[\textsuperscript{125}I]α-Bungarotoxin binding marks primary sensory areas of developing rat neocortex

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The postnatal ontogeny of [\textsuperscript{125}I]α-bungarotoxin (α-Btx) binding distribution in rat neocortex was described and quantified using autoradiography of in vitro labeled brain sections. During the first two weeks, distinctive transitory radial and laminar patterns emerged. Dense columnar bands of α-Btx binding extended through the depth of primary sensory cortex, including somatosensory, visual and auditory areas. An association of α-Btx binding with thalamic input zones was further demonstrated within developing somatosensory cortex, where discrete radial bands appeared over the whisker barrels around the time that ingrowing thalamocortical fibers segregate as they selectively innervate the barrels. The early laminar distribution of α-Btx binding also resembled that of developing thalamocortical afferents. From P12 to P20, α-Btx radial distinctions faded and the laminar pattern changed further to achieve the adult distribution. The spatiotemporal ontogeny of α-Btx binding suggests a role for α-Btx binding sites in the development of cortical connectivity.

INTRODUCTION

The relationship between neurochemical parameters of the brain and the development of functional synaptic connections has been a major focus in developmental neurobiology. Although it is clear that a necessary component of synaptogenesis is the development of neurotransmitter receptors, the relation between these events has not been elucidated. Studies in brain homogenates reveal diverse developmental time courses of binding levels among various ligands. α-Bungarotoxin (α-Btx) binding sites, considered putative acetylcholine receptors\textsuperscript{7,55} (but, see Clarke et al.\textsuperscript{6}), apparently develop sooner than a number of neurotransmitter receptor types (e.g. muscarinic\textsuperscript{10,11}, dopaminergic\textsuperscript{2}, α\textsubscript{1}- and α\textsubscript{2}-adrenergic\textsuperscript{41}). [\textsuperscript{125}I]α-Btx binding reaches nearly adult levels in neonatal rodent brain\textsuperscript{10,11,54}. Such early-appearing binding sites are of particular interest as candidate regulators of concurrent developmental processes, particularly synaptogenesis.

The importance of α-Btx binding sites and other putative cholinergic markers has been suggested in previous developmental studies. An autoradiographic analysis of developing rat hippocampus showed transitory changes in α-Btx binding, suggesting that the binding sites may have a special role in ontogeny\textsuperscript{20}. In addition, α-Btx binding sites were found to be necessary for the development and subsequent maintenance of retino-tectal connections in fish and toad species\textsuperscript{13}. Acetylcholinesterase (AChE) activity in the neocortex exhibits transient developmental changes\textsuperscript{32,47}, perhaps reflecting involvement of acetylcholine systems in the formation of neural connections\textsuperscript{12}. In the present study, α-Btx binding site distribution was characterized in developing rat using quantitative receptor autoradiography. The neocortex was chosen as the focus of this study because of its prolonged postnatal development and fairly well characterized anatomical organization. α-Btx binding showed major reorganization during the first 3 postnatal weeks, with transitory radial and laminar-specific patterns resembling the distribution of thalamocortical innervation.
These results were previously reported in abstract form\textsuperscript{14}.

MATERIALS AND METHODS

Subjects. The subjects were male Long-Evans hooded rats (Simonsen, Gilroy, CA). At least two rats were used at each of the following postnatal ages in days, where P0 is the day of birth: P0, 2, 4, 6, 8, 10, 12, 14, 17, 20 and 31. Adult rats 3 months of age were also examined. Each animal was taken from a litter of 6–10, and each member of the same age group was taken from a different litter. Animals were kept on a 12:12 light–dark cycle and were sacrificed between 09.00 and 13.00 h.

