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Authors

Bina, K G
Guzman, P
Broide, R S
et al.

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Localization of α7 Nicotinic Receptor Subunit mRNA and α-Bungarotoxin Binding Sites in Developing Mouse Somatosensory Thalamocortical System

KESHAVAN G. BINA, PATRICIA GUZMAN, RON S. BROIDE, FRANCES M. LESLIE, MARTIN A. SMITH, AND DIANE K. O'DOWD
Departments of Anatomy and Neurobiology (K.G.B., P.G., M.A.S., D.K.O.), Developmental and Cell Biology (K.G.B., P.G., D.K.O.), and Pharmacology (R.S.B., F.M.L.), University of California, Irvine, California 92717

ABSTRACT

Previous studies in rat, showing a transient pattern of expression of the α7 nicotinic acetylcholine receptor in the ventrobasal thalamus and barrel cortex during the first 2 postnatal weeks, suggest that these receptors may play a role in development of the thalamocortical system. In the present study, in situ hybridization and radiolabeled ligand binding were employed to examine the spatiotemporal distribution of α7 mRNA and α-bungarotoxin binding sites in the thalamocortical pathway of mouse during early postnatal development. As in the rat, high levels of α7 mRNA and α-bungarotoxin binding sites are present in the barrel cortex of mouse during the first postnatal week. Both α7 mRNA and its receptor protein are observed in all cortical laminae, with the highest levels seen in the compact cortical plate, layer IV, and layer VI. When viewed in a tangential plane, α7 mRNA and α-bungarotoxin binding sites delineate a whisker-related barrel pattern in layer IV by P3–5. Quantitative analysis reveals a dramatic decrease in the levels of expression of α7 mRNA and α-bungarotoxin binding sites in the cortex by the end of the second postnatal week. Unlike in the rat, only low levels of α7 mRNA or α-bungarotoxin binding sites are present in the ventrobasal complex of the mouse thalamus. The broad similarities between the thalamocortical development of rat and mouse taken together with the present results suggest that α7 receptors located on cortical neurons, rather than on thalamic neurons, play a role in mediating aspects of thalamocortical development.

Indexing terms: barrel cortex, ventrobasal complex, acetylcholine, basal forebrain, development

In the mature rodent, the face region of the somatosensory cortex is characterized by aggregates of neurons within layer IV, called barrels, that are organized in a somatotopic pattern reflecting the distribution of facial vibrissae on the contralateral snout (Woolsey and Van der Loos, 1970; Welker and Woolsey, 1974). Neurons within a barrel are innervated by discrete clusters of thalamocortical fibers arising from individual barreloids in the ventrobasal thalamus (Agmon et al., 1995). This highly organized thalamocortical system emerges during early postnatal development. Cortical barrels in both mouse and rat are first observed on postnatal day 3 (P3) as the neurons of layer IV are differentiating from the overlying compact cortical plate (Rice et al., 1985). Thalamocortical fibers also invade the barrel cortex during this first postnatal week, forming two distinct tiers of terminations; a lower tier at the border between layers V and VI and whisker-specific clusters in layer IV (Senft and Woolsey, 1991; Agmon et al., 1993 Functional synaptic connections between thalamocortic axons and neurons in the barrel cortex have been observe by P2 in layer IV (Agmon and O'Dowd, 1992) and as early a P0 in layers V and VI (Agmon and O'Dowd, 1994).

In an effort to identify cues that may be involved i mediating the development of this highly precise structur and functional organization in the thalamocortical systen a number of studies have focused attention on molecule that show transiently elevated expression in the barr cortex, coincident with the development of both the pre and postsynaptic elements in the thalamocortical pathwa For instance, during the first 2 weeks of postnatal life, th

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Address reprint requests to Dr. Diane K. O'Dowd, Department of Anatom and Neurobiology, University of California at Irvine, Irvine, CA 92717.

pattern of serotonergic innervation (D'Amato et al., 1987; Rhoades et al., 1990) and the localization of serotonin receptors (Leslie et al., 1992; Bennett-Clarke et al., 1993) in the somatosensory cortex of the rat closely match the distribution of thalamocortical afferents and organization of cortical barrels. Consistent with the hypothesis that serotonin plays an important role in the development of thalamic innervation of the primary somatosensory cortex, a recent study has shown that serotonin depletion during early postnatal development decreases the cross-sectional areas of the patches of thalamocortical afferents corresponding to the long mystacial vibrissae in rat (Bennett-Clarke et al., 1994). Patterns of intense acetylcholine esterase (AChE) staining that occur transiently in the developing thalamocortical afferents and their terminal fields in the rat barrel cortex (Kristt, 1979), have led to the suggestion that this molecule, the degrading enzyme for acetylcholine (ACh), may also be important in development of the thalamocortical system (Robertson, 1987). Although there are also AChE-positive axons in the somatosensory cortex of early postnatal mouse, the organization and development of the pattern of staining differs between rat and mouse (Kristt and Waldman, 1982).

Recent studies have suggested that the neuronal α-bungarotoxin (α-BTX)-binding nicotinic acetylcholine receptor (nAChR) may also serve as a candidate for a protein that might be involved in the development of the thalamocortical system. First, in rat, α7 mRNA encoding a subunit of this nAChR (Couturier et al., 1990; Schoepfer et al., 1990; Séguela et al., 1993) and α-BTX binding sites, are developmentally regulated, with high levels found in several thalamic nuclei and sensory cortices during the first 2 postnatal weeks (Fuchs, 1989; Broide et al., 1995). Second, in chick, several studies suggest that α7 nAChR may play a role in development of neurons in the peripheral nervous system. In chick ciliary ganglion neurons this receptor functions as a ligand-gated ion channel that is activated by nicotinic agonists (Zhang et al., 1994). Activation of this receptor leads to elevated levels of intracellular free calcium (Vijayaraghavan et al., 1992) and induction of neurite retraction (Pugh and Berg, 1994). Third, a potential source of endogenous ACh that could activate α7 nAChR in the thalamocortical system is indicated by the presence of choline acetyltransferase (ChAT, the synthetic enzyme for ACh) activity in mouse somatosensory cortex as early as postnatal day 4 (Höhmann et al., 1988). Finally, neonatal lesions of the mouse basal forebrain, resulting in cortical cholinergic deafferentation (Höhmann et al., 1988), leads to an alteration in the projection of thalamocortical afferents into the somatosensory cortex (Höhmann et al., 1991). Taken together, these studies suggest that α7 nAChR may be involved in the development of the rodent thalamocortical system.

Given the many similarities in the morphological and electrophysiological development of the thalamocortical system between rat and mouse, proteins that play a major role in its development should show a similar distribution in both species. Therefore, in the present study we examined the distribution of α7 mRNA and α-BTX binding sites in the pre- and postsynaptic elements of the thalamocortical pathway of mouse during the first 2 postnatal weeks. We report areas of overlap in expression patterns between the two species in the cortex, consistent with the possibility that α7 nAChR located on cortical neurons are involved in development of the thalamocortical system in rodents. However, in contrast to the high levels of α7 mRNA and α-BTX binding sites observed in rat ventrobasal complex of the thalamus (VB), only low levels were observed in mouse, suggesting that α7 nAChR located presynaptically are unlikely to play a major role in development of the thalamocortical system in rodents. A preliminary report of this study has been presented (Bina et al., 1994).

