer VI) undergo their final mitosis around E15. Thus, we have concluded that the regional specification for PC3.1 neurons occurs before their final mitosis. In this connection, it would be of interest to consider the timing and mechanism of cellular commitment for the molecular phenotype (PC3.1 expression). One possibility is that the phenotypic decision occurs in particular cells even before their final mitosis by a cell-intrinsic mechanism. Another possibility is that certain tissue specification as a whole might occur very early (at or before E12–E13), after which the fate of uncommitted precursor cells is determined by interactions within the specified tissue. It has been proposed that certain identities of cortical and other neurons are determined at or after their final mitosis depending on their environment (38–41). The latter possibility might be supported by the recent finding that clonally related cells in the mouse cortex become dispersed widely over the medial-lateral cortical axis (37).

In conclusion, we generated a monoclonal antibody, designated PC3.1, that detects a neuronal entity confined to lateral but not dorsomedial neocortical regions. By using this antibody as a molecular marker, it was possible to show that a molecular phenotype restricted to the lateral neocortical regions is specified well before thalamocortical and corticothalamic interactions and even prior to cortical neurogenesis. Molecular markers of specification, such as PC3.1, will facilitate a better definition of the extent to which the adult brain is specified early or can be modulated later by connections and experience.

We thank Dr. C. J. Barnstable (Yale University) and Dr. J. R. Naegele (Wesleyan University) for their comments on the manuscript.

1. Rakic, P. & Singer, W., eds. (1988) *Neurobiology of Neocortex* (Wiley, New York).
2. Kolb, B. & Tees, R. C., eds. (1990) *The Cerebral Cortex of the Rat* (MIT Press, Cambridge, MA).
3. Finlay, B. L., Innocenti, G. & Scheich, H., eds. (1991) *The Neocortex: Ontogeny and Phylogeny* (Plenum, New York).
4. O'Leary, D. D. M. & Stanfield, B. B. (1989) *J. Neurosci.* **9,** 2230–2246.
5. Santacana, M., Heredia, M. & Valverde, F. (1990) *Dev. Brain Res.* **56,** 217–222.
6. Jhaveri, S., Erzurumlu, R. S. & Crossin, K. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4489–4493.
7. Schlaggar, B. L. & O'Leary, D. D. M. (1991) *Science* **252,** 1556–1560.
8. O'Leary, D. D. M. (1989) *Trends Neurosci.* **12,** 400–406.
9. Killackey, H. P. (1990) *J. Cog. Neurosci.* **2,** 1–17.
10. McConnell, S. K. (1991) *Annu. Rev. Neurosci.* **14,** 269–300.
11. Kuljis, R. O. & Rakic, P. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5303–5306.
12. Rakic, P., Suner, I. & Williams, R. W. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2083–2087.
13. Rakic, P. (1988) *Science* **241,** 170–176.
14. Johnson, J. G. & Van Der Kooy, D. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1066–1070.
15. Bayer, S. A. & Altman, J. (1991) *Neocortical Development* (Raven, New York).
16. Campbell, M. J., Lewis, D. A., Benoit, R. & Morrison, J. H. (1987) *J. Neurosci.* **7,** 1133–1144.
17. Stephenson, D. T. & Kushner, P. D. (1988) *J. Neurosci.* **8,** 3035–3056.
18. McGuire, P. K., Hockfield, S. & Goldman-Rakic, P. S. (1989) *J. Comp. Neurol.* **288,** 280–296.
19. Zilles, K., Wree, A. & Dausch, N.-D. (1990) in *The Cerebral Cortex of the Rat*, eds. Kolb, B. & Tees, R. C. (MIT Press, Cambridge, MA), pp. 113–150.
20. Arimatsu, Y., Naegele, J. R. & Barnstable, C. J. (1987) *J. Neurosci.* **7,** 1250–1263.
21. Arimatsu, Y. & Miyamoto, M. (1991) *Dev. Brain Res.* **58,** 189–201.
22. Paxinos, G. & Watson, C. (1986) *The Rat Brain in Stereotaxic Coordinates* (Academic, North Ryde, Australia).
23. Hicks, S. P. & D'Amato, C. J. (1968) *Anat. Rec.* **160,** 619–634.
24. Miller, M. W. (1988) in *Cerebral Cortex*, eds. Jones, E. G. & Peters, A. (Plenum, New York), Vol. 7, pp. 133–175.
25. Valverde, F., Facal-Valverde, M. V., Santacana, M. & Heredia, M. (1989) *J. Comp. Neurol.* **290,** 118–140.
26. Rockel, A. J., Hiorns, R. W. & Powell, T. P. S. (1980) *Brain* **103,** 221–244.
27. Winfield, D. A., Gatter, K. C. & Powell, T. P. S. (1980) *Brain* **103,** 245–258.
28. Sofroniew, M. V., Campbell, P. E., Cuello, A. C. & Eckenstein, F. (1985) in *The Rat Nervous System*, ed. Paxinos, G. (Academic, North Ryde, Australia), Vol. 1, pp. 471–486.
29. Hendry, S. H. C., Schwark, H. D., Jones, E. G. & Yan, J. (1987) *J. Neurosci.* **7,** 1503–1519.
30. Bjorklund, A., Hokfelt, T., eds. (1985) *Handbook of Chemical Neuroanatomy* (Elsevier, Amsterdam), Vol. 4, Part I.
31. Bjorklund, A., Hokfelt, T. & Kuhar, M. J., eds. (1990) *Handbook of Chemical Neuroanatomy* (Elsevier, Amsterdam), Vol. 9, Part II.
32. Lund, R. D. & Mustari, M. J. (1977) *J. Comp. Neurol.* **173,** 289–306.
33. Catalano, S. M., Robertson, R. T. & Killackey, H. P. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2999–3003.
34. Blakemore, C. & Molnar, Z. (1990) *Cold Spring Harbor Symp. Quant. Biol.* **55,** 491–503.
35. Barbe, M. F. & Levitt, P. (1991) *J. Neurosci.* **11,** 519–533.
36. Levitt, P. (1984) *Science* **223,** 299–301.
37. Austin, C. P. & Cepko, C. L. (1990) *Development* **110,** 713–732.
38. McConnell, S. & Kaznowski, C. E. (1991) *Science* **254,** 282–285.
39. Renfranz, P. J., Cunningham, M. G. & McKay, R. D. G. (1991) *Cell* **66,** 713–729.
40. Iacovitti, L. (1991) *J. Neurosci.* **11,** 2403–2409.
41. Reh, T. A. & Tully, T. (1986) *Dev. Biol.* **114,** 463–489.