Histology. Rats were killed by decapitation, and the brains were immediately dissected out and frozen in $-30\,^\circ\text{C}$ isopentane. The rats were not perfused, as perfusion might introduce age-related variance. Coronal brain sections $16\,\mu\text{m}$ thick were then cut in a cryostat and thaw-mounted onto acid-cleaned, gelatinized slides. Generally, every 4th section through the neocortex was saved for $\alpha$-Btx autoradiography. The remaining series were stained for AChE activity by a modified Koelle method\textsuperscript{4} or were later used for examination of other ligands.

Additional brains from P10 rats were sectioned tangentially through the somatosensory cortex. Sections were air-dried for 0.5–3 h and then stored desiccated at $-70\,^\circ\text{C}$ for up to 5 weeks. Sections can be stored under these conditions without evident effect on $\alpha$-Btx binding for at least 3 months (personal observations). Following the binding and autoradiography, the sections were Nissl-stained with Cresyl violet.

Radioactivity standards. Five iodine-125 standards were prepared by mixing $[^{125}\text{I}]\alpha$-Btx with brain paste\textsuperscript{52} to achieve isotope concentrations ranging from 41 to 7177 dpm per mg wet wt. of tissue. For each brain paste standard, 16-$\mu\text{m}$ cryostat sections were collected on microscope slides, and alternate sections were weighed and counted on a gamma counter (Beckman LS 8000). Radioactivity values in dpm were converted to molar quantities of $\alpha$-Btx bound (fmol/mg wet wt. of tissue) based on the initial specific activity of the $[^{125}\text{I}]\alpha$-Btx and the 60-day half-life of iodine-125. In this experiment, 1 fmol of $[^{125}\text{I}]\alpha$-Btx was equivalent to 506 dpm.

$[^{253}]\alpha$-Bungarotoxin binding. The $\alpha$-Btx binding procedure was based on methods described previously\textsuperscript{6,8}. Brain sections were incubated at room temperature for 60 min in the ligand solution, consisting of 5 nM of $[^{253}]$iodo-$\alpha$-bungarotoxin (initial spec. act. 230 Ci/mmol, Amersham), 0.12 M NaCl and 0.05 M Tris-HCl (pH 7.4), with 3 mg/ml bovine serum albumin added. Sections were then washed at 4 $^\circ\text{C}$ sequentially in four 15-min buffer rinses (0.12 M NaCl and 0.05 M Tris-HCl, pH 7.4) and two 30-s distilled water rinses. Brain sections were drained and dried in a stream of dehydrated, cooled air\textsuperscript{57}.

Sections for assessing non-specific binding were taken from P10 and adult rats. These sections were pre-incubated for 30 min in buffer with 4 concentrations of unlabeled competitor, either nicotine ($10^{-7}, 10^{-6}, 10^{-5}$ or $10^{-4}$ M) or the ACh analogue carbamyl chloride ($10^{-6}, 10^{-5}, 10^{-4}$ or $10^{-3}$ M) (Sigma). Sections were further processed as above, but with unlabeled competitor in the ligand solution.

Autoradiography. The brain sections and radioactivity standards were exposed to tritium-sensitive Ultrasensitive film (LKB, Gaithersburg, MD) for 7 days. The film was processed according to the manufacturer's instructions.

Data analysis. The autoradiographs were quantified using a video-based computerized image analysis system (MCID, Imaging Research, St. Catharines, Ont., Canada). A digitized image of the transilluminated film autoradiograph was displayed on a color monitor, and regions to be measured were outlined using a mouse. Average transmittance values for the pixels within each outlined region were automatically calibrated with reference to a best-fit equation based on the iodine-125 standards. Readings were typically obtained from the right and left sides of at least 3 sections per animal. Quenching corrections were not used because iodine-125 shows little or no differential quenching between gray and white matter\textsuperscript{17}.

To verify the location of autoradiographic features with respect to cytoarchitectonic landmarks, features of autoradiographic and Nissl sections were traced separately using a projection microscope, and the drawings were then superimposed. In addition, the image analysis system was used to do ‘redirected sampling’, in which the autoradiographic and corre-
sponding Nissl sections were initially superimposed and separately stored so that areas outlined on the Nissl section could then be used to designate the autoradiographic region to be analyzed. Cortical areas in animals of ages P10 through adult were identified by cytoarchitectonics and topography as described elsewhere. Cortical areas for pups P8 and younger were identified by comparing series of autoradiographic and Nissl sections from progressively younger animals.