MATERIALS AND METHODS

Animals and tissue preparation

Timed pregnant HSD:ICR mice (Harlan Sprague-Dawley, San Diego, CA) were housed under normal light-dark cycles (LD12:12) with food and water ad libitum. The day of birth was designated as P0. A total of 48 pups, aged P0–P14 and four adults, representing seven experiments, were used in this study. A single experiment consisted of tissue processed in parallel with the same probe.

Animals were anesthetized, either by cooling on ice (ages P0–P4) or by halothane inhalation (P5 and older). Following decapitation, brains were rapidly removed and sliced at a thickness of 800 μm on a Vibroslicer (Campden Instruments) in a plane that preserves thalamocortical connectivity (Agmon and Connors, 1991). Slices were fixed in 2% paraformaldehyde in 0.1 M phosphate buffer (PB) for 1 hour at room temperature, cryoprotected in 20% sucrose in 0.1 M PB at 4°C for 2–4 hours, embedded in O.C.T. compound (Tissue-Tek) on dry ice, and stored at −80°C. In order to view the barrel cortex in its entirety, some brains were sectioned in a tangential plane. To obtain tangential sections, cortices were peeled off, flattened between two glass slides, frozen by immersion in isopentane at −20°C, and stored at −80°C until ready for sectioning. Thalamocortical and tangential slices were resectioned on a cryostat at 20 μm and collected directly on Vectabond (Vector Labs.) coated slides that were then processed for Nissl staining, in situ hybridization, or binding studies. Tangential sections were postfixed in 4% paraformaldehyde for 1 hour at room temperature before being processed for in situ hybridization.

In situ hybridization

Complementary RNA riboprobes were synthesized from 2.1 kb cDNA (the generous gift from Dr. Jim Boulter) of the rat α7 nAChR subunit using [^{35}S]-labeled UTP followed by alkaline hydrolysis after the method previously described (Cox et al., 1984). In initial studies, tissue hybridized to a full-length non-hydrolyzed α7 probe was compared with those hybridized to hydrolyzed subclones. Both probes showed a similar pattern of labeling, but a higher signal-to-noise ratio was obtained with the hydrolyzed probes. In addition, since the various α subunits of the nicotinic receptor share a high degree of sequence homology, the specificity of the rat probe was tested by comparing the pattern of hybridization with that of mouse cRNA probe generated from a short α7 cDNA (333 bp) obtained by the reverse transcriptase-polymerase chain reaction method. These experiments showed an identical pattern of hybridization for both rat and mouse probe. The higher specificity activity of the hydrolyzed rat probe resulted in the highest signal-to-noise ratio and was therefore used for all experiments reported in the present study.

Slide-mounted sections were processed for in situ hybridization using a modification of a method previously described (Simmons et al., 1989). Briefly, sections were pre-incubated in proteinase K solution (0.1 μg/ml) at 25°C for 30 minutes, acetylated for 10 minutes, dehydrated in an

ascending alcohol series, and air dried for 1 hour. Sections were incubated in [^{35}S]-labeled sense or antisense cRNA probe (1×10^7 cpm/ml in a buffer containing 50% formamide, 10% dextran sulfate, 0.02% Ficoll, 0.02% polyvinyl pyrolidone, 0.02% bovine serum albumin, 500 μg/ml tRNA, 10 mM DTT, 0.3 M NaCl, 10 mM Tris, pH 8.0, and 1 mM EDTA, pH 8.0) for 18 hours at 60°C. Slides were then washed in SSC buffer (20 minutes in 4× solution), subjected to RNase digestion (20 μg/ml) for 30 minutes at 37°C, and subjected to further washes in SSC buffer (3 times in 2× solution) at room temperature and a 10 minute wash in 0.5× SSC solution. Subsequently, the sections were dehydrated through an ascending alcohol series, air dried, and exposed to β-max film for 1–2 days at 4°C. Finally, slides were incubated in NTB emulsion (Kodak) for 1–2 weeks, developed in Kodak D-19 developer, fixed, counterstained with cresyl violet, and coverslipped in Permount.

Receptor binding with [^{125}I]BTX

Slide-mounted sections were preincubated in a buffer containing 0.12 M NaCl and 0.05 M Tris-HCl (pH 7.4), air dried, and then incubated in the same buffer containing 5 nM iodo-α-BTX ([^{125}I]BTX; Amersham) for 2 hours in darkness at room temperature. The sections were subsequently washed twice for 10 minutes in ice cold buffer, rinsed for 30 seconds in distilled water, and then air dried. Sections used for non-specific binding were processed as above except that tissues were incubated in a radiolabeled ligand solution containing 10^{-4} M nicotine. All slides along with [^{14}C] brain paste standards were exposed for 1–2 days at 4°C on tritium-sensitive Ultrafilm.

Quantitative analysis of α7 mRNA expression and α-BTX binding

The density of labeling in all layers of the somatosensory cortex, VB, hippocampus, and basal forebrain was quantified from digitized images of the autoradiographic film for in situ hybridization and receptor binding studies. Digitized images were obtained using a video-based image analysis system (Microcomputer Image Device, Imaging Research Inc., Canada). For both in situ hybridization and radioligand binding experiments, a calibration curve of optical density versus ligand concentration (dpm) was constructed using [^{14}C] brain paste standards of known radioactivity. Mean levels of mRNA and protein in all laminae of the somatosensory barrel cortex, VB, hippocampus, and basal forebrain were quantified by direct comparison to the calibration curve. Measurements within each layer in the barrel cortex were made by determining the density profile along a rectangular transept that was 50 μm wide and extended from the pial surface to the white matter border. Cortical layer boundaries were determined by direct comparison with the adjacent Nissl section. Levels of mRNA and binding sites in the basal forebrain and thalamus were measured within a circle 100 μm in diameter. Levels in the hippocampus were determined using a rectangular transept passing through the pyramidal cell layer of the CA2 region, an area that showed the highest levels of expression within the hippocampus. Specific labeling was determined by subtracting non-specific binding from total binding in receptor binding studies and labeling seen with the sense probe was subtracted from that seen with the antisense probe in the in situ hybridization studies.

We also quantified changes in level of α7 mRNA expression from emulsion-dipped slides. This was done by counting the number of neurons and determining the average size of neurons within a fixed area in layer IV or V (65 × 98 μm), from which a total neuronal surface area was calculated. The number of silver grains within the same rectangular area was counted and expressed as grains/total neuronal surface area (grains/μm^2). Due to its relatively rapid dissociation rate in aqueous solutions we were unable to examine emulsion-dipped sections labeled with [^{125}I]α-BTX and therefore grain/μm^2 determinations were not done for α-BTX binding sites.

RESULTS

Localization of α7 mRNA and α-BTX binding sites

The expression of α7 mRNA and α-BTX binding sites was examined in thalamocortical slice preparations (Fig. 1A) obtained from early postnatal animals. The distribution of α-BTX binding sites was coincident with that of α7 mRNA in all of the areas studied (Fig. 1B,C). In somatosensory cortex, both the α7 mRNA and α-BTX binding sites were expressed at high levels; low or undetectable levels were found in the immediately adjacent cortical areas, resulting in a sharp medial boundary (Fig. 1B,C). In contrast, VB, the thalamic nucleus containing the thalamocortical projection neurons, had extremely low levels of α7 mRNA in its entire rostrocaudal extent. The caudal aspects of the basal forebrain, another area that projects to the barrel cortex and is contained within this plane of section, showed a patchy distribution of α7 mRNA (Fig. 1B). Low levels of α-BTX binding sites were also seen at this age (Fig. 1C); however, higher levels were seen in older animals (Fig. 3B). Levels of hybridization and α-BTX binding observed with the sense probe and 10^{-4} M nicotine, respectively, were low and similar to that seen in the tissue sections (data not shown).

Mean levels of specific α7 mRNA expression and density of α-BTX binding sites in layer IV of the barrel cortex, basal forebrain, hippocampus (pyramidal cell layer in CA2), and VB were determined within single slices obtained from animals aged P3–P5 (n = 4). As shown in Table 1, the level of α7 mRNA was similar in layer IV and the hippocampus. Levels seen in basal forebrain and VB, however, were significantly lower than those in cortex. The density of α-BTX binding sites was greatest in cortical layer IV, with significantly lower levels seen in hippocampus.

The laminar distribution of α7 mRNA within the somatosensory cortex was examined at higher resolution in emulsion-dipped tissue sections (Fig. 2). Comparison with adjacent sections processed for Nissl staining (Fig. 2A) revealed that α7 mRNA was found in all cortical layers of the somatosensory cortex. The highest levels of expression were observed in the cortical plate, layer IV, upper half of layer VI, and subplate, with moderate to low levels in layer V, the lower half of layer VI, and layer I (Fig. 2B). Due to dissociation of the probe, we were unable to examine emulsion-dipped sections labeled with [^{125}I]α-BTX. However, the laminar distribution of α-BTX binding sites observed in film autoradiograms was similar to that seen for α7 mRNA (Fig. 1C).

Developmental regulation of α7 mRNA and α-BTX binding sites in the barrel cortex

To determine whether α7 mRNA expression and α-BTX binding sites within the somatosensory cortex were develop-

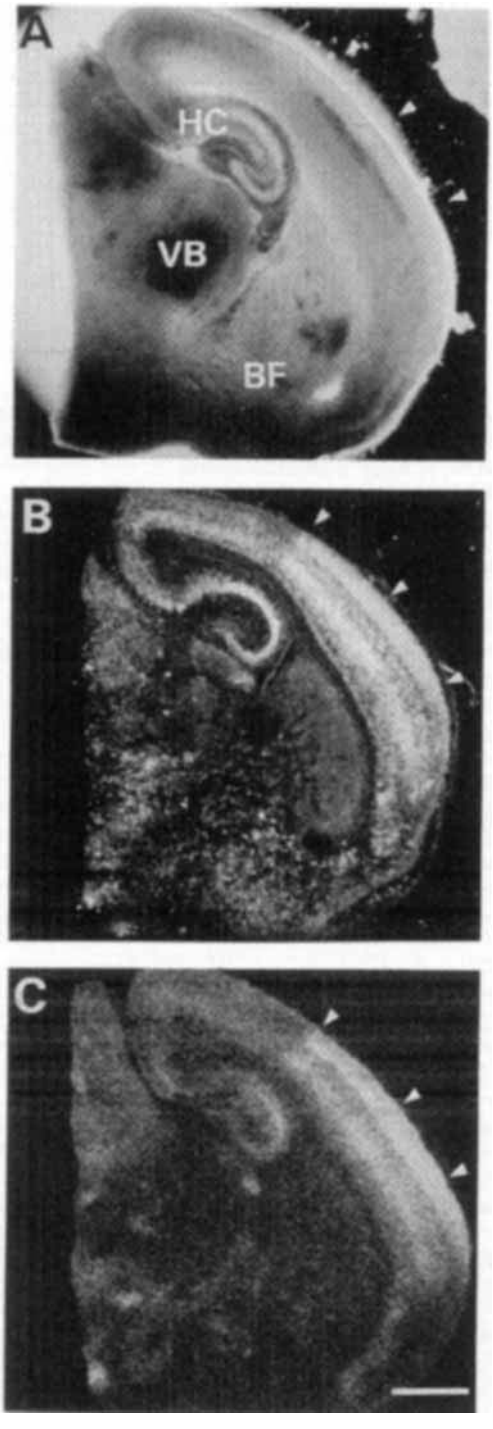

Fig. 1. Localization of α7 mRNA and α-bungarotoxin (α-BTX) binding sites in the thalamocortical slice. **A:** A 500 μm thick, transilluminated, unstained, thalamocortical slice preparation obtained from a P4 animal showing the location of the ventrobasal nucleus of the thalamus (VB), somatosensory barrel cortex (region indicated by white arrowheads), hippocampus (HC) and basal forebrain (BF). **B:** Darkfield photomicrograph showing in situ hybridization with an α7 antisense ^{35}S cRNA probe in a thalamocortical section obtained from a P4 animal. **C:** Photograph of an autoradiogram of an adjacent section showing a very similar pattern in the binding of ^{125}I-labeled α-BTX. Scale bar = 500 μm.

TABLE 1. Levels of α7 mRNA and α-BTX Binding Sites in Four Areas Within Thalamocortical Slices of Animals Aged P3–5 (n = 4)[1]

	Areas in the thalamocortical slice			
	Ctx IV	HC	BF	VB
α7 mRNA (mean dpm ± SEM)	646 ± 68	758 ± 88	212 ± 23***	113 ± 6***
α-BTX binding sites (mean dpm ± SEM)	4,159 ± 887	1,634 ± 285*	1,028 ± 159**	367 ± 65**

[1]Ctx IV, layer IV in barrel cortex; HC, CA2 region of hippocampus; BF, basal forebrain; VB, ventrobasal complex of the thalamus. Asterisks represent levels that were significantly different from those observed in Ctx IV by student's t-test.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

mentally regulated, we examined the distribution and levels of expression of both α7 mRNA (Fig. 3A) and α-BTX binding sites (Fig. 3B) at daily intervals during the first 2 postnatal weeks. In animals aged P0–P2 a superficial band of intense labeling was associated with the compact cortical plate, whereas a deep band was located in upper layer VI. A slightly lower level of labeling was also seen in layer V. At P3–P5, labeling was associated with all the laminae labeled at P0–P2 and with the newly differentiated layer IV. By P5–P6 (not shown), both mRNA and receptor protein expression in the superficial band was highest in layer IV, with lower levels occurring on either side in the supragranular layers (layers II and III) and infragranular layer (layer V). Between P6 and P14, there was a reduction in the intensity of α7 mRNA and α-BTX binding sites in both the superficial and deep bands. Cortical expression of α7 mRNA and α-BTX binding sites was comparatively low and more homogeneously distributed throughout the barrel cortex by P10 and P14, respectively. This contrasts with the high but constant levels of expression of both α7 mRNA and α-BTX binding sites seen in the hippocampus during the same developmental period (Figs. 3, 9).