RESULTS

During the first 2–3 postnatal weeks, $[^{125}\text{I}]\alpha$-Btx binding in the rat neocortex showed distinctive transitory radial and laminar patterns. From P2 to P18, radial stripes extended in columnar fashion through the cortical laminae. Highest binding levels appeared mainly in primary sensory neocortex: somatosensory (SI), visual (area 17) and auditory (Tel) (see Figs. 1 and 2). Labeling was also dense in granular insular cortex, which receives somatosensory information from the tongue. Lower binding levels were characteristic of remaining neocortical regions, such as motor cortex and secondary sensory or association areas. The radial pattern was in general more clearly demarcated in laminae II–III, IV, and deep V–superficial IV, than in I and deep VI.

While area 17 and Tel were densely labeled uniformly across their horizontal extents, SI contained discrete radial zones of dense $\alpha$-Btx binding in register with granular sensory fields representing the forelimb, hindlimb, and trunk. In the SI barrel field, dense $\alpha$-Btx bands were situated over whisker barrels, which were identified as granular IV aggregations in Nissl-stained coronal sections (Fig. 1) or as dark patches of AChE staining in tangential sections through layer IV (P10 shown in Fig. 3). Less dense labeling characterized the zones in between the whisker barrels. The $\alpha$-Btx patches appeared slightly larger, with less distinct borders, as compared with the AChE patches, which are localized in the barrel centers. At least some of this difference may be attributable to slight diffusion of the $[^{125}\text{I}]\alpha$-Btx ligand during rinse procedures or to the spread of radioactive decay.

The radial pattern of $\alpha$-Btx binding increased in complexity and contrast from P0 (the day of birth) to around P10, and then faded to the simpler, lower contrast adult distribution (see Fig. 2), which was reached around P20. SI was the first neocortical area to show higher levels of $\alpha$-Btx binding, and could be identified in autoradiographs of P0 rats (Fig. 4). By P2, area 17 and Tel also had slightly higher binding than adjacent areas. Over the next few postnatal days, the contrast increased between primary sensory areas and adjacent cortex, and a greater number of discrete radial bands could be distinguished within the SI barrel field.

An index of radial contrast was calculated based on the measurement of the density of dark bands in the SI barrel field and in areas 17 and Tel at each age. The index was calculated as the ratio of the density of dark bands in the SI barrel field to the density in areas 17 and Tel. The index increased from P0 to around P10, and then faded to the adult distribution. The index was highest in SI, and lower in areas 17 and Tel. This suggests that the radial pattern of $\alpha$-Btx binding is more developed in SI than in areas 17 and Tel.

Fig. 1. $[^{125}\text{I}]\alpha$-Btx binding in coronal sections through a 10-day-old rat brain. A: dark radial bands mark granular areas of primary somatosensory (SI) cortex. In the SI barrel field (b, between the asterisks) dark bands tend to overlap lamina IV granular aggregations, seen in the same Nissl-stained section. Also densely labeled is a cortical area (T) representing the tongue. B: at more caudal levels, high levels of $\alpha$-Btx binding are coextensive with primary visual (A 17) and primary auditory cortex (Tel). The hippocampal formation (H) and ventral posterior nucleus of the thalamus (VP) are also designated to aid in their localization in Fig. 2.
Fig. 2. Ontogeny of [125I]a-Btx binding in rat from P0 (the day of birth) through adult (3 months). Relatively dense radial bands appear in the somatosensory cortex (rostral level, left) and in primary visual and auditory cortex (caudal level, right). Laminar and radial patterns appear in cortex as early as P0–P2 and undergo changes throughout the first 3 postnatal weeks. The radial pattern differentiates progressively until P8–P12 and subsequently fades. Note also changes in the hippocampal formation and the thalamus, particularly in the thalamic ventralposterior nucleus (see Fig. 1 for localization of brain regions). Bar = 3 mm.