To quantify changes in the expression of α7 mRNA and α-BTX binding sites, computer-generated density profiles spanning the cortical depth were obtained from film autoradiographic images (Fig. 4). The younger animals (P4, P6) showed peak levels of expression of both α7 mRNA and α-BTX binding sites in layers IV and upper layer VI, with intermediate levels seen in the more superficial layers and layer V. In older animals (P14) the density profiles showed low levels of expression in all of the cortical laminae. The average level of expression for both α7 mRNA and density of α-BTX binding sites in layers IV, V, and upper VI was determined using density profiles from two to three sections for each animal. To pool data obtained from separate experiments, one or more animals in the P0–P2 age group were included in each experiment and the levels of expression in P3 and older animals were expressed as a percentage of the levels seen in P0–P2 animals run in the same experiment. Data from seven and five experiments were used to describe the developmental changes in expression of α7 mRNA (Fig. 5) and α-BTX binding sites (Fig. 6), respectively.

The α7 mRNA levels in layer IV of animals P3 and older were expressed as a percentage of the levels of labeling seen in compact cortical plate, since layer IV has not differentiated by P0–P2 (control group). Over the first 2 postnatal weeks there was a dramatic reduction in the intensity of labeling in layer IV, with adult levels of expression (26 ± 8% of the newborns) reached by P12–14 (Fig. 5A). A steady decline in α7 mRNA expression was also seen in layer V during this same time period (Fig. 5B). Expression levels in

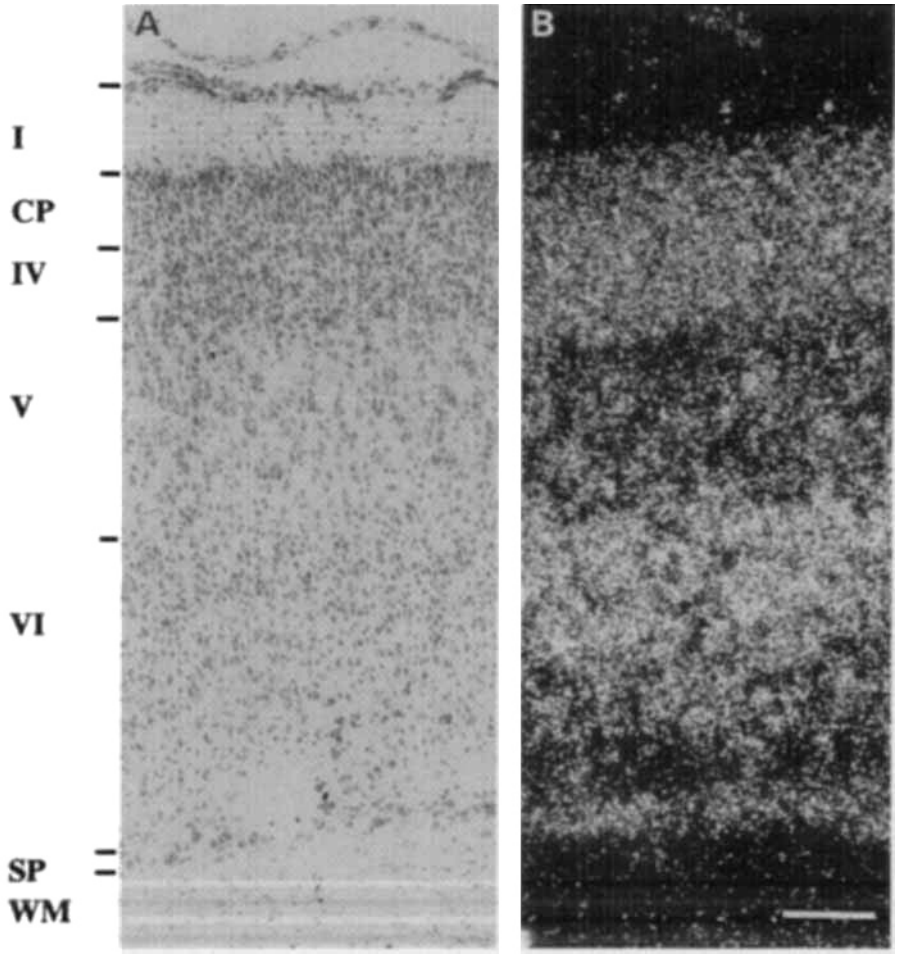

Fig. 2. Laminar distribution of α7 mRNA in the barrel cortex. **A:** High magnification photomicrograph of a Nissl-stained section through the somatosensory barrel cortex of a P4 animal. Layer boundaries are indicated to the left. CP, cortical plate; SP, subplate; WM, white matter. Scale bar = 100 μm. **B:** Darkfield photomicrograph of an adjacent section showing α7 mRNA localization.

layer VI remained high for a longer time, with the decline beginning only in the second postnatal week (Fig. 5C).

Parallel analysis of the density of α-BTX binding sites demonstrated a peak level in layer IV at P3–P5 (153 ± 23% of the levels seen in compact cortical plate of P0–P2 animals; Fig. 6A) with a steady decline during the first 2 postnatal weeks. Adult levels, approximately 30% of the P0–P2 level, were reached sometime after the end of the second postnatal week. In contrast to the mRNA levels, there was a remarkably constant level of α-BTX binding sites in layers V and VI during the first 2 postnatal weeks, with lower levels only observed in adults (Fig. 6B,C).

Developmental increases in cell size and decreases in cell packing density in the different cortical laminae during the first 2 weeks of postnatal development may have contributed to the developmental decrease in density of α7 mRNA and α-BTX binding sites determined from the film autoradiographic images. Therefore we examined the change in α7 mRNA expression, in layers IV and V, at higher resolution in tissue sections that had been processed for in situ hybridization and dipped in autoradiographic emulsion. A qualitative analysis revealed that, although there was a small decrease in neuron density and an increase in cell size between P4 and P12, a dramatic decrease in grain density throughout both layers IV and V was apparent (Fig. 7A,B). Quantitative analysis, in sections from four animals each in the P3–P5 and P12–P14 age groups, demonstrated a significant fourfold decrease in grains/μm^2 between P3–P5 and P12–P14 in layer IV (Fig. 7C). There was a smaller but still significant twofold decrease in grains/μm^2 in layer V. These data suggest that there is a reduction in α7 mRNA expression/neuron during the first 2 postnatal weeks.

To determine the organization of α7 nAChR in the horizontal plane we examined α7 mRNA and protein expression in tangential sections at the level of layer IV of the somatosensory cortex between P0 and P5. At P0, both α7 mRNA and α-BTX binding sites were present and appeared to be homogeneously distributed in the compact cortical plate (Fig. 8A,B). The pattern of α7 mRNA and α-BTX binding sites in layer IV started to display a patchy appearance by P3 (not shown). At P5, the α-BTX binding sites, and to a lesser extent α7 mRNA expression, in layer IV exhibited a pattern of localization that corresponded to individual barrel centers with clearly defined and unlabeled septal areas (Fig. 8C,D).

To determine whether the expression of α7 mRNA or the density of α-BTX binding sites changed during early postnatal development, in areas other than in the barrel cortex, we

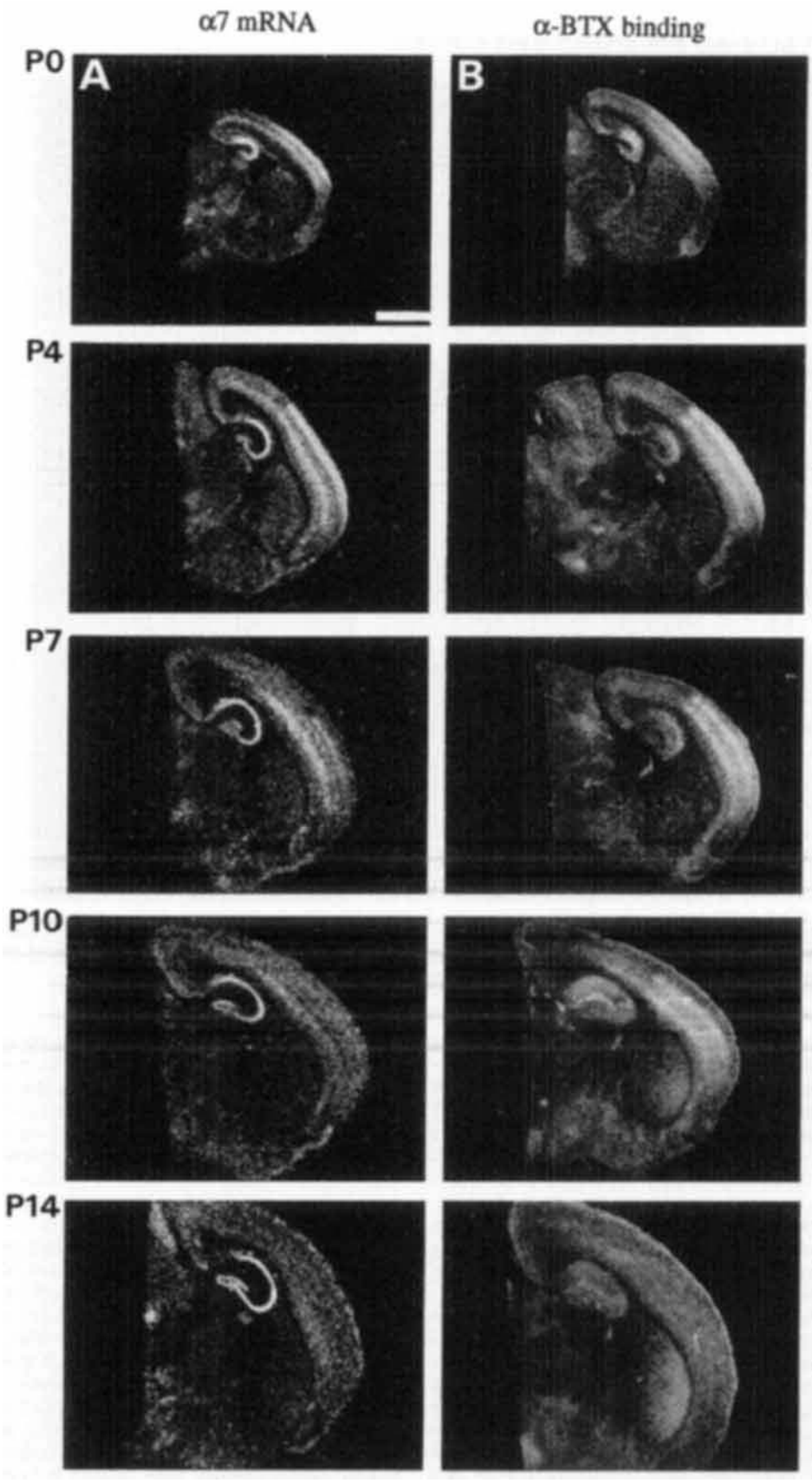

Fig. 3. Qualitative analysis of developmental changes in α7 mRNA expression and α-BTX binding in the somatosensory cortex. Low magnification autoradiograms of thalamocortical slices illustrating developmental changes in the distribution and levels of expression of α7 mRNA (**A**) and α-BTX binding (**B**) sites. Scale bar = 2 mm.

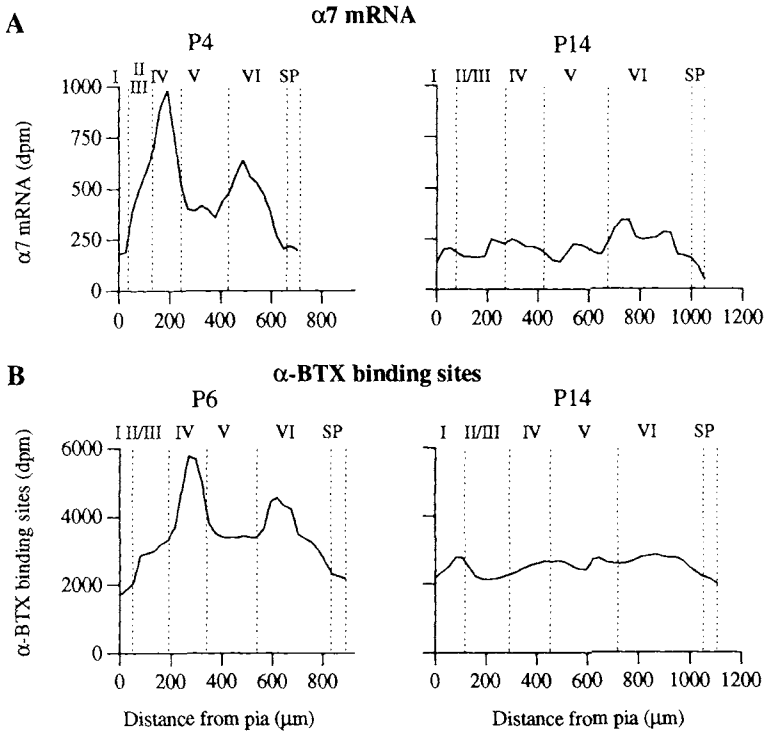

Fig. 4. Density profiles of α7 mRNA and α-BTX binding sites in the barrel cortex. **A:** Levels of α7 mRNA expression (dpm) in the somatosensory cortex of a P4 and P14 animal with layer boundaries determined from adjacent Nissl sections, marked by the dotted lines. **B:** Levels of α-BTX binding sites (dpm) in a P6 and P14 animal.

quantitatively compared the levels of expression of both mRNA and protein in layer IV of the barrel cortex, CA2 region of the hippocampus, basal forebrain, and VB obtained from animals early in the first postnatal week, with those obtained from animals at the end of the second postnatal week from film autoradiograms. As illustrated in Figure 9, in layer IV of the barrel cortex there was a two to fourfold reduction in the level of α7 mRNA expression (Fig. 9A) and number of α-BTX binding sites (Fig. 9B) of P12–P14 animals when compared with P3–P5 animals. Although there was also a twofold decrease observed in VB, the absolute levels in this nucleus were very low at both ages. In contrast, no significant difference was observed in the levels of α7 mRNA or the number of α-BTX binding sites in either hippocampus or basal forebrain (Fig. 9A,B).

DISCUSSION

This study demonstrates that high levels of α7 mRNA and α-BTX binding sites are expressed in the mouse somatosensory cortex during early postnatal development. Levels of both α7 mRNA and α-BTX binding sites are developmentally regulated, with the highest levels found in the first postnatal week. This transient elevation in levels of α7 nAChR coincides with the initial formation and maturation of functional thalamocortical synapses and with the morphological development of cortical neurons, consistent with the possibility that α7 nAChRs may be involved in one or more aspects of this developing system.