on binding levels in the posteromedial barrel field compared with the more sparsely labeled dysgranular zones lateral and medial to the barrel field5 (Fig. 5A), measuring across all laminae. Values were taken from the whole extent of posteromedial barrel field rather than just the whisker barrel zones, in order to permit comparable measurements even where individual barrels were not readily distinguishable (in autoradiographs of P0–P2 and P20 or older). The ratio of binding in the posteromedial barrel field divided by that in nearby dysgranular cortex increased from an average of 1.2 at birth to a peak of 1.9 on P8, and then declined to a low of 1.1 by P20 (Fig. 5B). The developmental gain in contrast
Fig. 2 continued.
Fig. 3. Correspondence between [125I]α-Btx binding (A) and AChE activity (B) at P10. High levels of both are located in the whisker barrels, shown here in adjacent tangential sections through the flattened SI barrel field cortex.

primarily reflected a nearly 3-fold increase in α-Btx binding in the barrel field (Fig. 5A). The subsequent fading of the radial pattern during the second and third weeks reflected a fairly steep decline in α-Btx binding in the barrel field and a small, more gradual rise in nearby dysgranular neocortex.

Laminar development of α-Btx binding is illustrated in Fig. 6, which shows the autoradiographs next to the same section stained with a Nissl stain. On P0, the marginal zone (lamina I) was the most densely labeled lamina. The remainder of the neocortex showed fairly light labeling, with slightly higher label in deep VI and, in some regions, on the V–VI border. Compared with rostral levels, e.g. at the level of SI, caudal levels were delayed by a couple of days in attaining increased binding on the V–VI border. P2 lamination was generally more
distinct and consistent than at P0. The dense line along the V–VI border had widened considerably, particularly at anterior levels and in primary sensory areas. On P4, when layer IV became cytoarchitectonically distinguishable from the rest of the cortical plate, high α-Btx binding levels also first appeared in layer IV of SI. In autoradiographs of area 17 and TeI, lamina IV did not become distinct from the rest of the cortical plate until P4–P8.

Subtle variations in the laminar pattern as a function of cortical area grew more distinct over the first couple of postnatal weeks and then subsided with the fading of the radial pattern. For example, in SI cortex around P10, a transitory ‘doublet’ lamina appeared, consisting of a band in lamina IV separated from one in upper V (see Fig. 1). These two laminar lines appeared as one in other cortical areas, and were no longer distinguishable in SI by the end of the second postnatal week.

The phenomenon of transitory α-Btx binding patterns was not limited to the neocortex. For example, the thalamus and hippocampal formation showed marked developmental change, as can be seen in Fig. 2. Within the ventralposterior thalamic nucleus, the major source of input to SI, the VPM division (representing the head region) showed dense α-Btx binding in neonates (6.9 fmol/mg at P0), then declined after around P14 to low values (1.3

Fig. 5. A: the ontogeny of [125I]α-Btx binding in SI posterior-medial barrel field vs dysgranular cortex medial and lateral to SI, measured across all laminae. Data points show averages from subjects in each age group. B: the ratio of binding in the barrel field to that in nearby dysgranular cortex serves as a measure of contrast in the radial pattern. A data point is shown for each subject. Values were obtained under the binding condition described in the text and do not represent $B_{max}$.

Fig. 6. Development of the α-Btx laminar pattern. Just above each autoradiographic section is the same region Nissl-stained, with laminae indicated. The early postnatal α-Btx laminar pattern unfolds with a time course and distribution resembling that of the ingrowing thalamocortical axons (see text). CP, cortical plate (presumptive laminae II–IV).
fmol/mg) in adulthood. In contrast, the VPL (trunk regions) maintained very low levels throughout development (1.0 fmol/mg at P0, 0.7 fmol/mg at 9 weeks), as did the medial geniculate nucleus (see Fig. 2). Levels in the LGN were high at birth (7.5 fmol/mg), decreasing to moderately low by the end of the first week (2.2 fmol/mg, adults). Note that the above values were determined under the binding conditions described, and do not represent $B_{max}$.