Previous studies in rat have shown high levels of α7 mRNA and α-BTX binding sites in both pre- and postsynaptic elements of the thalamocortical pathway (Fuchs, 1989; Broide et al., 1995), suggesting that α7 nAChR, located on cortical neurons and/or on axons or terminals of VB neurons, may play a role in thalamocortical development in rodents. Several lines of evidence from the present study suggest that in mouse, α7 nAChR are located primarily on cortical neurons rather than on thalamocortical afferents. First, unlike rat, only very low levels of both α7 mRNA and α-BTX binding sites are expressed in the VB, thus making it unlikely that α7 nAChR associated with these thalamocortical axons or their terminals make a significant contribution to the expression observed in the cortex. Second, high levels of expression of both α7 mRNA and α-BTX binding sites in the compact cortical plate of the neonatal barrel cortex are observed at an age prior to the arrival of the majority of thalamocortical afferents (Senft and Woolsey, 1991; Agmon et al., 1993). Finally, by the end of the first postnatal week, both α7 mRNA and α-BTX binding sites are found in layers (I, II, and III) in which thalamocortical axons are either sparse or excluded. Therefore, it is unlikely that expression of α7 nAChR receptors on thalamocortical axons or their terminals plays a critical role in the development of the thalamocortical pathway in rodents.

Another region that shows distinctly different expression patterns in mouse and rat is the compact cortical plate. In mouse very high levels of α7 mRNA and α-BTX binding sites are observed in the compact cortical plate between P0 and P4. However, only low levels of both the α7 mRNA and α-BTX binding sites are reported in rat (Fuchs, 1989; Broide et al., 1995). These findings suggest that expression of the nAChR in this region is not likely to be of fundamental importance to developmental processes common to both species.

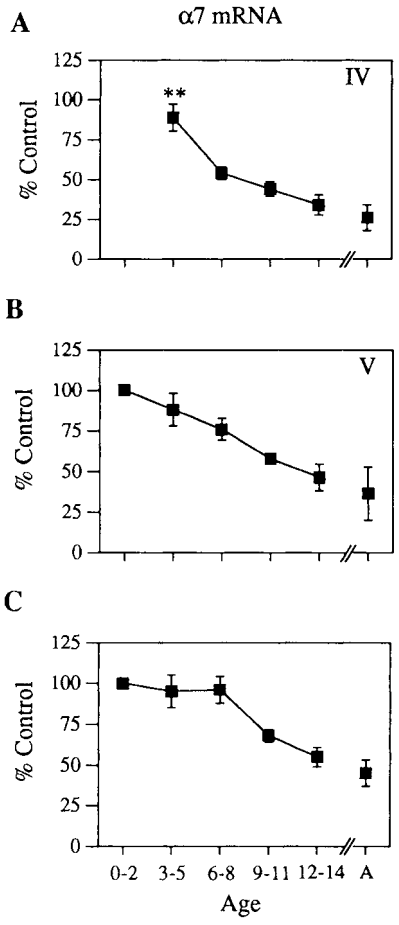

Fig. 5. Decline in the levels of α7 mRNA in cortical layers IV, V, and VI over the first 2 postnatal weeks. Mean levels of expression in layers IV (**A**), V (**B**), and VI (**C**) of the somatosensory cortex were determined from the autoradiographic density profiles obtained from a total of 6–11 animals in each age group, with the exception of adults, in which data were obtained from 4 animals. Tissue was processed in seven experiments, each containing sections from three or more age groups. To control for possible variability in the specific activity of the ^{35}S cRNA probes used in the different experiments, the levels of α7 mRNA in layers IV, V, and VI in older animals were expressed as a percentage of those seen in cortical plate, layer V, and layer VI, respectively, in P0–P2 (control) animals included in each experiment. Error bars indicate standard error of the mean. **, $P < 0.01$ significantly different from all older age groups (Scheffe's post hoc analysis).

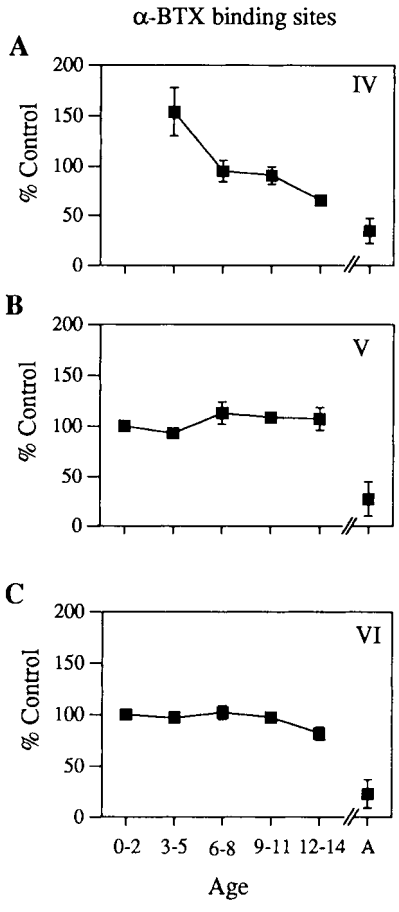

Fig. 6. Decline in α-BTX binding sites in cortical layers IV, V, and VI. Mean levels of α-BTX binding expression in layers IV (**A**), V (**B**), and VI (**C**) of the somatosensory cortex were determined from the autoradiographic density profiles obtained from a total of 6–11 animals in each age group, with the exception of adults, in which data were obtained from 4 animals. Sections were processed in five experiments and levels of α-BTX binding sites in animals P3 and older are expressed relative to the 100% control value obtained from the cortical plate (for layer IV comparison), layer V (for layers V comparison), or layer VI (for layer VI comparison) in animals P0–P2 included in each experiment.

In contrast to the differences in expression patterns detailed above, there are cortical laminae that exhibit similar expression patterns of α7 mRNA and α-BTX in both species. For instance, high levels of α7 mRNA expression are observed in the subplate and upper layer VI during early postnatal development, with significantly lower levels seen in the adult, in both rat (Broide et al., 1995) and mouse. High levels of α-BTX binding sites are also associated with upper layer VI as early as P2 and with the newly differentiating layer IV at P4 in rat (Fuchs, 1989) and mouse. In addition, when examined in the tangential plane of section, the α-BTX expression in both species exhibits a barrel-like distribution in layer IV of the primary somatosensory cortex.

The high levels of expression of α7 nAChR in upper layer VI and layer IV of the somatosensory cortex during the first postnatal week in both species is consistent with their potential involvement in development of two components of the thalamocortical system. First, the elevated levels of α7 mRNA and receptor protein coinciding temporally and spatially with the topological organization of thalamocortical afferents in layer VI and formation of whisker-specific clusters in layer IV (Senft and Woolsey, 1991; Agmon et al., 1993) suggest that these receptors may play a role in the organization of thalamocortical afferent fiber terminals. Consistent with this hypothesis is the finding that neonatal lesions of basal forebrain neurons, a source of ACh that might activate the α7 nAChR in the barrel cortex, resulting in extensive cortical cholinergic deafferentation (Höhmann et al., 1988), leads to altered projection of thalamocortical afferents into the somatosensory cortex (Höhmann et al., 1991). Whereas thalamocortical afferents are not themselves cholinergic, a possible mechanism by which cholinergic depletion might affect the development of these afferents would be through a population of cortical neurons that are activated by both ACh released from the basal forebrain

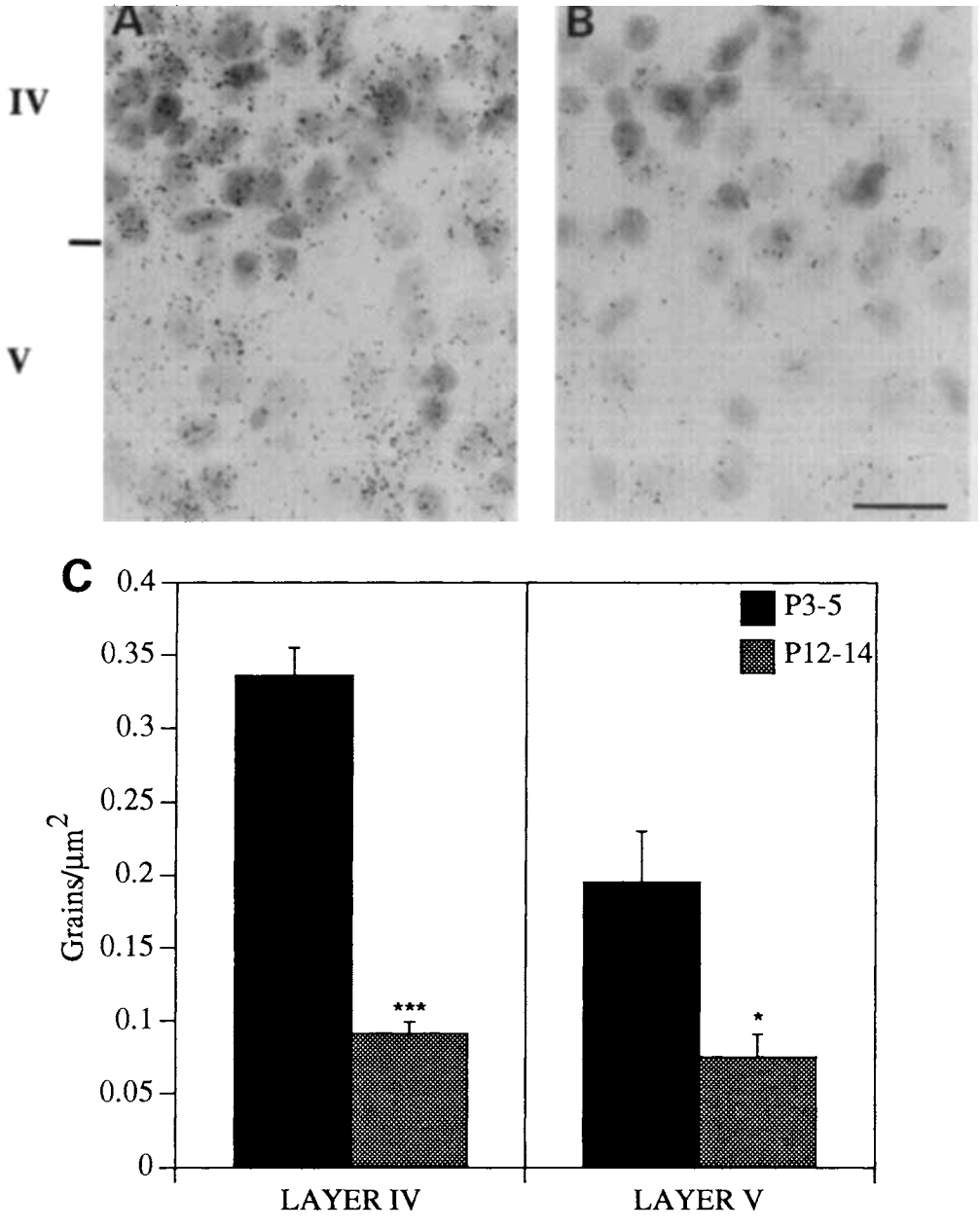

Fig. 7. Developmental decrease in α7 mRNA expression in layer IV and V neurons. High magnification brightfield photomicrographs showing localization of α7 mRNA in layers IV and V of the barrel cortex from P4 (**A**) and P12 (**B**) animals. There is a large decrease in the density of grains seen in both of these layers between P4 and P12. A small decrease in the density of neurons (nuclei are clearly counterstained by cresyl violet) over this same time period is also evident. Scale bar = 20 μm. **C:** To estimate the change in α7 mRNA expression/neuron the average number and size of neurons were determined and mean total neuronal area within a fixed region of each layer determined for tissue obtained from four animals each at P3–5 and P12–14. Grain counts performed within the same area in the same animals were then expressed as a function of mean total neuronal area (grains/μm^2). ***, $P < 0.001$; *, $P < 0.05$; Student's t test.

neurons and glutamate released from VB afferents, which in turn produce retrograde signals that influence the development of thalamocortical afferents. Evidence for the activity of cortical neurons being affected by both ACh and glutamate comes from electrophysiological studies in cat somatosensory cortex showing that basal forebrain stimulation can modify the responses of cortical neurons to pulses of glutamate (Tremblay et al., 1990). In addition, evidence for a role for ACh in mediating developmental plasticity through its interactions with another neurotransmitter system exists within the visual cortex. Combined destruction of cholinergic and noradrenergic inputs to the visual cortex from the basal forebrain and the dorsal adrenergic bundle, respectively, early in development, results in the reduction of monocular deprivation-induced developmental plasticity in kittens (Bear and Singer, 1986). Further experiments, however, will be needed to determine whether signals produced by cortical neurons receiving both cholin-