The P10 and adult control sections treated with increasing concentrations of nicotine or carbamyl chloride showed progressively lower levels of $\alpha$-Btx binding, and there was no obvious difference in percent displacement between the two ages. Incubation with nicotine yielded average regional decreases from 15% in $10^{-7}$ M nicotine, up to 82% in $10^{-4}$ M. Binding decreased from 15% in $10^{-6}$ M carbachol to 77% in $10^{-3}$ M.

DISCUSSION

The present study is the first autoradiographic analysis of the ontogeny $\alpha$-Btx binding in the neocortex. A distinctive transitory radial pattern emerged in the rat neocortex during the first few postnatal days, revealing a neurochemical distinction between neocortical areas even before presumptive laminae II–IV of the cortical plate are distinguishable and before most cortical synapses have formed. The early association of $\alpha$-Btx binding with primary sensory cortex suggests that $\alpha$-Btx could be a useful marker for sensory cortical areas which are otherwise difficult to delineate based on immature cytoarchitecture or subcortical landmarks. Both radial and laminar features of $\alpha$-Btx binding resembled the spatiotemporal pattern of the concurrently developing thalamocortical innervation. During the third postnatal week the radial pattern faded, and the resemblance to the thalamocortical projection diminished.

Evidence for an association of $\alpha$-Btx binding sites with developing thalamocortical projections

As early as P0–P2, primary sensory areas showed denser $\alpha$-Btx binding than the surrounding neocortical areas. These primary sensory areas, which receive projections from specific sensory thalamic nuclei, include primary visual cortex (area 17), primary somatosensory cortex (SI) and primary auditory cortex (Tel). A dense radial band also appeared in granular insular cortex, which has been considered gustatory cortex, but which responds to tongue tactile and thermal stimuli. This area would be an exception to the association of $\alpha$-Btx stripes with primary sensory areas, unless the area can be classified as primary sensory cortex based on its thalamic input from the ventral posteromedial thalamic nucleus. Correspondence between $\alpha$-Btx binding and specific thalamocortical projection fields was further revealed by the organization within sensory areas. For example, SI contained discrete stripes of dense $\alpha$-Btx labeling over the whisker barrel zones, which receive specific thalamic projections, and lighter stripes over the interdigitated dysgranular zones, which are callosally interconnected (the perigranular zones of Chapin and colleagues). Primary sensory cortical areas which do not have this markedly disjunctive organization of thalamic input, such as area 17 and Tel, accordingly showed more uniform $\alpha$-Btx label across their areal extents.

The emergence of radial $\alpha$-Btx bands in immature SI cortex coincides with or perhaps slightly foreshadows the reported anatomical segregation of thalamocortical terminals into distinct radial zones. Autoradiographic studies of tritiated amino acid transport have indicated that neonatal thalamic axons from the VPM enter SI in a fairly uniform sheet, and that discrete radial clusters of afferents do not become evident until about P4, when the layer IV whisker barrels also emerge as disjunctive granular aggregations. In the present study, radial organization of $\alpha$-Btx within SI could be distinguished by P2 and was quite distinct by P4. The exact timing of the appearance of radial $\alpha$-Btx zones relative to segregation of the thalamic afferents has not yet been determined, in part due to the limited temporal resolution of the anatomical tracing methods. Development of the $\alpha$-Btx pattern progressed slightly faster in anterior (SI) than in posterior (area 17, Tel) cortical areas, an observation compatible with an anteroposterior gradient of neocortical development.

The development of laminar organization also suggests an association between $\alpha$-Btx binding and developing thalamocortical projections. On the day
of birth (P0), some thalamic axons have begun to penetrate the deepest layers of the neocortex (SI\textsuperscript{156}, area 17\textsuperscript{39}). During the first and second postnatal days, axon terminals entering laminae V and VI begin to form synapses and soon thereafter, the outer part of layer I becomes innervated. Around P2–3 in SI\textsuperscript{23,36} and P4 in area 17\textsuperscript{39}, thalamic terminals reach the base of the cortical plate (future lamina IV). This corresponds fairly well with the time course of laminar \(\alpha\)-Btx development, shown in Fig. 6 (SI). However, some discrepancies between the early laminar distribution of \(\alpha\)-Btx and thalamic input were observed. For example, whereas \(\alpha\)-Btx label appeared dense throughout the depth of lamina I, thalamic projections are primarily to superficial I\textsuperscript{144,56}. In addition, while the dense band in lamina IV did not extend up into III, thalamic axons terminate in IV and deep III\textsuperscript{56}. SI cortex on P10 and P12 had one dense \(\alpha\)-Btx band in superficial V and another in deep V, whereas thalamic innervation in V is largely confined to deep V. During the third postnatal week, the maturing laminar \(\alpha\)-Btx pattern diverged further from that of the thalamocortical pattern. In the adult, the highest levels of \(\alpha\)-Btx labeling were in deep V to upper VI, with less in upper I and least in IV. In contrast, the major thalamic sensory innervation is to IV, followed by deep V to upper VI, and then I\textsuperscript{16}.

**Methodological considerations**

The resolution of the method, about 25 \(\mu\)m (based on the smallest resolvable tear in the tissue), is adequate to study laminar distribution in newborn as well as adult neocortex. The laminar distribution of \(\alpha\)-Btx binding in the neocortex of adult rat was consistent with that shown by others using similar procedures\textsuperscript{6,19}. Results from the control sections concur with previous demonstrations that low levels of non-specific binding are present but that nicotine and acetylcholine analogues are not particularly potent displacers of \(\alpha\)-Btx\textsuperscript{6,19}. It remains to be determined whether the developmental changes in \(\alpha\)-Btx binding reported here reflect changes in binding site numbers or affinity. Arguing against the likelihood that binding affinity is the major factor are the observations that the displacement controls did not reveal an obvious difference between P10 and adult, and that in the closely related mouse, the \(K_d\) for \([^{125}\text{I}]\alpha\)-Btx binding did not change from birth through 60 days of age\textsuperscript{11}.

**Relations of \(\alpha\)-Btx binding sites to neurotransmitter systems**

At the neuromuscular junction, \(\alpha\)-Btx binds to ACh receptors having pharmacological properties similar, but not identical, to \(\alpha\)-Btx binding sites in mammalian brain\textsuperscript{7,24,38,55}. However, in adult rat brain, high affinity \([^{125}\text{I}]\alpha\)-Btx and \([^{3}\text{H}]\)nicotine binding have disparate distributions, and high affinity \([^{3}\text{H}]\)ACh binding shows the nicotine rather than the \(\alpha\)-Btx distribution\textsuperscript{6}. Whether \(\alpha\)-Btx binds to cholinergic or another functionally relevant receptor in developing cortical neurons remains to be determined.

The comparison between patterns of thalamic innervation and \(\alpha\)-Btx binding indicates substantial overlap, especially in early ontogeny, but also suggests that \([^{125}\text{I}]\alpha\)-Btx may mark only a subset of thalamic terminal regions and that additional anatomical substrates for \(\alpha\)-Btx binding should be considered. It is not yet known which cortical elements contain \(\alpha\)-Btx binding sites. In the adult, thalamocortical neurons are apparently not cholinergic\textsuperscript{22,36,37,50} (but, see Stone\textsuperscript{51}). In young rats, however, evidence has been reported for the presence of AChE activity in thalamocortical terminals\textsuperscript{34,46}. Although the extrinsic cholinergic afferents to adult neocortex originate in basal forebrain nuclei, there are as yet no reports of laminar or radial similarity between the basal forebrain\textsuperscript{45} and thalamocortical projections. As has been suggested for AChE\textsuperscript{12,33}, \(\alpha\)-Btx binding sites could appear transiently in non-cholinergic neurons or in cholinergic neurons which later switch their neurotransmitter identity. Events underlying postnatal alterations in specific laminar or radial patterns have not been identified, but might include changes in the distribution of binding sites among or within cells, growth of cortical connections, ongoing synaptic remodeling, and differential growth in laminar thickness.