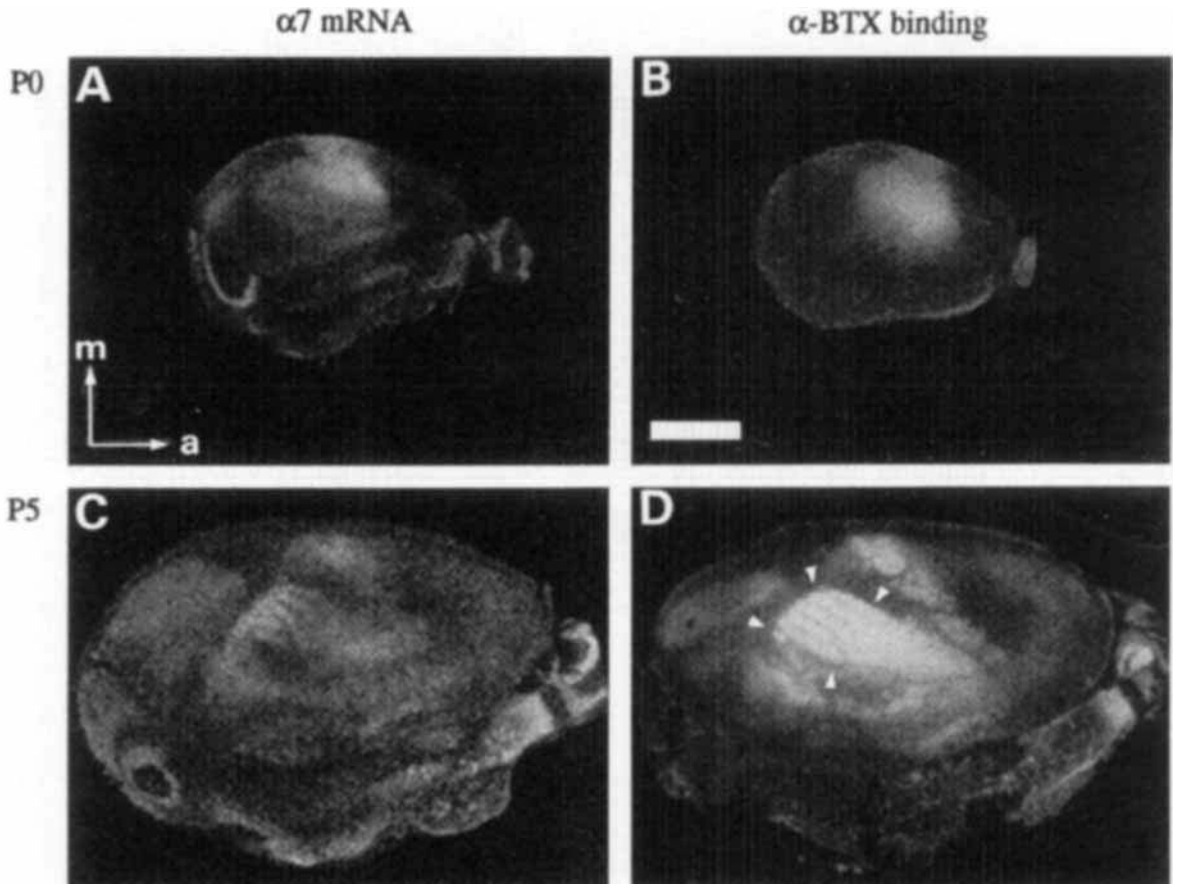

Fig. 8. Changes in the distribution of α-BTX binding and α7 mRNA expression in the tangential plane during development. Digitized autoradiograms of cortical tangential sections. **A:** Expression of α7 mRNA in somatosensory region of cortex at P0 at the level of the cortical plate. **B:** Adjacent section showing α-BTX binding in P0 somatosensory cortex. **C:** Expression of α7 mRNA in a section from a P5 animal through layer IV of the somatosensory cortex. **D:** Adjacent section showing α-BTX binding at P5. Note the distinct barrel-like pattern of expression in somatosensory region indicated by the white arrowheads. a, anterior; m, medial. Scale bar = 2 mm.

ergic and glutamatergic inputs can influence development of thalamocortical afferents.

A second possibility is that α7 nAChRs may play a role in the morphological development of the oriented dendritic fields of neurons located within barrels. During early postnatal development the number of dendrites oriented towards the barrel hollow increases with age and those oriented away from the hollow and toward barrel septae decrease (Greenough and Chang, 1988). Receptors that modulate intracellular calcium levels have been implicated in mediating developmental plasticity (Lipton and Kater, 1989). Previous studies demonstrating that activation of α7 nAChR results in an increase in intracellular calcium levels (Vijayaraghavan et al., 1992) and altered neurite outgrowth (Chan and Quik, 1993; Pugh and Berg, 1994) suggest the possibility that this receptor may play a similar role in the somatosensory cortex. ACh released from the terminals of basal forebrain fibers known to be located in the barrel septae in adult animals (Lysakowski et al., 1989) could potentially activate α7 nAChRs on dendrites of cortical neurons facing barrel septae and result in directed growth of the dendrites toward barrel centers. However, it will be important to determine whether basal forebrain fibers are in the appropriate position and capable of releasing ACh, during this early developmental period.

The onset of the second postnatal week, which marks the end of the critical period for barrel formation in the cortex (Killackey et al., 1990), is coincident with several significant changes in the thalamocortical system that may work toward reducing calcium entry into the cortical neurons through N-methyl-D-aspartate (NMDA) receptors, α7 nAChR, and voltage-gated calcium channels. Although the numbers of NMDA receptors in the neocortex continue to increase well into adulthood (Franklin et al., 1993; Kumar et al., 1994), the onset of inhibition in the second postnatal week reduces the likelihood for agonist-mediated calcium influx into cortical cells through a voltage-dependent blockade of the NMDA receptor (Agmon and O'Dowd, 1992). The decline in levels of α7 nAChR reported in the present study may result in a similar decrease in the likelihood for calcium influx into the cell through a second pathway. Finally, transiently elevated levels of AChE located on thalamocortical afferents that also occur during this second postnatal week (Kristt, 1979; Kristt and Waldman, 1982) may contribute toward reducing the activation of α7 nAChR and thus influx of calcium into the cells. Additional studies will be necessary to determine the functional role of α7 nAChR located on cortical neurons of early postnatal mice, and whether blockade of these receptors during the critical

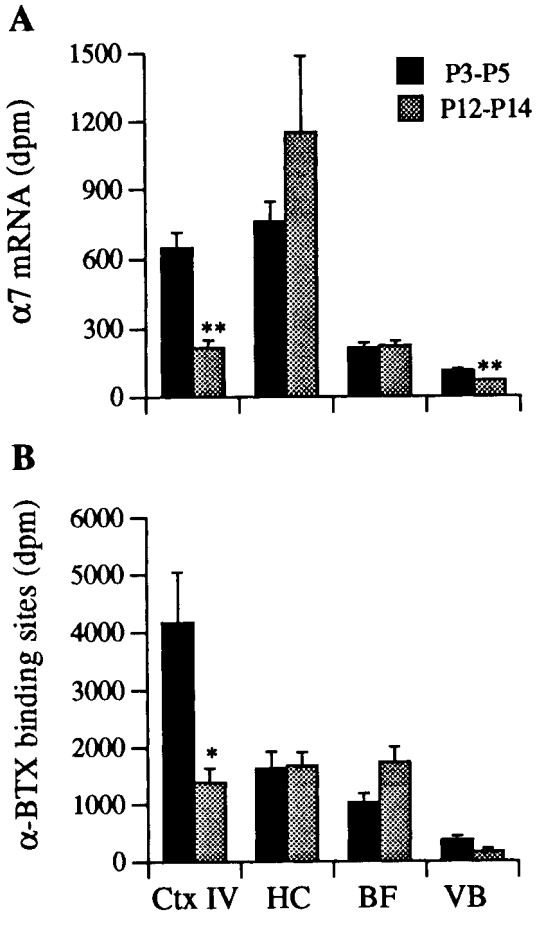

Fig. 9. Quantitative comparison of α7 mRNA expression and α-BTX binding sites in four different regions in slices obtained from animals at P3–5 and P12–14. Mean levels of α7 mRNA expression (**A**) and density of α-BTX binding sites (**B**) in layer IV of the barrel cortex (txIV), basal forebrain (BF), CAZ region of hippocampus (HC), and ventrobasal complex of the hypothalamus (VB) were determined within single slices obtained from animals aged P3–5 (n = 4) and compared with values obtained from animals at P12–14 (n = 3). All of the tissue sections included in these analyses were processed within the same experiment. Level of significance was examined with a Student's t-test. *, $P < 0.05$; **, $P < 0.01$.

period affects normal development of cortical neurons and/or thalamocortical afferents.

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