Similarities between developing \(\alpha\)-Btx binding and AChE activity in immature neocortex suggest a developmental association between the two markers. Like \(\alpha\)-Btx binding, AChE activity increases transiently in the whisker barrels and in other areas of primary sensory neocortex (rat\textsuperscript{32,47}, primates\textsuperscript{39}).
Nevertheless, discrepancies in the ontogeny of these two markers suggest some differences in their distribution across cell populations or within neurons. For example, the increase in AChE activity occurs later, around P7 in area 17 and P3–6 in SI. While AChE activity increases primarily in lamina IV, dense α-Btx binding was distributed in columnar fashion, extending through the laminae. This difference was particularly obvious in tangential sections through supragranular and infragranular layers, where the uniform AChE staining across the barrel field area contrasted with the patchy distribution of α-Btx. While AChE activity essentially disappears from the whisker barrel centers by P18–20, the decline in α-Btx binding was more modest. Although a relation has been suggested between AChE activity in thalamic nuclei and sensory cortical areas, the presence or absence of transitory α-Btx binding in thalamic nuclei did not predict α-Btx levels in the respective cortical projection areas.

**Possible roles in development**

The spatiotemporal development of α-Btx binding sites suggests that they may play a unique role in shaping the developing rat neocortex, particularly its columnar organization. α-Btx can be added to the list of ligand binding sites that show changing laminar patterns indicative of dynamic developmental processes in the neocortex. However, [125I]α-Btx is unusual among markers studied so far in that it was distributed in radial zones extending through all cortical laminae. Transient, radially disjunctive patterns in rat neocortex have also been observed for some other ligands (e.g. [3H]citalopram; [3H]imipramine, personal observations; [3H]muscimol, personal observations; [3H]nicotine), but in those cases, the transient binding was primarily restricted to lamina IV and in some cases also the V–VI border. α-Btx remains distinctive among ligands reported thus far in showing a columnar pattern extending through all laminae. A functional role for such columnar organization in rodents is suggested by the radial, spindle shaped column of enhanced [14C]-2-deoxyglucose uptake in mouse SI cortex upon stimulation of a vibrissa. In keeping with the suggestion of a directive role for α-Btx binding sites in development, α-Btx radial columns appeared before the 2-deoxyglucose columns, which did not reach to infragranular layers until P6–P12.

The observation that radial organization of α-Btx distribution accompanies or precedes the radial segregation of cortical afferents suggests that these sites may influence developing cortical circuitry, particularly if α-Btx binding sites act as neurotransmitter receptors at developing cortical synapses. The emergence of radial organization apparently involves selective synapse loss during development, as evidenced by the refinement of axonal distribution of the corpus callosum. Synapse survival likely depends upon synaptic efficacy, which should require an appropriate match between the presynaptic neurotransmitter and the postsynaptic receptor type. Accordingly, the presence of α-Btx binding sites may favor the establishment or persistence of thalamocortical over cortico-cortical synapses, thereby shaping the development of columnar organization in the rodent neocortex. Characterization of the role of α-Btx binding sites in brain development depends upon ultrastructural localization of the binding sites as well as the elucidation of their physiological significance. Nevertheless, the distinctive, transitory relation between α-Btx binding site distribution and patterns of thalamocortical connectivity is compatible with the suggestion that these sites may have a role unique to developing brain.

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